

Effect of Nitrogen Fertilization and Residual Nitrogen on Biomass Yield of Switchgrass

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Abstract Switchgrass, *Panicum virgatum* L., grown for biomass has been extensively researched where the annual precipitation >760 mm and the climate varies from humid to moist-subhumid. Research is needed for areas that receive <700 mm of precipitation, where the climate varies from dry-subhumid to semiarid. The objectives were to determine (1) the effect of nitrogen fertilization on biomass production, (2) the effect of residual nitrogen on biomass production, (3) the nitrogen yield from harvested biomass, and (4) the concentration of soil organic carbon (SOC) from switchgrass plots. Plots were fertilized annually with nitrogen at the rates of 0, 40, 80, and 120 kg ha⁻¹ from 2008 to 2011 and unfertilized from 2012 to 2015. The biomass yield varied with N rate × production year interactions ($P < 0.05$), and biomass yield as a function of N rate was either linear or curvilinear depending upon production year. When fertilized, the biomass yield averaged 4.4, 9.4, 11.6, and 13.2 ± 0.4 Mg ha⁻¹ for the 0, 40, 80, and 120 kg ha⁻¹ N rates, respectively. Residual nitrogen sustained high biomass yields for 1 year after fertilization ceased. The nitrogen harvested in biomass varied with N rate × production year interactions ($P < 0.05$), and the harvested nitrogen yield as a function of N rate was linear each year. Fertilization increased the concentration of SOC an average of 1.0 ± 0.2 mg g⁻¹ of soil. The data suggest that producers could occasionally skip a year of nitrogen fertilization without detrimentally impacting the production of switchgrass biomass.

Keywords Biomass · Switchgrass · N fertilization · Residual nitrogen · Soil organic carbon

Abbreviations

USDA	US Department of Agriculture
SOC	Soil organic carbon
N	Nitrogen
P	Phosphorus
K	Potassium

Introduction

Panicum virgatum L., commonly known as switchgrass, is a tall, coarse bunchgrass, adapted to a variety of prairie habitats primarily east of the Rocky Mountains extending from Canada to Mexico in North America [1]. Switchgrass is one of the “big four” dominant species of the North American tall-grass prairie and it along with big bluestem (*Andropogon gerardii* Vit.), little bluestem [*Schizachyrium scoparium* (Michx.) Nash], and indiagrass [*Sorghastrum nutans* (L.) Nash] produces greater than 70% of the biomass of tall-grass prairie sites [2]. Some of the earliest research with switchgrass was reported in 1944 on the revegetation of cropland to native grasses [3]. That same year, “Blackwell,” one of the first switchgrass cultivars, was released [4]. Blackwell was developed from seed harvested in 1934 from a single plant growing on a native prairie site near Blackwell, OK, USA [4]. From 1940 to circa 1990, much of the research on switchgrass has revolved around the development of new cultivars for erosion control, wildlife habitat, surface mine

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vegetation, and cropland revegetation [4, 5], forage production and quality [5–11], seed production and quality [6, 12–17], and pastureland grazing [18–21]. Since circa 1990, much of the switchgrass research has focused on biomass production when grown in differing environments [22–31], nutrient utilization [31–33], carbon sequestration [34–38], and economic cost of biomass production and delivery to biomass conversion plants [39–41].

Biomass yields of switchgrass vary significantly due to cultivar grown and the growth environment, i.e., location, soil, fertilization, and precipitation. This is known as genotype \times environment (G \times E) interactions [26]. To limit G \times E interactions, Casler et al. (2004) stated that “Upland populations should not be moved south of their origin by more than one hardiness zone and lowland populations should not be moved north of their origin by more than one hardiness zone without expecting severe losses in biomass yield and survival” [42]. The limiting factors, however, that drive most biomass production are precipitation and soil fertility [43]. Many of the references cited above have addressed soil fertility in the form of N and P management, and the recommended N and P application rates were found to vary among locations. Variations in application rates are dependent upon precipitation, cultivar, and harvest management [5]. On the basis of forage analysis, switchgrass grown for biomass (a single harvest after frost) removes less N than was applied [32, 33, 44] and stores more N in belowground plant tissues, suggesting translocation of N from aboveground plant tissues to belowground tissues [44]. Similarly, other research has shown that switchgrass has a stronger response to N than to water [45]; however, with adequate soil moisture, switchgrass is capable of forming an extensive root system that may extend more than 3 m deep [46].

Over the past 20 years, switchgrass has been extensively evaluated for its bioenergy aspects. Switchgrass was chosen by US Department of Energy’s Bioenergy Feedstock Development Program, as a model species due to its high forage yields, high nutrient use efficiency, and adaptation over a wide geographic range [47]. However, much of this research has been conducted in areas where the mean annual precipitation exceeds 760 mm and where the climate varies from humid to moist-subhumid [48, 49]. Research is lacking for switchgrass biomass production in areas that receive less than 600 mm of annual precipitation where the climate varies from dry-subhumid to semiarid [50]. Furthermore, it is unknown how switchgrass biomass production will respond once fertilization ceases in a dry environment. Thus, the objectives of this study were to determine (1) the effect of nitrogen fertilization on biomass production, (2) the effect of residual nitrogen on biomass production, (3) the nitrogen yield from

harvested biomass, and (4) the concentration of soil organic carbon (SOC) from switchgrass plots.

Materials and Methods

This experiment was conducted at the USDA Agricultural Research Service Southern Plains Range Research Station, Woodward, OK (36° 25′ N, 99° 24′ W, and elevation 586 m), on a Carey silt loam soil (fine-silty, mixed, superactive, thermic Typic Argiustolls). “Alamo” switchgrass was drill planted at the rate of 4.0 kg ha⁻¹ of pure live seed into a clean seedbed on 5 June 2007. The row spacing of the drill was 20 cm and the depth of coulter was 15 mm. The plot was irrigated to assure establishment of the switchgrass but was not irrigated in subsequent years. After establishment, the three N fertility treatments and an unfertilized check treatment were arranged in a randomized complete block design replicated five times. Prior to fertilization in 2008, a baseline soil sample was collected from each plot. Six random sub-samples were collected within each plot with a T-bar soil sampling probe to a depth of 15 cm. The sub-samples were mixed in a bucket and the bulk sample was placed in a soil sample bag and dried at 40 °C and stored in sealed buckets until laboratory analysis. All samples were dried at 60 °C and ground to pass a 2-mm sieve. Samples were analyzed for pH, NO₃-N, plant available P and K, and SOC. Soil pH was measured with a glass electrode in a one-part soil to one-part water suspension and SMP buffer solution, respectively [51]. Soil NO₃-N and NH₄-N were extracted with 1 M KCl solution and quantified by the cadmium reduction method on a Lachat QuikChem 8000 (Lachat Instruments, Loveland, CO, USA). Soil available P and K was extracted using Mehlich 3 solution [52]. Mehlich 3 P was quantified colorimetrically using a Lachat, while K was analyzed by a Spectro CirOs ICP [51]. The concentration of SOC was determined using an Elementar vario MAX CN analyzer (Elementar Americas, Inc., Mt. Laurel, NJ, USA). Subsequent soil samples were collected after the last forage harvest in 2011 and 2015 and prepared and analyzed as outlined above. Nitrogen was applied to the entire plot by hand in late March (2008–2011) in the form of urea. Nitrogen application rates were 0, 40, 80, and 120 kg N ha⁻¹.

A single forage harvest was made each year after frost from 2008 to 2015 for biomass production. For each harvest, the forage dry matter (DM) yield of each plot was determined by harvesting a 1.2- \times 6.0-m area from the center of the plot to a stubble height of 10 cm. After the forage data was collected, the remaining standing crop (plot borders and edges) was harvested and removed. The harvested material of each plot was weighed, and a 250- to 300-g sub-sample of forage was collected for dry matter determination. Forage sub-samples were oven-dried at 60 °C. The DM yield of each plot was calculated by multiplying the percentage DM of the oven-

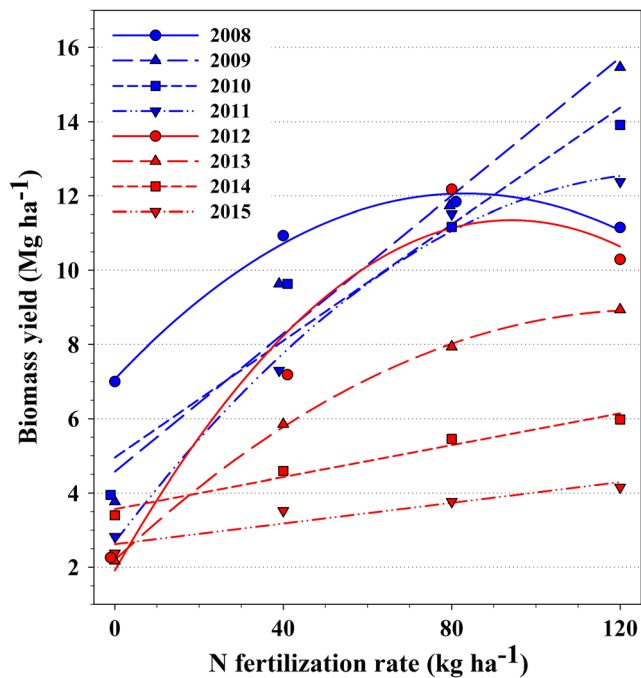


Fig. 1 The relationship of N fertilization rate on the biomass yield of “Alamo” switchgrass (*Panicum virgatum* L.) grown at Woodward, OK, USA, from 2008 to 2015. Plots were fertilized at the rates of 0, 40, 80, and 120 kg ha⁻¹ from 2008 to 2011. Plots were unfertilized from 2012 to 2015. Regression equations were as follows: $Y = -0.00072x^2 + 0.12x + 7.07$, $r^2 = 0.64$, root mean square error (RMSE) = 1.55 for 2008; $Y = 0.093x + 4.57$, $r^2 = 0.88$, RMSE = 1.61 for 2009; $Y = 0.079x + 4.95$, $r^2 = 0.71$, RMSE = 2.36 for 2010; $Y = -0.00056x^2 + 0.15x + 2.67$, $r^2 = 0.89$, RMSE = 1.46 for 2011; $Y = -0.0011x^2 + 0.20x + 1.91$, $r^2 = 0.86$, RMSE = 1.58 for 2012; $Y = -0.00042x^2 + 0.106x + 2.195$, $r^2 = 0.85$, RMSE = 1.17 for 2013; $Y = 0.021x + 3.57$, $r^2 = 0.61$, RMSE = 0.81 for 2014; and $Y = 0.014x + 2.62$, $r^2 = 0.45$, RMSE = 0.72. Some means are slightly offset for clarity

dried sub-sample by the harvested weight of the plot and converted to megagrams per hectare. Oven-dried sub-samples were ground to pass through a 1-mm screen. The nitrogen concentration of the forage was determined using an Elementar Vario MAX CN analyzer (Elementar Americas, Inc., Mt. Laurel, NJ, USA).

Data for biomass DM yield and N yield of harvested biomass were analyzed separately by year of production as mixed model analyses of variance with block and block by fertilization rate as random effects and N rate as a fixed effect [53]. Linear regression equations were fit separately for biomass DM yield and N yield of harvested biomass by least squares to obtain the best unbiased estimators of the regression parameters using N rate as the independent variable [53].

The change in SOC for fertilized years was calculated by subtracting the SOC value from each plot in 2008 from its corresponding SOC value in 2011. The change in SOC for unfertilized years was calculated by subtracting the SOC value from each plot in 2015 from its corresponding value in 2011, and the change in SOC over the life of the experiment was calculated by subtracting the SOC value from each plot in

2008 from its corresponding SOC value in 2015. Data for the changes in SOC were analyzed separately by fertilized years, unfertilized years, or life of experiment as a mixed model analyses of variance with block and block by fertilization rate as random effects and N rate as a fixed effect [53]. Nonlinear regression equations were separately fit for fertilized years, unfertilized years, and life of the experiment for the SOC data by nonlinear least squares to obtain the best unbiased estimators of the regression parameters using N rate as the independent variable [53].

Results and Discussion

For several years prior to initiating this research, the field plot was used to produce an annual hay crop of wheat (*Triticum aestivum* L.). The soil fertility of the field was minimally maintained, fertilizing with 40 kg ha⁻¹ of urea once every 2 or 3 years. The wheat crop was used mostly to reduce wind erosion of the soil, and once the crop was harvested, the stubble remained in the field until the next field planting. In March 2008, prior to fertilization, the soils sampled to a depth of 15 cm in each plot had an average pH of 7.4 ± 0.3 , NO₃-N of 1.5 ± 0.4 kg ha⁻¹, P of 11.5 ± 1.2 mg kg⁻¹, K of 114 ± 8 mg kg⁻¹, and SOC of 4.9 ± 0.1 mg g⁻¹.

Biomass Yields When Plots Were Fertilized (Years 2008–2011)

Though switchgrass biomass increased as the rate of nitrogen fertilization increased, different response patterns emerged among the years 2008–2011. In 2008, switchgrass biomass yield, as a function of N rate, was curvilinear ($P < 0.01$; Fig. 1). The biomass yield peaked at a N rate of 80 kg ha⁻¹, rising to 12.1 ± 0.5 Mg ha⁻¹ of biomass before declining. The regression equation for 2008 data was $Y = -0.00072x^2 + 0.12x + 7.07$, $r^2 = 0.64$, and root mean square error (RMSE) = 1.55, where Y is the biomass yield in megagrams per hectare and x is the rate of applied N in kilograms per hectare. The unfertilized plots, a nitrogen rate = 0, in 2008 averaged 7.1 ± 0.7 Mg ha⁻¹ of biomass. In 2008, plots received 421 mm of precipitation during the growing season (April through September). The long-term average growing season precipitation for Woodward, OK, is 434 mm.

In 2009 and 2010, the biomass yield of switchgrass responded linearly to increasing rates of nitrogen ($P < 0.01$; Fig. 1). The rate of increase was 93 ± 8 kg ha⁻¹ of biomass per unit of N in 2009 and 79 ± 11 kg ha⁻¹ of biomass per unit of N in 2010. The biomass yield of unfertilized plots decreased to 4.7 ± 0.5 Mg ha⁻¹ (an average of 2009 and 2010) suggesting a

decline in soil N. The regression equation for 2009 biomass yield was $Y = 0.093x + 4.57$, $r^2 = 0.88$, and $RMSE = 1.61$, and for 2010 biomass yield was $Y = 0.079x + 4.95$, $r^2 = 0.71$, and $RMSE = 2.36$, where Y is the biomass yield in megagrams per hectare and x is the rate of applied N in kilograms per hectare. The growing season precipitation in 2009 and 2010 was 354 and 499 mm, respectively, producing a precipitation deficit of 80 mm in 2009 and a precipitation surplus of 65 mm in 2010. Although the rate of biomass increase was lower in 2010 compared to 2009, the slopes of the two equations were homogeneous (N rate \times production year interaction, $P > 0.56$), and the effect of production year was insignificant ($P > 0.53$).

In 2011, switchgrass biomass yield was again curvilinear ($P < 0.01$; Fig. 1). The regression equation for 2011 data was $Y = -0.00056x^2 + 0.15x + 2.67$, $r^2 = 0.89$, and $RMSE = 1.46$, where Y is the biomass yield in megagrams per hectare and x is the rate of applied N in kilograms per hectare. The regression equation predicts that biomass yield would peak at 12.6 Mg ha^{-1} at a N rate of 133 kg ha^{-1} . This was outside the range of the values for N rate in this experiment; thus, the biomass yield effectively peaked at 12.5 Mg ha^{-1} at an N rate of 120 kg ha^{-1} . The biomass yield of unfertilized plots continued to decline in 2011 to $2.7 \pm 0.6 \text{ Mg ha}^{-1}$, a decline of 4.4 Mg in 4 years. The growing season precipitation in 2011 was 194 mm, 240 mm below the long-term average growing season precipitation.

The variation observed from 2008 to 2009 is not uncommon during the growth phase of perennial forage grasses. The growth phase is characterized by root, shoot, and crown development, and the expansion of roots, shoots, and crowns into available space both above and belowground level [54]. Once the growth phase is complete, the plants transition into an equilibrium phase where growth is limited by competition for light and available nutrients. A similar result was found for biomass yield of switchgrass at Stephenville, TX, for the first harvest year after establishment [24], and it has been found that full biomass production may not be achieved until the third growing season [40].

Once plants reached an equilibrium phase, the response of N fertilization was similar in 2009 and 2010. The 2008 biomass harvest of unfertilized plots removed approximately 15 kg ha^{-1} of N from the soil reducing the biomass yields of unfertilized plots in 2009 and 2010. The average N removed in the biomass in 2009 and 2010 was 4.7 ± 2.0 and $6.2 \pm 1.9 \text{ kg ha}^{-1}$, respectively. These values are similar to the average annual N received in precipitation at Woodward, OK, of $6.1 \pm 0.5 \text{ kg ha}^{-1}$ [55].

Except for the 80 kg ha^{-1} N rate in 2011, the biomass yield of N fertilization treatments was reduced due to drought. In 2011, there was a 55% reduction in growing season precipitation and a 24% reduction in dormant season precipitation. This resulted in a 15% reduction in annual biomass yield (averaged across N fertilization treatments) in 2011 (a drought

year) compared with the average of the previous 3 years. Similarly, Harlan and Ahring [6] showed a 33% reduction in switchgrass biomass yield for dryland production (5.9 Mg ha^{-1}) compared with irrigated production (8.8 Mg ha^{-1}) in OK. Heaton et al. [45] reported that switchgrass responds more to N than to water, and I agree with their statement as 73% of the variation in the present experiment was attributed to N fertilization rate compared with 3% for the environment. However, Harlan [43] stated that the first limiting factor in native plant biomass production is rainfall, and Sanderson et al. [22] verified their statement at Beeville, TX, where switchgrass failed to produce a crop under severe drought.

Biomass Yields When Plots Were Unfertilized (Years 2012–2015)

The effects of residual nitrogen on switchgrass biomass yield were present for 3 of the 4 years that plots were not fertilized. In 2012, the year nitrogen fertilization ceased, switchgrass biomass yield, as a function of residual N rate, was curvilinear ($P < 0.01$; Fig. 1). The regression equation for 2012 data was $Y = -0.0011x^2 + 0.20x + 1.91$, $r^2 = 0.86$, and $RMSE = 1.58$, where Y is the biomass yield in megagrams per hectare and x is the rate of previous applied (residual) N in kilograms per hectare. The regression equation predicts that biomass yield will peak at $11.4 \pm 0.6 \text{ Mg ha}^{-1}$ at a nitrogen rate of 94 kg ha^{-1} , but for all practical purposes, the yield peaked at $11.1 \pm 0.6 \text{ Mg ha}^{-1}$ at a residual nitrogen rate of 80 kg ha^{-1} . In 2012, the biomass yield of the plots previously fertilized at 40 and 80 kg N ha^{-1} overlapped with the biomass yields of the previous three fertilized years. Also in 2012, the biomass yield of unfertilized plots (previous nitrogen rate = 0) fell below 2.0 Mg ha^{-1} suggesting a continued loss of N in the soil (Fig. 1). Not fertilizing in 2012 reduced the biomass yield of plots that were previously fertilized at 120 kg N ha^{-1} . In 2011, the plots receiving 120 kg ha^{-1} of N fertilizer had an average biomass yield of $12.5 \pm 0.6 \text{ Mg ha}^{-1}$. In 2012, the same plots, with only residual nitrogen, produced an average biomass yield of $10.6 \pm 0.7 \text{ Mg ha}^{-1}$. Although it is not known what specifically caused this decline in biomass yield, it is possible that the culm density declined due to drought during the 2011 and 2012 growing seasons. The average growing season precipitation was reduced by 55% in 2011 and 40% in 2012. Culms have been shown to comprise the majority of the aboveground biomass in switchgrass [44] and lower culm densities would greatly reduce switchgrass biomass yield.

Although residual nitrogen effects were present, the switchgrass biomass yield began to decline in 2013. There was a curvilinear response to biomass yield as a function of residual N (rate) in 2013 ($P < 0.01$; Fig. 1). The regression equation for 2013 data was $Y = -0.00042x^2 + 0.106x + 2.195$, $r^2 = 0.85$, and $RMSE = 1.17$, where Y is the biomass yield in

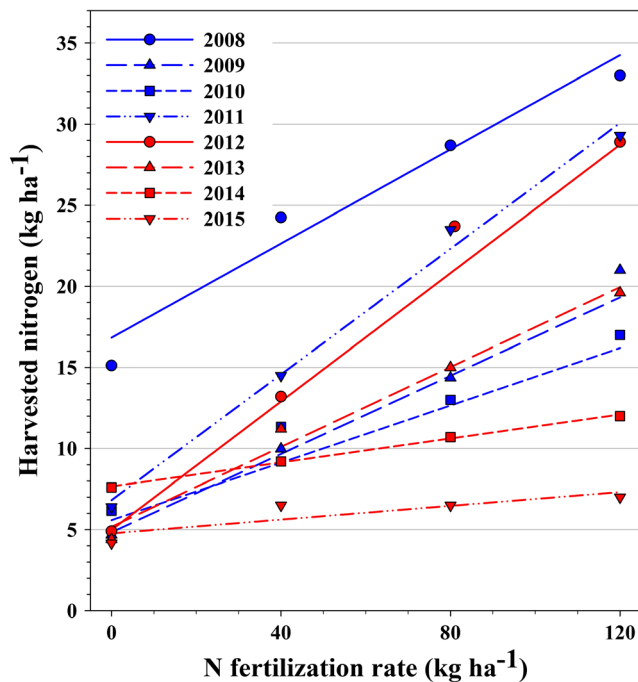


Fig. 2 The relationship of N fertilization rate on harvested nitrogen in the biomass yield of “Alamo” switchgrass (*Panicum virgatum* L.) grown at Woodward, OK, USA, from 2008 to 2015. Plots were fertilized at the rates of 0, 40, 80, and 120 kg ha⁻¹ from 2008 to 2011. Plots were unfertilized from 2012 to 2015. Regression equations were as follows: $Y = 0.15x + 16.8$, $r^2 = 0.50$, RMSE = 6.7 for 2008; $Y = 0.12x + 4.8$, $r^2 = 0.87$, RMSE = 2.2 for 2009; $Y = 0.09x + 5.6$, $r^2 = 0.67$, RMSE = 3.0 for 2010; $Y = 0.19x + 6.8$, $r^2 = 0.91$, RMSE = 2.9 for 2011; $Y = 0.20x + 5.0$, $r^2 = 0.93$, RMSE = 2.7 for 2012; $Y = 0.12x + 5.2$, $r^2 = 0.79$, RMSE = 3.0 for 2013; $Y = 0.04x + 7.7$, $r^2 = 0.59$, RMSE = 1.4 for 2014; and $Y = 0.02x + 4.8$, $r^2 = 0.31$, RMSE = 1.5 for 2015. Some means are slightly offset for clarity

megagrams per hectare and x is the rate of previously applied (residual) N in kilograms per hectare. The growing season precipitation in 2013 was 423 mm, only 11 mm below the long-term average growing season precipitation.

The biomass yield also continued to decline without fertilization in 2014. In 2014, the biomass yield of switchgrass responded linearly to residual N rates ($P < 0.01$; Fig. 1). The regression equation for 2014 data was $Y = 0.021x + 3.57$, $r^2 = 0.61$, and RMSE = 0.81, where Y is the biomass yield in megagrams per hectare and x is the rate of previous applied (residual) N in kilograms per hectare. The rate of increase was 21 ± 5 kg ha⁻¹ of biomass per unit of residual N. This rate is four times smaller than the rates of increase in 2009 and 2010. The growing season precipitation in 2014 was 412 mm, 22 mm below the long-term average growing season precipitation.

Biomass yield decreased again in 2015. The regression equation for 2015 data was $Y = 0.014x + 2.62$, $r^2 = 0.45$, and RMSE = 0.71, where Y is the biomass yield in megagrams per hectare and x is the rate of previous applied N in kilograms per hectare. Four years after fertilization ceased, the effects of residual N were still present. The growing season precipitation

in 2015 was 573 mm, 139 mm greater than the long-term average growing season precipitation.

In an attempt to determine when the effects of residual N rate would become insignificant, a linear regression model was used on a subset of the data for the unfertilized years (2012–2015). Using only the 120 kg ha⁻¹ N rate data, a regression equation was fit for biomass yield as the dependent variable and the number of years from 2011 as the independent variable. The regression equation for these data was $Y = -2.13x + 12.7$, $r^2 = 0.82$, and RMSE = 1.17, where Y is the biomass yield in megagrams per hectare and x is the number of years from 2011, i.e., for 2012, $x = 1$; for 2013, $x = 2$; ...; for 2016, $x = 5$. Substituting 2.55 Mg ha⁻¹ in the equation above for the value of Y (2.55 is the minimum biomass yield of the check plots (0 kg ha⁻¹) for the years 2012–2015) and solving for x , the value of $x = 4.8$ years. Thus, it is estimated that it would take approximately 5 years to lose the effects of the residual nitrogen.

Nitrogen Removed in Harvested Biomass (Years 2008–2015)

For the fertilized years (2008–2011), the amount of nitrogen removed in biomass varied with N rate \times production year interactions, and the harvested nitrogen yield as a function of N rate was linear each year (Fig. 2; $P < 0.05$). In 2008, the growth phase in the life of the plant stand, there was considerable variation among plots for the concentration of nitrogen in the plant tissue which affected the amount of nitrogen removed in biomass. The N removal rate in 2008 was 0.15 ± 0.04 kg per unit of applied N. The regression equation for 2008 data was $Y = 0.15x + 16.8$, $r^2 = 0.50$, and RMSE = 6.7, where Y is the amount of nitrogen removed in biomass in kilograms per hectare and x is the rate of applied N in kilograms per hectare (Fig. 2). The April–September precipitation in 2008 was 421 mm, 13 mm below average.

The plant stand would have been in a state of equilibrium in 2009 and 2010. The rate of nitrogen removal in 2009 was slightly lower than in 2008, but the intercept was significantly lower. The regression equation for 2009 data was $Y = 0.12x + 4.8$, $r^2 = 0.87$, and RMSE = 2.2, where Y is the amount of nitrogen removed in biomass in kilograms per hectare and x is the rate of applied N in kilograms per hectare (Fig. 2). The April–September precipitation in 2009 was 354 mm, 80 mm below average. The regression equation for 2010 data was $Y = 0.09x + 5.6$, $r^2 = 0.67$, and RMSE = 3.0, where Y is the amount of nitrogen removed in biomass in kilograms per hectare and x is the rate of applied N in kilograms per hectare (Fig. 2). The growing season precipitation in 2010 was 499 mm, 65 mm above average. Although the rate of nitrogen removal was lower in 2010 compared to 2009, the slopes of the two equations were homogeneous (N rate \times production

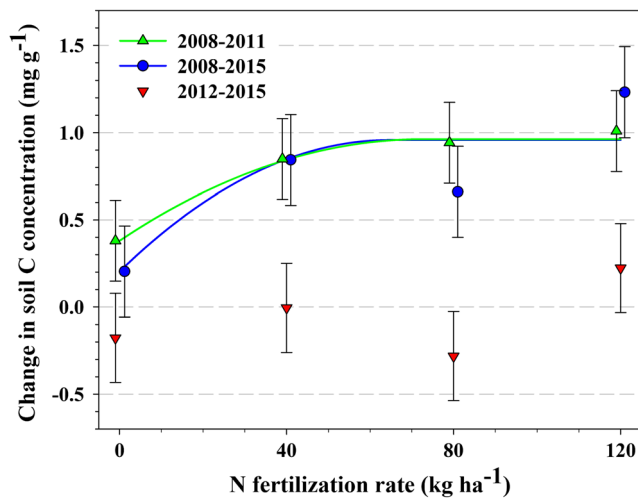


Fig. 3 The relationship of N fertilization rate on the change in soil organic carbon (SOC) for plots planted to “Alamo” switchgrass (*Panicum virgatum* L.) grown at Woodward, OK, USA, from 2008 to 2015. Plots were fertilized at the rates of 0, 40, 80, and 120 kg ha⁻¹ from 2008 to 2011, and plots were unfertilized from 2012 to 2015. For plots sampled in 2011, after 4 years of fertilization, the regression equation was as follows: $Y = -0.00011x^2 + 0.016x + 0.38$, $r^2 = 0.22$, RMSE = 0.50, where the equation plateaued at 1.0 ± 0.2 mg g⁻¹ at a N rate of 74 kg ha⁻¹ ($P < 0.13$, green triangle = mean \pm SE). For plots sampled in 2015, after 4 years of fertilization and followed by 4 years without fertilization, the regression equation was as follows: $Y = -0.00018x^2 + 0.023x + 0.20$, $r^2 = 0.23$, RMSE = 0.60, where the equation plateaued at 0.9 ± 0.2 mg g⁻¹ at a N rate of 64 kg ha⁻¹ ($P < 0.10$, blue circle = mean \pm SE). No relationship was found between N fertilization rate and SOC during the period 2012–2015 (unfertilized years, $P > 0.60$, red inverted triangle = mean \pm SE). Some means are slightly offset for clarity

year interaction, $P > 0.10$), and the effect of year was insignificant ($P > 0.60$).

In 2011, a drought year, the rate of nitrogen removal was 0.19 ± 0.01 kg per unit of applied N. This was approximately 111% higher than the rate of nitrogen removal in a wet year, 2010. The regression equation for 2011 data was $Y = 0.19x + 6.8$, $r^2 = 0.91$, and RMSE = 2.9, where Y is the amount of nitrogen removed in biomass in kilograms per hectare and x is the rate of applied N in kilograms per hectare (Fig. 2). The growing season precipitation in 2011 was 194 mm, 240 mm below average.

The exact cause of the interactions among years is unknown, but the environment may be a large contributor to the variation. Rainfall has been shown to enhance the removal of nutrients from senescing plant tissue [56], and switchgrass leaf litter has been shown to contain a significant amount of nitrogen [44]. Moisture stress has also been shown to increase leaf nitrogen concentration in switchgrass [57]. This was evident in 2011, a drought year, where the N concentration in biomass (pooled over N rates) was 69% greater than in 2010, a wet year.

For the unfertilized years (2012–2015), the amount of nitrogen removed in biomass also varied with previous N rate \times

production year interaction (Fig. 2; $P < 0.05$). The nitrogen removal rate in 2012 was 0.20 ± 0.01 kg per unit of residual N, and it was similar to that of 2011. A test of the homogeneity of the two equations found that the slopes were homogeneous (N rate \times production year interaction, $P > 0.80$), and the effect of year was insignificant ($P > 0.20$). As mentioned earlier, 2012 was a drought year, receiving only 60% of its average growing season precipitation. The regression equation for 2012 data was $Y = 0.20x + 5.0$, $r^2 = 0.93$, and RMSE = 2.7, where Y is the amount of nitrogen removed in biomass in kilograms per hectare and x is the rate of previously applied (residual) N in kilograms per hectare (Fig. 2). Not fertilizing in 2012 had little effect on switchgrass biomass yield, as described above, and virtually no effect on the rate of nitrogen removal in the biomass.

The nitrogen removal rate in 2013 was similar to that of 2009 and 2010. The rate of nitrogen removal in 2013 was 0.12 ± 0.01 kg per unit of previously applied N. The regression equation for 2013 data was $Y = 0.12x + 5.2$, $r^2 = 0.79$, and RMSE = 3.0, where Y is the amount of nitrogen removed in biomass in kilograms per hectare and x is the rate of previously applied (residual) N in kilograms per hectare (Fig. 2). Again, the slopes of the three equations for 2009, 2010, and 2013 were homogeneous (N rate \times production year interaction, $P > 0.15$), and the effect of year was insignificant ($P > 0.88$). Although the biomass yields of plots in 2013 were lower than those in 2009 or 2010, the rates of nitrogen removal remained the same. This may also suggest that switchgrass plants in 2013 were trying to maintain the equilibrium between nutrient availability and above- and belowground biomass.

