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Rebecca A Arundale, University of Illinois at Urbana-Champaign
Frank G Dohleman, University of Illinois at Urbana-Champaign
Emily A. Heaton, University of Illinois at Urbana-Champaign
Justin M Mcgrath, University of Illinois at Urbana-Champaign
Thomas B Voigt, University of Illinois at Urbana-Champaign, et al.
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REBECCA A. ARUNDALE*, †, ¶, FRANK G. DOHLEMAN*, †, §, EMILY A. HEATON‡, †, JUSTIN M. MCGRATH‡, †, THOMAS B. VOIGT†, ‡ and STEPHEN P. LONG*, †

*Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, IA, USA, †Energy Biosciences Institute, University of Illinois at Urbana-Champaign, Urbana, IA, USA, ‡Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IA, USA

Abstract

For the C4 perennial grasses, Miscanthus × giganteus and Panicum virgatum (switchgrass) to be successful for bioenergy production they must maintain high yields over the long term. Previous studies under the less conduci ve climate for productivity in N.W. Europe found little or no yield decline in M. × giganteus in the long term. This study provides the first analysis of whether yield decline occurs in M. × giganteus under United States Midwest conditions in side-by-side trials with P. virgatum over 8–10 years at seven locations across Illinois. The effect of stand age was determined by using a linear regression model that included effects of weather. Miscanthus × giganteus produced yields more than twice that of P. virgatum averaging 23.4 ± 1.2 Mg ha⁻¹ yr⁻¹ and 10.0 ± 0.9 Mg ha⁻¹ yr⁻¹, respectively, averaged over 8–10 years. Relationships of yield with precipitation and growing degree days were established and used to estimate yields corrected for the stochastic effects of weather. Across all locations and in both species, yield initially increased until it reached a maximum during the fifth growing season and then declined to a stable, but lower level in the eighth. This pattern was more pronounced in M. × giganteus. The mean yields observed over this longer term period of 8–10 years were lower than the yields of the first 5 years. However, this decline was proportionately greater in M. × giganteus than in P. virgatum, suggesting a stronger effect of stand age on M. × giganteus. Based on the average yield over the period of this study, meeting the United States Renewable Fuel Standard mandate of 60 billion liters of cellulosic ethanol by 2022, would require 6.8 Mha of M. × giganteus or 15.8 Mha of P. virgatum. These appear manageable numbers for the United States, given the 16.0 Mha in the farmland Conservation Reserve Program in addition to another 13.0 Mha abandoned from agriculture in the last decade.

Keywords: bioenergy, biofuel, C4 photosynthesis, drought, nitrogen, stand age, temperature, yield decline

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Introduction

Production of biofuels from plant biomass provides the opportunity to reduce carbon emissions relative to conventional fossil fuels (Davis et al., 2012). To achieve production mandates while maintaining a positive carbon balance, biomass crops must achieve high yields with low inputs over the long term (Heaton et al., 2004a). Both Miscanthus × giganteus Greef and Deuter ex Hodkinson and Renvoize (Greef & Deuter, 1993; Hodkinson & Renvoize, 2001) and Panicum virgatum L. (switchgrass) have been suggested as high-yielding, low-input bioenergy feedstocks (Heaton et al., 2008; Dohleman & Long, 2009). Both of these species are C4 warm season perennial grasses that achieve high yields and high nutrient and water use efficiencies. An autumn or winter harvest, following retranslocation of nutrients to the underground perennating organs allows efficient interannual nutrient recycling (Beale & Long, 1997; Davis et al., 2009; Heaton et al., 2009; Propheter & Staggenborg, 2010). Both of these crops are planted in the spring and the shoots harvested annually in the autumn or early winter following senescence to allow for nutrient translocation to the perennial rhizomes while still achieving high harvestable yields (Beale & Long, 1995; Heaton et al., 2008, 2009). The resulting low-nitrogen concentration of the harvested material is

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desirable for both direct combustion and conversion to biofuel products (Beale & Long, 1997; Heaton et al., 2009). Aerial shoot regrowth of both species occurs the following spring directly from buds on the below-ground rhizomes. Both species are typically cited as achieving high stable ('ceiling') yields in the second or third year after planting (Clifton-Brown et al., 2001; Mclaughlin & Kszos, 2005; Wright & Turhollow, 2010). However, modeled results suggest it could take up to 5 years for Miscanthus × giganteus (Miguez et al., 2009), 2012). This prediction agrees with observations under cooler or poorer establishment conditions (Clifton-Brown et al., 2007; Heaton et al., 2008).

Miscanthus × giganteus is a sterile, naturally occurring hybrid which has been trialed extensively in Europe as a potential bioenergy crop (Hodkinson & Renvoize, 2001; Lewandowski, 2003; Heaton et al., 2004a) and is now grown commercially as a bioenergy crop (DEFRA, 2007). In parallel, P. virgatum has been trialed and developed as the major perennial grass feedstock for the United States, with several cultivars developed for different regions (Fuentes & Taliaferro, 2002; Mclaughlin & Kszos, 2005; Parrish & Fike, 2005). A heat and power or lignocellulosic ethanol operation is a significant investment, which requires a reliable supply of feedstock over many years for economic viability (Hamelinck et al., 2005). In addition, the establishment costs of Miscanthus × giganteus are high relative to seeded feedstocks (Lewandowski, 2003; Christian et al., 2005), and for the grower to make a profit, yield must be maintained for at least 5–10 years after planting (Heaton et al., 2004a). Stable and predictable yields are therefore necessary over a long period of time when used in bioenergy systems.

At present, there are only a few long-term continuous yield studies reported in the peer-reviewed literature, making it difficult to provide an accurate assessment of how long Miscanthus × giganteus will maintain ceiling yields (Miguez et al., 2008, 2012). Bullard (2001) and Lewandowski et al. (2000) propose that Miscanthus × giganteus will maintain ceiling yields for 15–20 years, but these predictions are based on the data from trials in N.W. Europe where reported annual yields of biomass have been about half of those observed thus far in the Midwest US (Heaton et al., 2008; Dohleman et al., 2012). These higher yields, and therefore higher nutrient off-takes, may lead to deficiencies or other yield decline drivers that could drive an earlier yield decline in the Midwest US.

Miscanthus × giganteus is a putative sterile hybrid of Miscanthus sacchariflorus and Miscanthus sinensis (Linde-Laursen, 1993; Rayburn et al., 2008). The growth cycle of the parent species may be indicative of Miscanthus × giganteus stand longevity. The parent species, Miscanthus sinensis and Miscanthus sacchariflorus, are both native to eastern Asia and at least one of these, Miscanthus sinensis, is a primary colonizer, invading bare land following fires or volcanic events and ultimately being replaced by trees. In Japan, grasslands dominated by Miscanthus sinensis are used for cattle grazing and may be under production for up to 6 decades (Stewart et al., 2009). Carbon isotope evidence suggests that some stands of Miscanthus sinensis, managed only by burning, have persisted for as long as 13 500 years (Stewart et al., 2009). These observations indicate that a stand might be maintained for many decades without fertilization. Currently, the longest continuous replicated trials of Miscanthus × giganteus in the peer-reviewed literature are four European trials spanning 12 years (Angelini et al., 2009), 14 years (Christian et al., 2008), 14 years (Gauder et al., 2012), and 15 years (Clifton-Brown et al., 2007). These studies did not use N-fertilization, excepting Angelini et al. (2009), which added 100 kg N ha⁻¹ yr⁻¹ and Clifton-Brown et al. (2007) provided small and intermittent additions of N.

In southern England over 14 years Christian et al. (2008) observed an initial 6 years of yield building, followed by 8 years of relative yield stability with no clear decline. However, no attempt was made to remove the interannual effects of differences in weather, which may be confounded with the result. In central Italy, Angelini et al. (2009) reported a ‘maturity phase’ of stable yields and then a ‘declining phase,’ although no description is given as to how they determined these phases. Gauder et al. (2012) reported that yields of fertilized Miscanthus × giganteus stands in S.W. Germany fluctuated significantly across years and did not comment on a relationship between yields and stand age, but instead reported a positive correlation between both ‘heat sum’ and precipitation during the growing season to yield. In western Ireland, Clifton-Brown et al. (2007) compared observed yields to those predicted by validated models and concluded that there was a decline in yield following the ninth growing season which could not be accounted for by interannual variation in weather. Clifton-Brown et al. (2007) described three phases: ‘yield building’ in years 2–4 followed by ‘stable yield’ (years 5–11) and then ‘reduced yield’ (years 12–14). It is perhaps not surprising that a yield decline was observed at the trial in western Ireland as it was on much poorer soils than the trials in southern England and therefore any deficiency caused by nutrient off-take would likely appear in western Ireland sooner than in southern England.

Although many studies examine the relationship of newly established (<3 years) to mature (≥3 years) stand yields of Panicum virgatum (Wang et al., 2010), few studies report continuous monitoring of yields in the long term (>4 years). Panicum virgatum stands that are annually harvested are expected to maintain productivity for 10–20 years (Hopkins et al., 1995), and it has been suggested that with harvest following complete senescence
and consistent nutrient replenishment, stands could remain in production ‘ad infinitum’ (Parrish & Fike, 2005). In fact, *P. virgatum* was selected as the model herbaceous bioenergy species in the United States, because it produced ‘reliable yields over varying climate conditions’ and has a large land base for potential production (Wright & