

## Yield Potential and Nitrogen Requirements of *Miscanthus* × *giganteus* on Eroded Soil

Matt A. Yost,\* Bryan K. Randall, Newell R. Kitchen, Emily A. Heaton, and Robert L. Myers

### ABSTRACT

*Miscanthus* × *giganteus* yield and fertilizer N requirements have been well studied in Europe and parts of the United States, but few reports have investigated its production on eroded claypan soils economically marginal for grain crops. This study was conducted to evaluate yield potential and fertilizer N strategies for young and mature *M. × giganteus* on eroded soils. Constant (medium, high) and priming (low, medium, high) fertilizer N rates were applied during 2013 to 2015 at three locations in Missouri to young stands planted in 2012. Constant rates (low, medium, high) also were applied to one mature stand planted in 2007. Winter biomass yield (13.3–23.8 Mg ha<sup>-1</sup>) was comparable to more productive soils and increased with N fertilization at only two sites with young stands. At one location, yield increased by 44, 48, and 82% with fertilization in 2013, 2014, and 2015, respectively, while the other location increased by 43% only in 2015. At both locations, the medium constant rate (67 kg N ha<sup>-1</sup> yr<sup>-1</sup>) was the lowest rate that maximized total yield across 3 yr. This rate was efficient as it did not increase N removal in harvested biomass. Fertilization always increased leaf chlorophyll concentration (LCC) and site-years with relative LCC <0.85 had yield response to N, while those at or above this level did not. Nitrogen fertilization is not always needed for *M. × giganteus* on eroded soils with low organic matter content, and relative LCC can accurately identify when fertilization is required.

### Core Ideas

- *Miscanthus*'s performance on eroded claypan soils was comparable to other more productive soil.
- Only 4 of 12 site-year combinations required fertilizer N to maximize *Miscanthus* yield.
- Nitrogen priming had no benefit and the constant rate of 67 kg N ha<sup>-1</sup> yr<sup>-1</sup> was the lowest that maximized total yield.
- Relative leaf chlorophyll concentration in June is a reliable indicator of yield response to N.

**M**ISCANTHUS × GIGANTEUS (subsequently referred to as *Miscanthus*) is a plausible renewable energy feedstock option because it is more productive than most C<sub>4</sub> perennial grasses, is more efficient than almost all crops at converting solar energy into biomass (Dohleman et al., 2012), typically requires few agrochemical inputs (Cadoux et al., 2012), can potentially improve soil quality or health, and can reduce negative environmental impacts of agriculture (McLaughlin and Walsh, 1998; Heaton et al., 2004, 2008). Other markets in the United States, besides producing second generation ethanol, have begun to emerge for *Miscanthus*, including use as poultry bedding, flooring material, general absorbent, and soluble fiber (MFiber, 2016). These markets are good for local economies and provide growers with other options in land use, especially in areas that are marginally profitable with arable crops.

Marginal or eroded soils are primary targets for fuel and fiber crop development because they avoid displacement of productive cropland currently used for food and feed production (Robertson et al., 2008; Searchinger et al., 2008). Eroded soils, particularly as they pertain to some parts of the southern U.S. Corn Belt, have major concerns of water erosion, flooding, limited plant available water capacity, and maintenance of organic matter (USDA-NRCS, 2006). Water erosion, accelerated by modern agricultural practices, has severely degraded cropland productivity of these sensitive soil areas and resulted in significant topsoil loss (USDA-NRCS, 2006; Lerch et al., 2008). These issues on eroded soils are generally further exacerbated when restrictive claypans are present at shallow depths. As a result, eroded claypan soils can often have marginal profitability due to suppressed grain yield or greater risk of crop failure, especially for corn (*Zea mays* L.) (Massey et al., 2008).

*Miscanthus* is generally well-suited for a range of soil conditions, but may perform best in soils with high water-holding capacity and adequate soil moisture (Heaton et al., 2004, 2010). Pyter et al. (2009) noted that yield in Europe showed no apparent relationship to soil type but required 3 to 5 yr to obtain maximum yield on poor soil in contrast to 2 to 3 yr on

M.A. Yost and N.R. Kitchen, Cropping Systems and Water Quality Research Unit, U.S. Department of Agriculture-Agricultural Research Service, University of Missouri, 269 Agric. Eng. Bldg., Columbia, MO 65211; B.K. Randall, Dow AgroSciences, Billings, MT 59102; E.A. Heaton, Department of Agronomy, 1403 Agronomy Hall, Iowa State University, Ames, Iowa 50010; R.L. Myers, N.C. Sustainable Agriculture Research and Education, University of Missouri, 238 Agric. Eng. Bldg., Columbia, MO 65211. \*Corresponding author (yostma@missouri.edu).

**Abbreviations:** LCC, leaf chlorophyll concentration; PMN, soil potentially mineralizable nitrogen; SPAD, soil plant analysis development.

Published in *Agron. J.* 109:684–695 (2017)

doi:10.2134/agronj2016.10.0582

Received 12 Oct. 2016

Accepted 6 Jan. 2017

Supplemental material available online

Copyright © 2017 by the American Society of Agronomy  
5585 Guilford Road, Madison, WI 53711 USA  
All rights reserved

more fertile soils. Slower establishment on poorer soils also was confirmed in trials across Illinois where two sites with poorer soil (land capability class 3w) reached maximum yield slower than five others with more productive soils (Arundale et al., 2013). Slower establishment on poorer soils has not, however, consistently led to lower maximum or ceiling yields in the United States. At these same seven Illinois locations, the maximum yield of mature stands (5–7-yr old) varied across locations and did not consistently improve as soil productivity or capability improved. Although some studies in the United States have evaluated production capacity of *Miscanthus* on eroded soils, few trials have been conducted on eroded soils with a claypan. These estimates of productivity are critical because claypan soils occupy more than 3 million ha in Missouri and Illinois, and this land base could contribute significantly to future bioenergy feedstock production.

An important ongoing question regarding the production and sustainability of *Miscanthus* is whether fertilizer N is required to increase and maintain yield. In general, stands in North America have had higher yield and greater frequency of yield response to N than European stands (e.g., Christian et al., 2008; Arundale et al., 2013; Finnan and Burke, 2014; Haines et al., 2014; Davis et al., 2015). However, studies in both continents have shown that yield response to fertilizer N can vary considerably among sites and among years within sites. This variation has not corresponded directly to *Miscanthus* stand age, and it may be even more rampant on eroded claypan soils because they are generally low in organic matter, high in N loss, and have highly variable plant available water capacity. The lack of N response in some young and mature stands has been attributed to the ability of the plant to recycle nutrients back into the rhizome before senescence (Heaton et al., 2009; Dohleman et al., 2012), high soil N levels or N mineralization rates (Himken et al., 1997; Clifton-Brown et al., 2007; Christian et al., 2008; Davis et al., 2013), and/or atmospheric N deposition from possible symbiotic relationships of free-living N fixing organisms associated with *Miscanthus* roots (Eckert et al., 2001; Miyamoto et al., 2004; Christian et al., 2008). Although *Miscanthus* can effectively recycle N, stands in the United States remove between 20 and 116 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Heaton et al., 2009; Haines et al., 2014;

Singh et al., 2015; Masters et al., 2016), which may necessitate maintenance fertilizer be added in the long-term (Miguez et al., 2008) if soil N mineralization or possible N fixation is less than removal.

The yield and amount of fertilizer N required by *Miscanthus* is influenced by its ability to move N and carbohydrates between aboveground and belowground portions of the plant during key moments of the growing season. Beale and Long (1997) found that in the spring, N moved from the rhizome and roots into the aboveground biomass until July when maximum levels were attained. From July until the end of the season, N content then reversed as N remobilized from the aboveground biomass back to the belowground biomass until senescence. This same trend in aboveground biomass N content was confirmed using LCC (Finnan and Burke, 2014), which is strongly related to N content (Evans, 1989), and can be measured accurately with chlorophyll meter sensing (Markwell et al., 1995) such as soil plant analysis development (SPAD). Leaf chlorophyll concentration has been successfully used to predict fertilizer N requirements of several crops (e.g., Bullock and Anderson, 1998), but has not been evaluated for this purpose in *Miscanthus*.

All previous *Miscanthus* studies have evaluated annual fertilizer N requirements. As a perennial crop that efficiently recycles applied N through remobilization and storage in belowground tissue (Christian et al., 2006), N fertilization strategies that are “maintenance-like” or multi-year in nature may be beneficial. Priming the N cycle by adding more N upfront to help the crop establish then reducing later application rates to maintain growth and removal may be effective. These types of fertilization strategies need to be evaluated for *Miscanthus* to most efficiently manage this potentially profitable bioenergy and industrial crop on eroded claypan soils. Furthermore, tools are needed to annually predict when fertilizer N will be required. Therefore, the objectives of this research were to: (i) determine the yield potential of *Miscanthus* on eroded claypan soils; (ii) evaluate the effects of N fertilization strategies (constant vs. priming rates) on seasonal LCC, biomass yield, biomass N concentration and content at harvest and soil N dynamics in both young and mature *Miscanthus* stands on eroded soils; and (iii) determine whether LCC is a reliable tool for identifying responsiveness to N.

**Table 1. Geographic location, official soil series descriptions from USDA-NRCS, and measured background soil fertility to 0.3-m depth in control plots in January 2015 at four Missouri sites.**

| Experiment | Site     | Latitude, longitude  | Taxonomy, drainage, slope, and land capability class  | Soil characteristics† |                          |                              |          |  |
|------------|----------|----------------------|---|-----------------------|--------------------------|------------------------------|----------|--|
|            |          |                      |   | pH                    | OM<br>g kg <sup>-1</sup> | P<br>— kg ha <sup>-1</sup> — | K<br>571 | CEC<br>mol <sub>c</sub> kg <sup>-1</sup> |
| Young      | Boone–LT | 38.9091,<br>–92.2769 | Mexico silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualf), poorly drained, slope 1%, 3e‡         | 6.1                   | 20                       | 73                           | 571      | 15.9                                     |
|            | Cooper   | 38.9426,<br>–92.7533 | Weller silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualf), moderately well drained, slope 5%, 3e | 6.2                   | 17                       | 56                           | 622      | 16.3                                     |
|            | Moniteau | 38.7001,<br>–92.3886 | Menfro silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualf), well drained, slope 5%, 3e            | 5.8                   | 20                       | 11                           | 510      | 17.7                                     |
| Mature     | Boone–JF | 38.9068,<br>–92.2696 | Mexico silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualf), poorly drained, slope 2%, 3e          | 6.7                   | 26                       | 255                          | 586      | 15.8                                     |

† OM, organic matter; P, Bray I P; K, ammonium acetate exchangeable K; CEC, cation exchange capacity.

‡ Class 3e soils have severe limitations (high risk of erosion) that require conservation practices.

## MATERIALS AND METHODS

### Site Descriptions

This research was defined by two different N rate experiments. The first experiment was conducted during 2013 to 2015 on young *Miscanthus* stands planted in 2012 at the University of Missouri South Farm lone tree field (denoted as Boone–LT) and two producer fields, one in Cooper County and one in Moniteau County, Missouri (denoted as Cooper and Moniteau). The second experiment was conducted during 2012 to 2015 on a mature *Miscanthus* stand planted in 2007 in Boone County, Missouri, at the Jefferson Farm, adjacent to the University of Missouri South Farm (denoted as Boone–JF). The sites were selected to represent marginal soils of the Midwest. All four sites had land capability classifications of 3e, which are soils with high risk of erosion, severe limitations on the choice of productive plants, and/or with special conservation practice requirements. A summary of site characteristics, soil descriptions, and general soil fertility in 2015 are reported in Table 1.

Daily weather data (Fig. 1) for Boone–LT and Boone–JF were obtained from a weather station at the University of Missouri South Farm, and for Cooper and Moniteau were obtained from the nearest National Weather Service station to the site from the Utah State University Climate Database Server. For Cooper, the weather station reporting air temperature and precipitation were at distances of 2.3 and 8.5 km

from the study site, respectively. For Moniteau, air temperature and precipitation were obtained from the Columbia Regional Airport, located 19 km from the study site. This station also was used to obtain 30-yr (1981–2010) normal air temperature and precipitation for all locations. A base temperature of 10°C was subtracted from the daily average air temperatures, and all resulting daily temperatures above 0°C were summed to determine cumulative growing degree days.

### Young *Miscanthus* Stand Experiment

Prior to *Miscanthus* establishment in 2012, Boone–LT was cropped with corn and soybean (*Glycine max* L.) that had annual tillage, Cooper was a long-term grass mixture not harvested as a part of the Conservation Reserve Program, and Moniteau was a long-term grass mixture with annual harvests. All three locations had no recent livestock grazing or manure applications. In the spring of 2012 at all three sites, plots were tilled with a disk chisel and *Miscanthus* rhizomes were planted. At Boone–LT, rhizomes were hand planted at 0.75-m spacing within and between rows to a depth of 10 cm. At the two sites on producer fields, rhizomes were planted with a field-scale planter at approximately 2 rhizomes m<sup>-2</sup> and to a depth of 10 cm. Rhizomes for all three sites were the Illinois clone cultivar, which has been certified by the Ohio Seed Improvement Association quality assurance program (USDA-FSA, 2012). In the spring of 2013, four replications of six

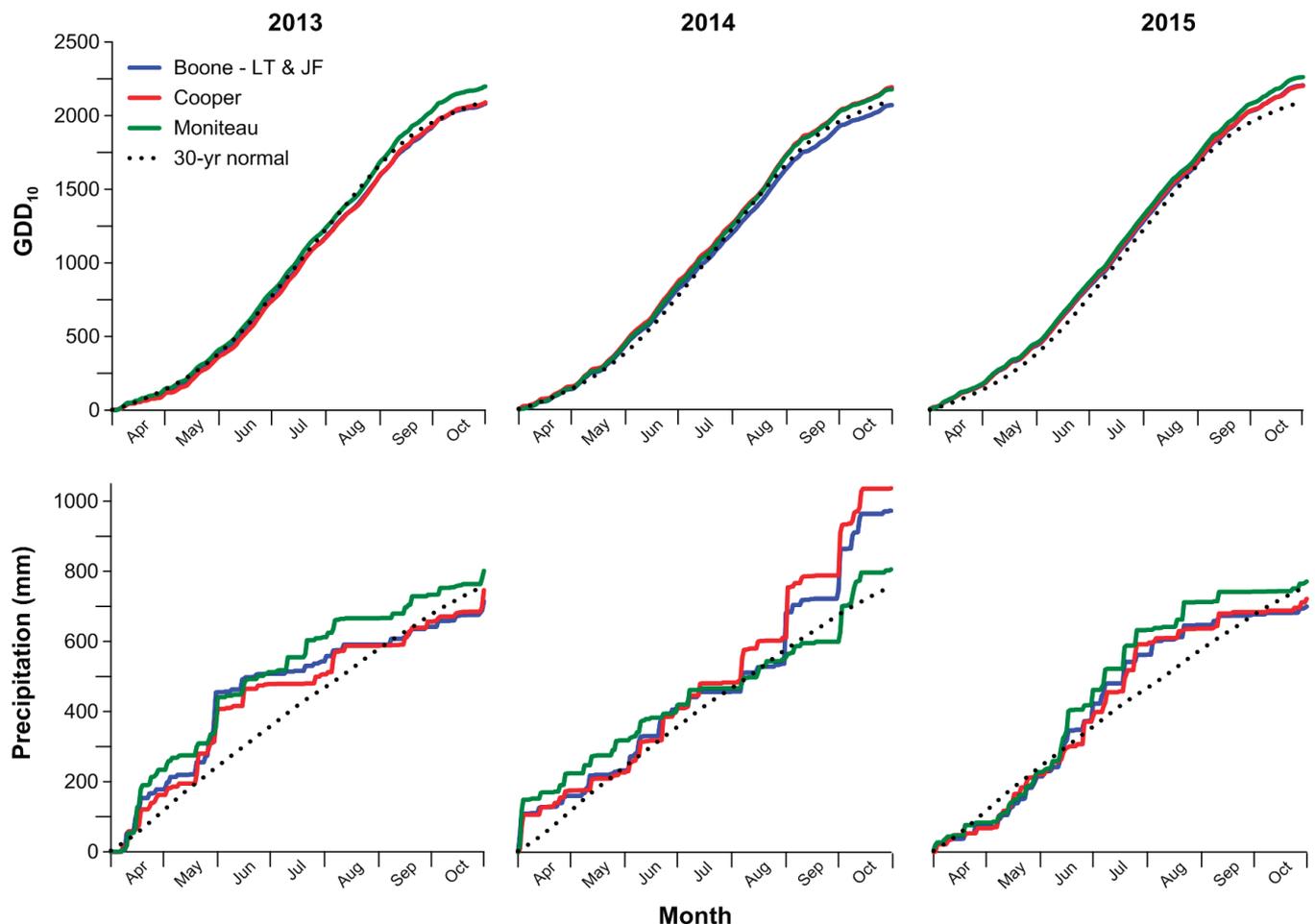


Fig. 1. (top) Growing season cumulative annual growing degree days using a base temperature of 10°C (GDD<sub>10</sub>) and (bottom) cumulative precipitation, along with their respective 30-yr (1981–2010) normals at four Missouri N trial sites in 2013, 2014, and 2015.

fertilizer N rates were established in a randomized complete block design. Fertilizer N plot dimensions varied between locations and were 3.8 by 9.1 m at Boone–LT and 3 by 4 m at Cooper and Moniteau. Fertilizer treatments of 0, 34, 67, 67, 134, or 134 kg N ha<sup>-1</sup> were broadcast by hand on 20 May using the stabilized granular urea SUPERU (Koch Agronomic Services, LLC, Wichita, KS). In both 2014 and 2015, the same application method, source, and date were used but previous fertilizer N treatments were staggered to create a range of constant and N priming rates over 3 yr (Table 2). No additional N as fertilizer or manure was applied to the plots by cooperating growers. Herbicides were used as needed to control weeds during *Miscanthus* establishment in 2012 and included 2,4-D [(2,4-dichlorophenoxy) acetic acid] and glyphosate [*N*-(phosphonomethyl) glycine in the form of its isopropylamine salt].

### Mature *Miscanthus* Stand Experiment

The Boone–JF site was established in 2007 by planting plugs from the *Miscanthus* clone FSC 2006, which was imported from Germany but originated from Southeast Asia during World War II. Plugs were planted at 1-m spacing into tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] sod that had been planted in the fall of 2006 and then killed with glyphosate in May prior to *Miscanthus* planting on 1 June. Water was applied to each plug by hand at planting and twice after planting due to dry summer conditions. The terminated tall fescue provided excellent weed control so the only herbicide applied after *Miscanthus* planting was spot-applied glyphosate in late summer of 2007.

From 2007 to 2010, 56 kg N ha<sup>-1</sup> yr<sup>-1</sup> was applied in April or early May as either broadcast NH<sub>4</sub>NO<sub>3</sub> or granular CH<sub>4</sub>N<sub>2</sub>O. During this time, no P and K were applied due to high soil test values caused by livestock manure applications prior to 2006. In 2008 and 2009, the plots were mowed in the early spring and residue was left in place. In 2010, plots were burned in early spring. In 2011, no N fertilizer was applied and the plots were harvested after senescence in the early winter. The present study began in 2012 with the establishment of three replications of four fertilizer N rates organized in a randomized complete block design. Fertilizer N rate plots measured 3.7 by 13.7 m and rates of 0, 45, 90, or 134 kg N ha<sup>-1</sup> were broadcast by hand on 17 May using SUPERU. In 2013, no fertilizer N was applied, and in 2014 and 2015, the same rates from 2012 were re-applied on the same plots on 20 May (Table 2).

### Response Measurements

Each year (2013–2015) in both experiments, SPAD chlorophyll meter (SPAD-502, Minolta Camera Co. Ltd., Osaka, Japan) readings were taken at all four sites in each N rate plot at 4-wk intervals from mid-June to mid-September in 2013 and from mid-May to mid-September in 2014 and 2015. The one exception was that no readings were collected at Cooper in September 2014. Where measured, the SPAD reading for each plot was the average of readings collected on the middle of 20 to 30 randomly selected leaves in the mid-canopy. Relative LCC or SPAD was calculated by year, location, month, and block as the SPAD value of the nonfertilized control divided by the SPAD value of the high N rate treatments (134 kg N ha<sup>-1</sup> yr<sup>-1</sup>).

After *Miscanthus* canes had senesced and the majority of the leaves had dropped in each year they were harvested in December by cutting a 1.32-m wide swath at 10 to 15 cm above the soil surface from the center of each plot using a sickle-bar mower. Cut canes were collected and weighed in the field and a three to five cane subsample was taken and dried at 60°C for 72 to 96 h to determine moisture and calculate dry matter yield. All yields are expressed on a dry matter basis. Maximum yield was the average yield across N rates at site-years with no response to N or the average yield across N rate treatments with the highest statistically equivalent yield at N responsive site-years. Dried subsamples were ground to pass through a 1-mm sieve using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and analyzed for total N concentration with combustion using a Vario Max CNS Analyzer (Elementar Americas Inc., Mt. Laurel, NJ). Nitrogen content was the product of N concentration and dry matter yield.

Residual soil NO<sub>3</sub>-N following *Miscanthus* harvest was measured in early April 2014, mid-January 2015, and mid-December 2015 at all four sites. In April 2014 and January 2015, three soil samples (20-mm i.d. by 0.9-m deep) were collected from each N rate plot and composited into single samples by 0.3-m segments. In December 2015, the same protocol was followed but larger diameter (41 mm) samples were collected using a hydraulic coring system (Giddings Machine Co., Fort Collins, CO). All soils were dried at 60°C for 48 h before being pulverized with a Dynacrush Soil Crusher (Custom Laboratory Equipment Inc., Orange City, FL). Pulverized soil was subsampled (10 g), extracted with 25 mL of 2 M KCl, and analyzed for NO<sub>3</sub>-N with cadmium reduction using an

**Table 2.** Annual and total fertilizer N rate treatments (N trt) rounded to the nearest kilogram N per hectare that were applied to *Miscanthus* from 2012 to 2015 at three locations (Boone–LT, Cooper and Moniteau) with young stands and from 2007 to 2015 at Boone–JF with a mature stand.

| Experiment | N trt | Description     | 2007–2010             | 2011 | 2012 | 2013 | 2014 | 2015 | Total |
|------------|-------|-----------------|-----------------------|------|------|------|------|------|-------|
|            |       |                 | kg N ha <sup>-1</sup> |      |      |      |      |      |       |
| Young      | 1     | Control         |                       |      | 0    | 0    | 0    | 0    | 0     |
|            | 2     | Low prime       |                       |      | 0    | 34   | 34   | 0    | 68    |
|            | 3     | Medium prime    |                       |      | 0    | 67   | 34   | 34   | 134   |
|            | 4     | Medium constant |                       |      | 0    | 67   | 67   | 67   | 201   |
|            | 5     | High prime      |                       |      | 0    | 134  | 67   | 67   | 268   |
|            | 6     | High constant   |                       |      | 0    | 134  | 134  | 134  | 402   |
| Mature     | 1     | Control         | 56                    | 0    | 0    | 0    | 0    | 0    | 224   |
|            | 2     | Low constant    | 56                    | 0    | 45   | 0    | 45   | 45   | 359   |
|            | 3     | Medium constant | 56                    | 0    | 90   | 0    | 90   | 90   | 494   |
|            | 4     | High constant   | 56                    | 0    | 134  | 0    | 134  | 134  | 626   |

automated flow-injection analyzer (QuikChem 8500 Lachat, Lachat Instruments, Loveland, CO). Soil  $\text{NO}_3\text{-N}$  concentration was converted to content using soil dry bulk densities for each 0.3-m segment from the USDA-NRCS soil survey geographic database (Soil Survey Staff, 2011) for each site. The sum of  $\text{NO}_3\text{-N}$  content from the surface to the 0.9-m depth was used to describe the soil profile.

A subset of the soil samples collected in January 2015 was used to determine soil potentially mineralizable nitrogen (PMN). Samples used from each site and replication were the control, medium constant, and high constant N rate treatments (Table 2). Three subsamples (10 g each) were taken from each pulverized soil sample. One subsample was submerged in 100 mL 2 M KCl, shaken for 45 min, then filtered and colorimetrically analyzed for  $\text{NH}_4\text{-N}$  following the methods of Rhine et al. (1998). The second and third subsamples were submerged with 50 mL ultrapure water and incubated at 40°C for 7 or 14 d. Following incubation,  $\text{NH}_4\text{-N}$  concentration was measured using the same extraction and analysis method as the nonincubated samples. For each sample, mineralizable N was calculated as the difference in  $\text{NH}_4\text{-N}$  concentration between incubated and nonincubated samples. Another subset of the samples collected in January 2015 were used to determine soil fertility levels. Dry, pulverized subsamples from the 0.3-m depth of each nonfertilized control treatment were composited by site and analyzed for soil pH, organic matter, P, K, and cation exchange capacity by the University of Missouri Soil Testing Laboratory (Grafton et al., 2015).

### Statistical Analysis

Data were analyzed by experiment (young and mature stands) and year using the GLIMMIX procedure of SAS (SAS Institute, 2011) at  $P = 0.05$ . Data were analyzed by year because N rate treatments were cumulative and differed each year. Location, N rate, month of SPAD measurement, and their interactions were considered fixed effects while block within location and interactions of block with fixed effects were considered random. Month of SPAD measurement also was treated as a repeated measure. Several covariance structures were evaluated and the selected structure, first-order autoregressive, had the lowest fit statistics or best fit. Dependent variables were biomass yield, LCC, relative LCC, biomass N concentration and content at harvest, residual soil  $\text{NO}_3\text{-N}$ , and PMN at 7 or 14 d. Leaf chlorophyll concentration was not collected at Cooper in September 2014 so data from this month for the other two locations with young stands were analyzed separately from May through August. The interaction between location  $\times$  N rate was significant for biomass yield in 2014 and 2015, but not 2013. Mean biomass yield in 2013 at Moniteau was numerically greater at medium N rates than the highest N rate. Therefore, three polynomial contrasts of location  $\times$  N between Moniteau and Cooper, Moniteau, and Boone-LT, and Cooper and Boone-LT were conducted for yield data in 2013 to ensure that locations were correctly identified as responsive or non-responsive to N. The assumptions of equal variance and normality were examined by visually inspecting the residuals vs. predicted and QQ-plots generated with the UNIVARIATE procedure of SAS and were met. When fixed effects were significant, means were compared using pairwise  $t$  tests at  $P \leq 0.05$  with the PDIFF option in the GLIMMIX procedure of SAS.

## RESULTS AND DISCUSSION

### Biomass Yield Increase with Stand Age

The increase in maximum biomass yield from 2013 to 2015 as stands aged was 32% at Cooper, 44% at Moniteau and Boone-JF, and 79% at Boone-LT the most N-responsive site (Table 3). These percentage yield increases observed during the first 3 yr (2013–2015) of production were similar to the range (12–100%) on young stands in England (Christian et al., 2006) and the midwestern and eastern United States (Davis et al., 2015). Furthermore, the range in maximum yield obtained in this study (13 to 16, 15 to 20, and 20 to 24  $\text{Mg ha}^{-1}$  for 1-, 2-, and 3-yr-old stands, respectively) were similar to those in other reports of young *Miscanthus* grown on a range of soils in the United States. Maximum yield of *Miscanthus* across five states (Kentucky, Illinois, Nebraska, New Jersey, and Virginia) ranged from 9 to 17, 11 to 27, and 10 to 31  $\text{Mg ha}^{-1}$  for 1-, 2-, and 3-yr-old stands, respectively, (Davis et al., 2015) and was 24  $\text{Mg ha}^{-1}$  across two sites with 3-yr-old stands in the Piedmont and Mountain regions of North Carolina (Haines et al., 2014). Therefore, eroded claypan soils may not negatively impact *Miscanthus* establishment or yield (Arundale et al., 2014; Randall et al., 2016).

### Biomass Yield Response to Nitrogen

Boone-LT was the only site where biomass yield was increased with fertilizer N each year (Supplemental Table S1). In 2013, biomass yield increased by 4.1  $\text{Mg ha}^{-1}$  (43%) only when the highest rate (134  $\text{kg N ha}^{-1}$ ) was applied. In 2014, yield increased by 6.5  $\text{Mg ha}^{-1}$  (48%) when cumulative rates of  $\geq 101 \text{ kg N ha}^{-1}$  (medium prime or greater; Table 2) were applied (Fig. 2). The percentage of yield increase with N was similar in 2014 as 2013, but occurred with much less fertilizer. In 2015, yield increased from 14.0  $\text{Mg ha}^{-1}$  for the control or the cumulative low prime N rate (Table 2) to 22.1  $\text{Mg ha}^{-1}$  for rates greater than the medium prime, or 23.8  $\text{Mg ha}^{-1}$  for rates greater than the medium (Fig. 2). The yield increase between the nonfertilized control and the high rate was 11.6  $\text{Mg ha}^{-1}$  (82%) and was nearly twice the percentage increase than the previous 2 yr. Total yield across all 3 yr was maximized with the cumulative medium rate (67  $\text{kg N ha}^{-1} \text{ yr}^{-1}$ ) or greater (Table 2, Fig. 2).

Cooper was the only other site besides Boone-LT where biomass yield increased with fertilizer N, but the increase only occurred in 1 yr. Yield in 2015 increased from 15.4  $\text{Mg ha}^{-1}$  with the low prime rate or less to 20.7  $\text{Mg ha}^{-1}$  when the cumulative

**Table 3.** *Miscanthus* annual maximum dry matter biomass yield† along with annual and cumulative percentage yield increases for two experiments in Missouri.

| Year      | Boone-LT                               | Cooper | Moniteau | Boone-JF |
|-----------|--|--------|----------|----------|
|           | Mg dry matter $\text{ha}^{-1}$         |        |          |          |
| 2013      | 13.3                                   | 15.7   | 13.6     | 14.2     |
| 2014      | 20.1                                   | 19.4   | 14.6     | 17.4     |
| 2015      | 23.8                                   | 20.7   | 19.6     | 20.4     |
|           | Annual or cumulative yield increase, % |        |          |          |
| 2013–2014 | 51                                     | 24     | 7        | 23       |
| 2014–2015 | 18                                     | 7      | 34       | 17       |
| 2013–2015 | 79                                     | 32     | 44       | 44       |

† Maximum yield was the average yield across N rates at site-years with no response to N (Moniteau and Boone-JF in all years, and Cooper in 2014) or the average yield across N rate treatments with the highest statistically equivalent yield at N responsive site-years.

medium rate or greater (Table 2) was applied (Fig. 2). The increase in yield between the nonfertilized control and the high N rate was 5.1 Mg ha<sup>-1</sup> (43%), which was similar to the level of N response at Boone-LT in 2013 and 2014. Although yield responded to N in 2013, only the 34 kg N ha<sup>-1</sup> produced less yield (2.4 Mg ha<sup>-1</sup>) than the other N rates, which indicated fertilizer additions would not be warranted. The minimum cumulative N rate needed to maximize the total yield across the 3 yr was the medium constant rate (67 kg N ha<sup>-1</sup> yr<sup>-1</sup>).

Moniteau had a marginal response to N in 2013 (polynomial contrasts indicated that the response to N was the

same for Cooper and Boone-LT [ $P = 0.63$ ], for Moniteau and Cooper [ $P = 0.21$ ], but not for Moniteau and Boone-LT [ $P = 0.033$ ]) and a lack of response in the other 2 yr or across all 3 yr (Supplemental Table S1, Fig. 2). Lack of yield response to N may have been related to low P fertility or slight differences in soil characteristics between it and the Cooper site (Table 2); soils at Moniteau had greater N supply (higher organic matter) and potentially less N loss (lower runoff and better drainage). Furthermore, cumulative precipitation levels were greater at Moniteau in most years (Fig. 1), which may have stimulated greater soil N mineralization. In contrast, the lack of yield

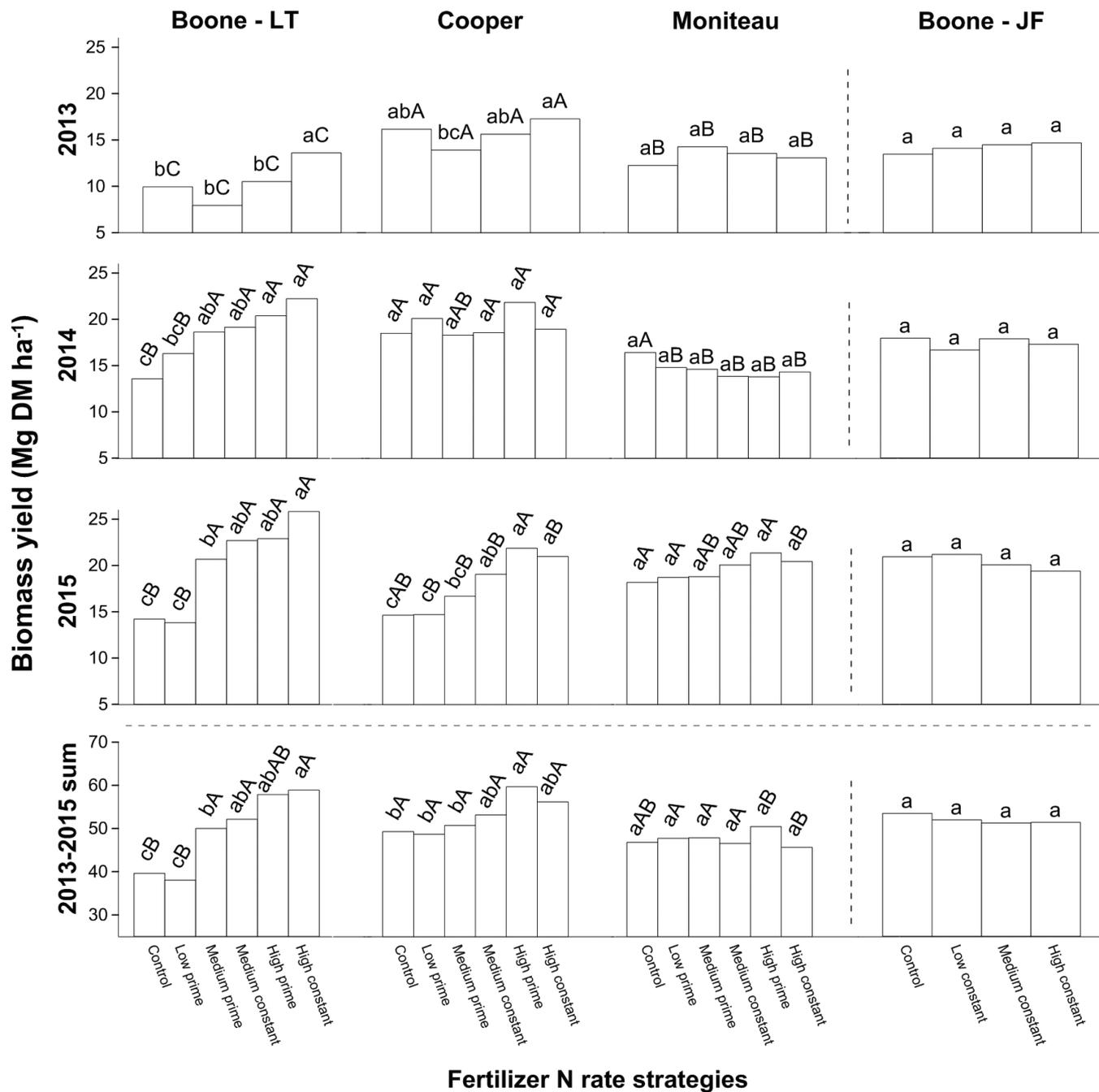


Fig. 2. Dry matter (DM) biomass yield of *Miscanthus* during and across 2013 to 2015 for young stands at Boone-LT, Cooper, and Moniteau and a mature stand at Boone-LT by fertilizer N rate treatment described in Table 2. Within location and year(s), lowercase letters above means denote significant differences among N rate treatments. Within year(s) for the three locations with young stands, uppercase letters above means denote differences among locations by N rate treatments. The 2013 results were combined for the low prime and medium prime, and for the medium constant and high prime because the same N rate was applied.

response to N in all 3 yr of the mature stand at Boone–JF could have been due to adequate N priming during the first 4 yr of production ( $56 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), different cultivar and enhanced soil fertility (especially P) compared to other locations (Table 2), enhanced soil N mineralization (discussed below in PMN results), lack of tillage prior to planting, or other factors not measured.

The contrasting strong response to fertilizer N at Boone–LT relative to the other young stands (i.e., Cooper and Moniteau) was probably partially due to its prior grain cropping history. Other research suggests that previous cropping history might influence N response in *Miscanthus* stands. Christian et al. (2008) noted that *Miscanthus* did not respond to N when following grassland, but did respond following cropland. In another study, nearly all young stands (1–4-yr old) in five states had no annual yield response to N fertilizer except a 3-yr-old stand in Illinois in 2012 (drought year; [Davis et al., 2015]). This Illinois site was previously conventionally row-cropped, which may have been a factor in its yield response to N, although one other site also cropped before *Miscanthus* did not respond to N in 2012. Furthermore, the two sites with previous row-crop history had no or less increase in N content of harvested biomass with fertilization than two other sites without prior cropping history (Masters et al., 2016). The potential impact of previous cropping on *Miscanthus* N requirements at some sites may be related to greater soil health (including improved nutrient availability and cycling) for long-term grassland than grain cropping systems (Veum et al., 2015). An additional reason why Boone–LT may have been responsive to N was greater N loss through denitrification caused by less water movement offsite (negligible slope) and higher soil water content (not measured) in comparison to Cooper and Moniteau.

The observed variance in the responsiveness of *Miscanthus* biomass yield to fertilizer N among sites and within sites among years is not uncommon to results from several other European and North American studies for both young (Ercoli et al., 1999; Strullu et al., 2011; Arundale et al., 2013; Haines et al., 2014; Davis et al., 2015) and mature stands (Christian et al., 2008; Larsen et al., 2013; Finnan and Burke, 2014). Results from a meta-analysis of 184 site-years of *Miscanthus* research from Europe prior to 2008 suggested that low maintenance fertilizer N is needed to maintain long-term ceiling or maximum yield for stands greater than 3-yr old (Miguez et al., 2008), yet individual, more recent studies mentioned above have found that some stands require no fertilizer N to maximize yield in the short or long term. Furthermore, stands in the United States have generally yielded higher and responded to N more frequently than European studies (Witzel and Finger, 2016).

The results of this study suggest that *Miscanthus* grown on eroded claypan soils may respond to N more frequently than other soils. Furthermore, they support the approach of medium constant ( $67 \text{ kg N ha}^{-1}$ ) fertilizer rates instead of priming rates to maximize production for young stands, but not at every site or site-year. Therefore, it is imperative that site-specific tools are developed to predict annual fertilizer N response in *Miscanthus* to avoid excessive fertilizer applications that waste resources, decrease ethanol quality (Arundale et al., 2015), and increase potential for N loss (Davis et al., 2015; Roth et al., 2015).

## Leaf Chlorophyll Concentration

At the three sites with young stands, LCC was influenced by all three of the two-way interactions (location  $\times$  N, location  $\times$  month, and month  $\times$  N) (Supplemental Table S1) in 2013 and by the three-way interaction among location  $\times$  N  $\times$  month in 2014 and 2015. At the mature stand at Boone–JF, LCC was influenced by only month in 2013, month and N in 2014, and month  $\times$  N in 2015. For brevity, select results concerning the seasonal trends in LCC, differences in LCC among sites with and without biomass yield response to N, and detection of apparent carryover N from year to year are presented.

### Seasonal Trends

At all four sites in all 3 yr, LCC across N rates usually peaked shortly after fertilization in June and then decreased as the season progressed (Supplemental Table S2, Supplemental Fig. S1, Supplemental Fig. S2). Similar patterns have been observed for leaf N concentration in a young stand (2–3 yr) in France (Strullu et al., 2011) or LCC in a mature stand (14–18 yr) in Ireland (Finnan and Burke, 2014). Declining LCC from June or July to September was likely due to the ability of *Miscanthus* to intercept some of the readily available N from applied fertilizer and mobilize it into the most photosynthetically active portions of the plant. Then, as the season progressed those reserves may have been increasingly reallocated to the belowground biomass in preparation for senescence (Dohleman et al., 2012). This reallocation pattern also has been observed in other studies from Europe with peak LCC or N uptake occurring as early as May (Himken et al., 1997) or as late as July (Beale and Long, 1997; Finnan and Burke, 2014). Other factors causing low LCC late in the season may be dilution of N from increased biomass or roots harvesting water deeper in the soil profile where soil N is generally diluted.

### Nitrogen Fertilization Trends

In almost every month after fertilization (June–September) in every site-year, N fertilization increased LCC (Supplemental Table S2, Supplemental Fig. S1, Supplemental Fig. S2). The only exceptions were for the mature stand in 2013 when no fertilizer was applied, and sometimes at the end of the season (August and/or September) at the two sites with no biomass yield response to N (Moniteau and Boone–JF). These results indicate that a portion of the fertilizer was always available and recovered by *Miscanthus*, regardless of whether it was needed to increase biomass yield. Site-years with biomass yield response to N (i.e., Boone–LT in 2013–2015 and Cooper in 2015) also had consistently less LCC in nonfertilized plots than the other site-years throughout the growing season, and the greatest LCC response to N.

### Apparent Nitrogen Carryover as Detected in May before Fertilization

At all three sites with young stands, residual N in the soil or stored in the rhizome was apparently available or remobilized to the aboveground plant tissue because LCC in May of 2014 before fertilization was greater in plots fertilized in 2013 than those that were not (Supplemental Fig. S1). In contrast, carryover N was apparently low or not available in 2015 because the fertilization in 2014 had minimal effects on LCC in May (Supplemental Fig. S2). Trends were similar for the mature stand at Boone–JF. No carryover from the 2012 N application was

apparent in 2013, possibly due to high amounts of stored N in the rhizome, and/or high soil N mineralization in 2013, but some was present in 2014 (Supplemental Fig. S1). Moreover, the N application in 2014 had no influence on LCC in May 2015.

### Relative Leaf Chlorophyll Concentration

Relative LCC was evaluated for its potential as an indicator of N fertilizer need in *Miscanthus*. The fact that relative LCC was lower at Boone–LT than other nonresponsive sites in May 2014, but not May 2015 (Supplemental Table S3, Fig. 3) suggests that early to mid-June might be the earliest time that relative LCC could reliably identify responsiveness to N fertilization. In June of all 3 yr, Boone–LT had relative LCC  $\leq 0.76$ , Moniteau had  $\geq 0.87$ , and Cooper had  $\geq 0.84$ . The site-years where yield increased with N by 40 to 50% had relative LCC between 0.72 and 0.84 (Boone–LT 2013 and 2014; Cooper 2015), and the site-year with 82% yield increase (Boone–LT 2015) had relative LCC of 0.61. Furthermore, yield increased with fertilization at Cooper in 2015 when relative SPAD was 0.84, but not in 2014 when it was 0.85. The contrasting outcome between two relative LCCs one unit apart makes it risky to define a critical relative LCC level that separates responsive and nonresponsive site-years. Few other studies have measured LCC, but a mature stand (14–18 yr) in France (Finnan and Burke, 2014) with no annual response to N fertilization had relative LCC in June that ranged from 0.81 in 2011 (warmest and driest year of study) to  $\geq 0.88$  in four other years (2008–2010, 2012). These collective findings support the use of relative LCC as a reliable indicator of N responsiveness, and suggest that *Miscanthus* with relative LCC  $< 0.85$  in early to mid-June, and especially  $< 0.70$ , likely will require N fertilization to maximize yield.

### Biomass Nitrogen Concentration and Content at Harvest

At the three sites with young stands, biomass N concentration at harvest across N rates was greatest at Moniteau and least at Boone–LT each year and across years (Supplemental Table S1, Fig. 4). This was not the case for N content; Boone–LT had less N content in 2013 or across 2013 to 2015 than the other two

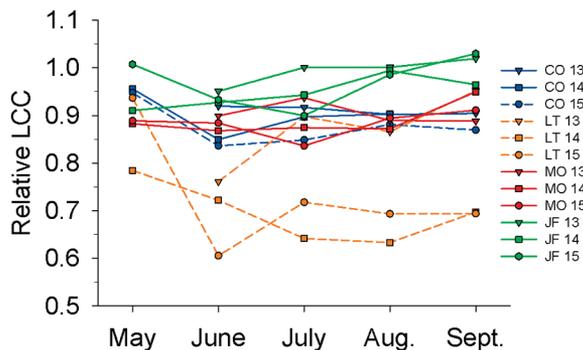


Fig. 3. Relative (nonfertilized/highest N rate) *Miscanthus* leaf chlorophyll concentration (measured with SPAD) in 2013 to 2015 by location (CO, Cooper; LT, Boone–LT; MO, Moniteau; JF, Boone–JF) and month of sampling for two experiments in Missouri. Dashed lines represent site-years where yield significantly increased with fertilizer N application. Yield at Cooper in 2013 did respond to fertilizer N, but not consistently as N application increased. Mean separations are shown in Supplemental Table S2.

sites, but equal content in 2014 and 2015. Nitrogen fertilization had no impact on N concentration or content of young stands in the first year (2013), but increased both measures in a similar way at all three sites in subsequent years and across years (Supplemental Table S1). Biomass N concentration increased by  $0.9 \text{ g kg}^{-1}$  (35%) with  $\geq$  high N prime rate in 2014 at all sites, by  $1.6 \text{ g kg}^{-1}$  (70%) with  $\geq$  medium prime rate only at Moniteau in 2015, and by  $0.5 \text{ g kg}^{-1}$  (17%) with  $\geq$  high N prime rate across years at all sites (Fig. 4). Likewise, N content increased at all sites by  $20 \text{ kg ha}^{-1}$  (45%) with  $\geq$  high N prime rate in 2014, by  $23 \text{ kg ha}^{-1}$  (61%) with  $\geq$  medium prime N rate in 2015, and by  $50 \text{ kg ha}^{-1}$  (35%) with  $\geq$  high N prime rate across years (Fig. 5). In contrast, the N concentration and content of the mature stand at Boone–JF never consistently increased as fertilizer N rates increased.

Aboveground biomass N concentration and content of young stands in 2013 may not have been influenced by fertilizer because it was mainly absorbed in the belowground biomass (Christian et al., 2006; Strullu et al., 2011) of plants establishing after a major drought year (Fig. 1). The uniformity in N content increases ( $20\text{--}23 \text{ kg N ha}^{-1}$ ) with fertilization in 2014 and 2015 among young stands with and without biomass yield increases showed that *Miscanthus* absorbed applied N, sometimes without increasing harvestable yield. Studies in United Kingdom and Ireland have found similar results; fertilization increased N content ( $18\text{--}23 \text{ kg N ha}^{-1}$ ) of non-yield-responsive mature stands in some but not all years (Christian et al., 2008; Finnan and Burke, 2014). Comparable results have occurred in young stands, but the increases in N content with fertilization were lower ( $9\text{--}10 \text{ kg N ha}^{-1}$ ) in New Jersey and North Carolina (Haines et al., 2014; Davis et al., 2015) or much higher ( $82 \text{ kg N ha}^{-1}$ ) in France (Strullu et al., 2011).

The only fertilizer treatments that consistently increased annual and cumulative N concentration and content were the high prime and high N rate (Table 2). Therefore, although the medium constant rate maximized total biomass yield across years at Boone–LT and Cooper, it almost never increased N concentration or content of harvested biomass. Moreover, average annual N content of harvested biomass rarely surpassed  $67 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . These results support the use of medium constant rates rather than priming or higher constant rates at responsive sites.

### Soil Nitrogen

Residual soil  $\text{NO}_3\text{-N}$  to 0.9 m in 2013 was  $13 \text{ kg ha}^{-1}$  (217%) less at Moniteau than Boone–LT and Cooper and increased from 14 to  $17 \text{ kg ha}^{-1}$  (21%) when the highest rate ( $134 \text{ kg N ha}^{-1}$ ) was applied (Supplemental Table S1). In subsequent years, residual soil  $\text{NO}_3\text{-N}$  was not impacted by location or fertilization in 2014 and 2015 and averaged 9 and  $19 \text{ kg ha}^{-1}$ , respectively. Likewise, residual soil  $\text{NO}_3\text{-N}$  following the mature stand at Boone–JF in 2013 and 2015 was not influenced by N rate and averaged 29 and  $34 \text{ kg ha}^{-1}$  in these respective years. However, following the 2014 harvest, the two highest cumulative N rates ( $179$  and  $269 \text{ kg N ha}^{-1}$ ) had  $16 \text{ kg ha}^{-1}$  (68%) more residual soil  $\text{NO}_3\text{-N}$  than the nonfertilized control and lowest N rate.

The low or lack of N fertilization effects on residual soil  $\text{NO}_3\text{-N}$  for the young stands suggests that *Miscanthus* was

efficient at recovering and recycling applied N or that a significant amount of N was lost through denitrification, or both. Fertilization at these three locations consistently increased LCC from June to September, usually increased N concentration or N content of harvested biomass, and increased yield at Boone-LT in all years and Cooper in 2015. Thus, fertilizer was absorbed by aboveground *Miscanthus* biomass and much of the unaccounted for N was either in belowground biomass (not measured) or was lost, probably through denitrification and leaching (Blevins et al., 1996; Christian and Riche, 1998). Results for the mature stand were similar to those of the young

stand except in 2014 when N loss was apparently lower than other years despite wet conditions during the latter part of the season (Fig. 1).

Potentially mineralizable N was measured in the winter of 2014 following harvest, and would be most representative of N available during the growing season of 2015. However, results may apply to other years because organic matter content should have changed none or little given the short time over which this study took place and the absence of cultivation. Potentially mineralizable N was not affected by location, N rate, or their interaction for the three young stands (Supplemental Table

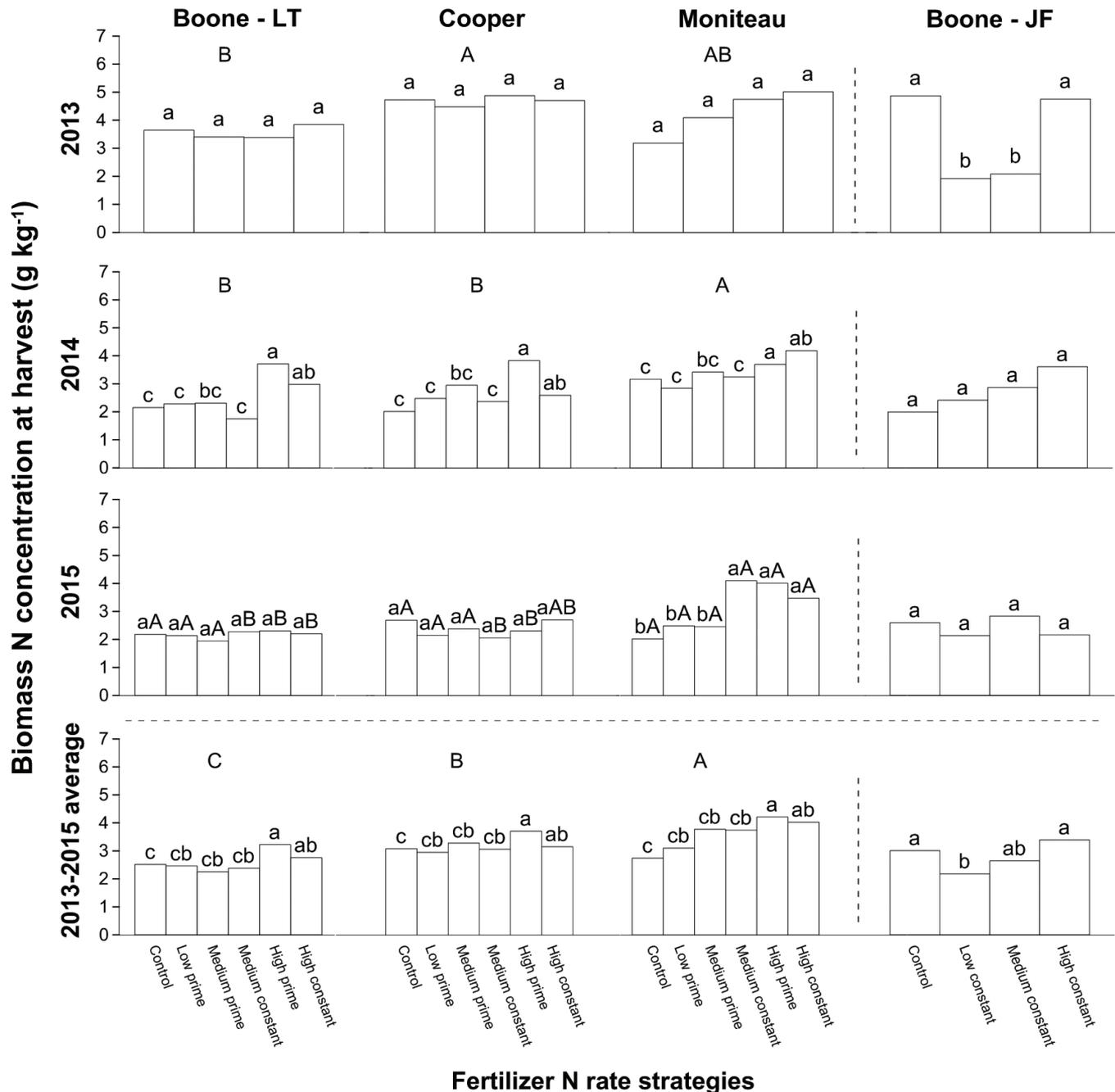


Fig. 4. Biomass N concentration of *Miscanthus* at harvest during and across 2013 to 2015 for young stands at Boone-LT, Cooper, and Moniteau and a mature stand at Boone-LT by fertilizer N rate treatment described in Table 2. Within location and year(s), lowercase letters above means denote significant differences among N rate treatments. Within years for the three sites with young stands, uppercase letters above means denote differences among sites by or across N rates. The 2013 results were combined for the low prime and medium prime, and for the medium constant and high prime because the same N rate was applied.

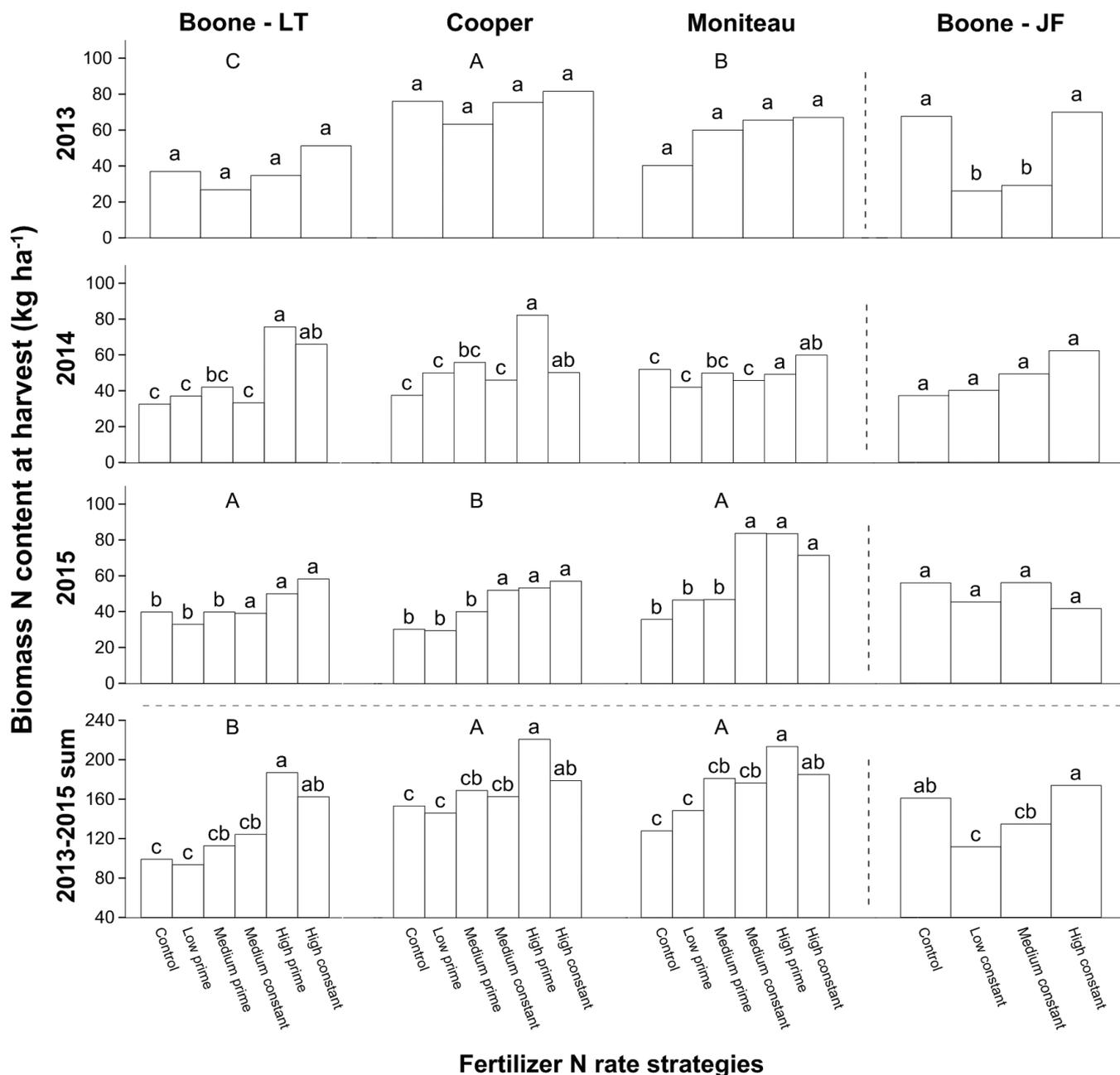


Fig. 5. Biomass N content of *Miscanthus* at harvest during and across 2013 to 2015 for young stands at Boone-LT, Cooper, and Moniteau and a mature stand at Boone-LT by fertilizer N rate treatment described in Table 2. Within location and year(s), lowercase letters above means denote significant differences among N rate treatments. Within years for the three sites with young stands, uppercase letters above means denote differences among sites across N rates. The 2013 results were combined for the low prime and medium prime, and for the medium constant and high prime because the same N rate was applied.

S1) and averaged 19 and 44 kg ha<sup>-1</sup> at 7 and 14 d, respectively, across treatments. This indicates that the three sites may have had similar amounts of soil N mineralization, and that mineralized N sometimes may be sufficient to meet N removal (37–58 kg N ha<sup>-1</sup>) in nonfertilized harvested biomass.

At Boone-JF with a mature stand, N application also did not affect PMN (Supplemental Table S1), though in comparison to young stands, PMN was 74 and 43% greater at 7 and 14 d, respectively. Greater PMN may have partially been caused by increased organic matter from manure application prior to the study and mature *Miscanthus*, but also may be due to inherent soil characteristic differences among locations. Few other studies have measured PMN in *Miscanthus*, but Davis et al.

(2015) found that fertilization did not influence PMN at 7 d at five sites across the midwestern and eastern United States, but PMN at a shallow depth (0–10 cm) did increase by 29 to 454% during the first four production years. Further, the 1 site-year that had a yield response to N (i.e., Illinois, 2012) did not have lower PMN than other nonresponsive site-year. These results combined with those in the present study indicate that PMN is likely not a reliable indicator of *Miscanthus* yield response to N fertilization.

## CONCLUSIONS

*Miscanthus* biomass yield of young and mature stands on eroded claypan soils were similar to those on many other eroded and some productive soils in the United States. Across

three locations, maximum biomass yield averaged 14, 18, and 21 Mg ha<sup>-1</sup>, during the first, second, and third production year, respectively. At one location, yield averaged 14, 17, and 20 Mg ha<sup>-1</sup> during the sixth, seventh, and eighth production year, respectively. Comparable yield on marginal and productive soils demonstrates *Miscanthus's* resiliency and suggests that it might be a suitable alternative to more vulnerable annual crops on marginal soils.

Biomass yield response to fertilizer N may be more frequent on eroded claypan soils than other soils as yield increased with fertilization in 4 of 12 site-year combinations. At two locations with yield response to N in young stands, priming N fertilization strategies had no advantage over constant annual rates of fertilizer. The medium constant rate of 67 kg N ha<sup>-1</sup> yr<sup>-1</sup> at these two sites was the lowest rate needed to maximize 3-yr production, and this rate did not increase removal of N in harvested biomass. Relative LCC in June may reliably indicate which site-years require fertilizer N because site-years with relative LCC < 0.85 had yield response to N, while those ≥ 0.85 did not. This and other site-specific tools aimed at predicting annual fertilizer N response in *Miscanthus* need to be further validated to avoid excessive fertilizer applications that waste resources, decrease ethanol quality, and impair environmental quality.

### ACKNOWLEDGMENTS

This research was made possible by funding from the USDA North Central Region-Sustainable Agriculture Research and Education Program, the USDA-NIFA South Central Region Sun Grant, and USDA-ARS. The authors would like to thank Rob Myers, Lance Conway, Kurt Holiman, Matt Volkmann, Kyle Ahart, Matt Copeland, Connor Drummond, Derek Volkmann, Mac Bean, Arndt Gossel, Chamera Weerasekara, and Jeremy Matson for their help in establishing, maintaining, and sampling from the trials and Dr. Kristen Veum for her assistance with the soil N analyses. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

### REFERENCES

- Arundale, R.A., S. Bauer, F.B. Haffner, V.D. Mitchell, T.B. Voigt, and S.P. Long. 2015. Environment has little effect on biomass biochemical composition of *Miscanthus × giganteus* across soil types, nitrogen fertilization, and times of harvest. *BioEnergy Res.* 8:1636–1646. doi:10.1007/s12155-015-9613-2
- Arundale, R.A., F.G. Dohleman, E.A. Heaton, J.M. Mcgrath, T.B. Voigt, and S.P. Long. 2014. Yields of *Miscanthus × giganteus* and *Panicum virgatum* decline with stand age in the Midwestern USA. *GCB Bioenergy* 6:1–13. doi:10.1111/gcbb.12077
- Arundale, R.A., F.G. Dohleman, T.B. Voigt, and S.P. Long. 2013. Nitrogen fertilization does significantly increase yields of stands of *Miscanthus × giganteus* and *Panicum virgatum* in multi-year trials in Illinois. *BioEnergy Res.* 7:408–416. doi:10.1007/s12155-013-9385-5
- Beale, C.V., and S.P. Long. 1997. Seasonal dynamics of nutrient accumulation and partitioning in the perennial C4-grasses *Miscanthus × giganteus* and *Spartina cynosuroides*. *Biomass Bioenergy* 12:419–428. doi:10.1016/S0961-9534(97)00016-0
- Blevins, D.W., D.H. Wilkison, B.P. Kelly, and S.R. Silva. 1996. Movement of nitrate fertilizer to glacial till and runoff from a claypan soil. *J. Environ. Qual.* 25:584–593. doi:10.2134/jeq1996.00472425002500030026x
- Bullock, D., and D. Anderson. 1998. Evaluation of the Minolta SPAD-502 chlorophyll meter for nitrogen management in corn. *J. Plant Nutr.* 21:741–755. doi:10.1080/01904169809365439
- Cadoux, S., A.B. Riche, N.E. Yates, and J.-M. Machet. 2012. Nutrient requirements of *Miscanthus × giganteus*: Conclusions from a review of published studies. *Biomass Bioenergy* 38:14–22. doi:10.1016/j.biombioe.2011.01.015
- Christian, D., P. Poulton, A. Riche, N. Yates, and A. Todd. 2006. The recovery over several seasons of 15N-labelled fertilizer applied to *Miscanthus × giganteus* ranging from 1 to 3 years old. *Biomass Bioenergy* 30:125–133. doi:10.1016/j.biombioe.2005.11.002
- Christian, D.G., and A.B. Riche. 1998. Nitrate leaching losses under *Miscanthus* grass planted on a silty clay loam soil. *Soil Use Manage.* 14:131–135. doi:10.1111/j.1475-2743.1998.tb00136.x
- Christian, D.G., A.B. Riche, and N.E. Yates. 2008. Growth, yield and mineral content of *Miscanthus × giganteus* grown as a biofuel for 14 successive harvests. *Ind. Crops Prod.* 28:320–327. doi:10.1016/j.indcrop.2008.02.009
- Clifton-Brown, J.C., J. Breuer, and M.B. Jones. 2007. Carbon mitigation by the energy crop *Miscanthus*. *Glob. Chang. Biol.* 13:2296–2307. doi:10.1111/j.1365-2486.2007.01438.x
- Davis, M.P., M.B. David, and C.A. Mitchell. 2013. Nitrogen mineralization in soils used for biofuel crops. *Commun. Soil Sci. Plant Anal.* 44:987–995. doi:10.1080/00103624.2012.747607
- Davis, M.P., M.B. David, T.B. Voigt, and C.A. Mitchell. 2015. Effect of nitrogen addition on *Miscanthus × giganteus* yield, nitrogen losses, and soil organic matter across five sites. *GCB Bioenergy* 7:1222–1231. doi:10.1111/gcbb.12217
- Dohleman, F.G., E.A. Heaton, R.A. Arundale, and S.P. Long. 2012. Seasonal dynamics of above- and below-ground biomass and nitrogen partitioning in *Miscanthus × giganteus* and *Panicum virgatum* across three growing seasons. *GCB Bioenergy* 4:534–544. doi:10.1111/j.1757-1707.2011.01153.x
- Eckert, B., O.B. Weber, G. Kirchhof, A. Halbritter, M. Stofels, and A. Hartmann. 2001. *Azospirillum doebereineriae* sp. nov., a nitrogen-fixing bacterium associated with the C4-grass *Miscanthus*. *Int. J. Syst. Evol. Microbiol.* 51:17–26. doi:10.1099/00207713-51-1-17
- Ercoli, L., M. Mariotti, A. Masoni, and E. Bonari. 1999. Effect of irrigation and nitrogen fertilization on biomass yield and efficiency of energy use in crop production of *Miscanthus*. *Field Crops Res.* 63:3–11. doi:10.1016/S0378-4290(99)00022-2
- Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia* 78:9–19. doi:10.1007/BF00377192
- Finnan, J., and B. Burke. 2014. Nitrogen dynamics in a mature *Miscanthus × giganteus* crop fertilized with nitrogen over a five year period. *Ir. J. Agric. Food Res.* 53:171–188.
- Grafton, K., M. Nathan, R. Gelderman, B. Joern, A. Mallarino, D. Mengel et al. 2015. In: M. Nathan and R. Gelderman, editors, Recommended chemical soil test procedures for the North Central Region. North Central Regional Res. Publ. 221 (rev.). Missouri Agric. Exp. Stn.
- Haines, S.A., R.J. Gehl, J.L. Havlin, and T.G. Ranney. 2014. Nitrogen and phosphorus fertilizer effects on establishment of giant *Miscanthus*. *BioEnergy Res.* 8:17–27. doi:10.1007/s12155-014-9499-4
- Heaton, E.A., J. Clifton-Brown, T.B. Voigt, M.B. Jones, and S.P. Long. 2004. *Miscanthus* for renewable energy generation: European Union experience and projections for Illinois. *Mitig. Adapt. Strategies Glob. Change* 9:433–451. doi:10.1023/B:MITI.0000038848.94134.be
- Heaton, E.A., F.G. Dohleman, A. Fernando Miguez, J.A. Juvik, V. Lozovaya, J. Widholm et al. 2010. *Miscanthus*: A promising biomass crop. In: J.-C. Kader and M. Delseny, editors, *Advances in botanical research*. Academic Press, San Diego, CA. p. 76–124.

- Heaton, E.A., F.G. Dohleman, and S.P. Long. 2008. Meeting US biofuel goals with less land: The potential of *Miscanthus*. *Glob. Change Biol.* 14:2000–2014. doi:10.1111/j.1365-2486.2008.01662.x
- Heaton, E.A., F.G. Dohleman, and S.P. Long. 2009. Seasonal nitrogen dynamics of *Miscanthus × giganteus* and *Panicum virgatum*. *GCB Bioenergy* 1:297–307. doi:10.1111/j.1757-1707.2009.01022.x
- Himken, M., J. Lammel, D. Neukirchen, U. Czypionka-Krause, and H.-W. Olf. 1997. Cultivation of *Miscanthus* under West European conditions: Seasonal changes in dry matter production, nutrient uptake and remobilization. *Plant Soil* 189:117–126. doi:10.1023/A:1004244614537
- Larsen, S.U., U. Jørgensen, J.B. Kjeldsen, and P.E. Lærke. 2013. Long-term *Miscanthus* yields influenced by location, genotype, row distance, fertilization and harvest season. *BioEnergy Res.* 7:620–635. doi:10.1007/s12155-013-9389-1
- Lerch, R., E. Sadler, N. Kitchen, K. Sudduth, R. Kremer, D. Myers et al. 2008. Overview of the Mark Twain Lake/Salt River Basin conservation effects assessment project. *J. Soil Water Conserv.* 63:345–359. doi:10.2489/jswc.63.6.345
- Markwell, J., J.C. Osterman, and J.L. Mitchell. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynth. Res.* 46:467–472. doi:10.1007/BF00032301
- Massey, R.E., D.B. Myers, N.R. Kitchen, and K.A. Sudduth. 2008. Profitability maps as an input for site-specific management decision making. *Agron. J.* 100:52–59. doi:10.2134/agronj12007.0057
- Masters, M., C. Black, and I. Kantola. 2016. Soil nutrient removal by four potential bioenergy crops: *Zea mays*, *Panicum virgatum*, *Miscanthus × giganteus*, and prairie. *Agric. Ecosyst.* 216:51–60. doi:10.1016/j.agec.2015.09.016
- McLaughlin, S.B., and M. Walsh. 1998. Evaluating environmental consequences of producing herbaceous crops for bioenergy. *Biomass Bioenergy* 14:317–324. doi:10.1016/S0961-9534(97)10066-6
- MFiber. 2016. Working with local farmers to grow a high-quality fiber ingredient. *Renew Biomass*, Springfield, MO. <http://mfiber.net/forFarmers/> (accessed 3 Jan 2017).
- Miguez, F.E., M.B. Villamil, S.P. Long, and G.A. Bollero. 2008. Meta-analysis of the effects of management factors on *Miscanthus × giganteus* growth and biomass production. *Agric. For. Meteorol.* 148:1280–1292. doi:10.1016/j.agrformet.2008.03.010
- Miyamoto, T., M. Kawahara, and K. Minamisawa. 2004. Novel endophytic nitrogen-fixing clostridia from the grass *Miscanthus sinensis* as revealed by terminal restriction fragment length polymorphism analysis. *Appl. Environ. Microbiol.* 70:6580–6586. doi:10.1128/AEM.70.11.6580-6586.2004
- Pyter, R., E. Heaton, F. Dohleman, T. Voigt, and S. Long. 2009. Agronomic experiences with *Miscanthus × giganteus* in Illinois, USA. In: J.R. Mielenz, editor, *Biofuels: Methods and protocols*. Springer, New York. p. 41–52.
- Randall, B.K., M.A. Yost, N.R. Kitchen, E.A. Heaton, H.E. Stelzer, and A.L. Thompson. 2016. Impact of rhizome quality on *Miscanthus* establishment in claypan soil landscapes. *Ind. Crops Prod.* 85:331–340. doi:10.1016/j.indcrop.2015.12.040
- Rhine, E.D., R.L. Mulvaney, E.J. Pratt, and G.K. Sims. 1998. Improving the Berthelot reaction for determining ammonium in soil extracts and water. *Soil Sci. Soc. Am. J.* 62:473–480. doi:10.2136/sssaj1998.03615995006200020026x
- Robertson, G.P., V.H. Dale, O.C. Doering, S.P. Hamburg, J.M. Melillo, M.M. Wander et al. 2008. Agriculture. Sustainable biofuels redux. *Science* 322:49–50. doi:10.1126/science.1161525
- Roth, B., J.M. Finnan, M.B. Jones, J.I. Burke, and M.L. Williams. 2015. Are the benefits of yield responses to nitrogen fertilizer application in the bioenergy crop *Miscanthus × giganteus* offset by increased soil emissions of nitrous oxide? *GCB Bioenergy* 7(1):145–152. doi:10.1111/gcbb.12125
- SAS Institute. 2011. Statistical analysis system. SAS Inst., Cary, NC.
- Searchinger, T., R. Heimlich, R.A. Houghton, F. Dong, A. Elobeid, J. Fabiosa et al. 2008. Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. *Science (Washington, DC)* 319:1238–1240. doi:10.1126/science.1151861
- Singh, M.P., J.E. Erickson, L.E. Sollenberger, K.R. Woodard, J.M.B. Vendramini, and R.A. Gilbert. 2015. Mineral composition and removal of six perennial grasses grown for bioenergy. *Agron. J.* 107:466–474. doi:10.2134/agronj14.0339
- Soil Survey Staff. 2011. Soil Survey Geographic (SSURGO) database. NRCS, Washington, DC. [http://www.nrcs.usda.gov/wps/portal/nres/detail/national/soils/?cid=nrcs142p2\\_053631](http://www.nrcs.usda.gov/wps/portal/nres/detail/national/soils/?cid=nrcs142p2_053631) (accessed 3 Jan. 2017).
- Strullu, L., S. Cadoux, M. Preudhomme, M.H. Jeuffroy, and N. Beaudoin. 2011. Biomass production and nitrogen accumulation and remobilisation by *Miscanthus × giganteus* as influenced by nitrogen stocks in belowground organs. *Field Crops Res.* 121:381–391. doi:10.1016/j.fcr.2011.01.005
- USDA Farm Service Agency. 2012. Biomass crop assistance program project area listing. Farm Service Agency, Washington, DC. <http://www.fsa.usda.gov/programs-and-services/energy-programs/BCAP/index> (accessed 3 Jan. 2017).
- USDA Natural Resources Conservation Service. 2006. Land resource regions and major land resource areas of the United States, the Caribbean, and the Pacific Basin. *USDA Handb.* 296. USDA, Washington, DC. p. 357–359.
- Veum, K.S., R.J. Kremer, K.A. Sudduth, N.R. Kitchen, R.N. Lerch, C. Baffaut et al. 2015. Conservation effects on soil quality indicators in the Missouri Salt River Basin. *J. Soil Water Conserv.* 70:232–246. doi:10.2489/jswc.70.4.232
- Witzel, C.-P., and R. Finger. 2016. Economic evaluation of *Miscanthus* production—A review. *Renew. Sustain. Energy Rev.* 53:681–696. doi:10.1016/j.rser.2015.08.063