The rate of nitrogen removal dropped to 0.04 ± 0.01 kg per unit of residual N in 2014 compared to 2013. The regression equation for 2014 data was $Y = 0.04x + 7.7$, $r^2 = 0.59$, and RMSE = 1.4, where Y is the amount of nitrogen removed in biomass in kilograms per hectare and x is the rate of applied (residual) N in kilograms per hectare (Fig. 2). With the drop in nitrogen removal rate and a corresponding decline in biomass yield across residual N rates (Fig. 1), the switchgrass plants were still trying to maintain equilibrium between biomass yield and plant nutrients.

The year 2015 was different than the previous 7 years in terms of harvested nitrogen ($P < 0.05$). The rate of nitrogen removal declined to 0.02 ± 0.01 kg per unit of residual N. The regression equation for 2015 data was $Y = 0.02x + 4.8$, $r^2 = 0.31$, and RMSE = 1.5, where Y is the amount of nitrogen removed in biomass in kilograms per hectare and x is the rate of applied (residual) N in kilograms per hectare (Fig. 2). At this point in the experiment, the effect to residual nitrogen on biomass yield was shown to be insignificant, and for all practical purposes, the amount of nitrogen recovered in biomass has reach its minimum for this site. The fact that precipitation was not a limiting factor in 2015 would lead one to conclude that nitrogen had become the limiting factor, and that switchgrass biomass yield was in equilibrium with nitrogen.

Soil Organic Carbon

Considerable variation existed among the experimental units for the concentration of SOC in 2008 (the baseline data). In 2008, the concentration of SOC among all plots averaged 4.9 ± 0.1 mg of carbon per gram of soil. Nitrogen fertilization was found to increase the concentration of SOC in this experiment. After 4 years of nitrogen fertilization (2008–2011), the change in SOC concentration (the concentration of SOC in 2011 minus that of 2008) followed the equation $Y = -0.00011x^2 + 0.016x + 0.38$, $r^2 = 0.22$, and RMSE = 0.50, where Y is the change in SOC concentration in milligrams per gram of soil and x is the rate of applied N in kilograms per hectare, and where the equation plateaued at 1.0 ± 0.2 mg g⁻¹ of soil at a nitrogen fertilization rate of 74 kg ha^{-1} ($P < 0.13$, Fig. 3). The accumulation of SOC near the soil surface was undoubtedly due to replacement of the annual cropping system with that of a perennial cropping system. Well-managed switchgrass has been shown to accumulate SOC in the upper 15 cm of the soil profile [37].

Four years after fertilization ceased (2011–2015), the change in SOC concentration (the concentration of SOC in 2015 minus that of 2011) was effectively zero (-0.06 ± 0.13 mg of carbon g⁻¹ of soil), and no relationship was found between SOC and the previous nitrogen rates (2012–2015, $P = 0.60$).

After 8 years of biomass harvests, the SOC concentration increased during 4 years of nitrogen fertilization and once fertilization ceased, the concentration of SOC over the next 4 years did not decline. So after 8 years, the SOC concentration was comparable to that of fertilized years (Fig. 3). The change in SOC concentration (the concentration of SOC in 2015 minus that of 2008) followed the equation $Y = -0.00018x^2 + 0.023x + 0.20$, $r^2 = 0.23$, and RMSE = 0.60, where Y is the change in SOC concentration in milligrams per gram of soil and x is the rate of applied N in kilograms per hectare, and where the equation plateaued at 0.9 ± 0.2 mg g⁻¹ of soil at a nitrogen fertilization rate of 64 kg ha^{-1} ($P < 0.10$, Fig. 3). Thus, the absence of nitrogen fertilization in years 2011–2015 resulted in little or no loss of SOC in fertilized treatments. Further research is needed to determine the rate of SOC accumulation throughout the soil profile for well-managed switchgrass grown in dry-subhumid to semiarid environments.

Conclusions

Switchgrass biomass yields in excess of 10 Mg ha^{-1} are possible in areas with 600 mm of annual precipitation and where the climate varies from dry-subhumid to semiarid. Annual nitrogen fertilization of switchgrass at 80 kg ha^{-1} provided for sustainable biomass yields that averaged $11.7 \pm 0.4 \text{ Mg ha}^{-1}$. This level of nitrogen fertilization was least affected by environmental conditions (such as drought in

2011) as indicated by the overlapping of annual biomass yields from 2008 to 2011 (Fig. 1). The switchgrass biomass yield the first year after nitrogen fertilization ceased in 2012 averaged $12.2 \pm 0.6 \text{ Mg ha}^{-1}$ for the plots that had received 80 kg ha^{-1} of N the previous 4 years. Residual nitrogen effects failed to sustain high forage biomass yields the second through fourth years after nitrogen fertilization ceased. The data suggest that producers could occasionally skip a year of nitrogen fertilization without detrimentally impacting the production of switchgrass biomass. Additional research is needed to determine the rate of SOC accumulation in the soil profile for well-managed switchgrass grown in dry-subhumid to semiarid environments.

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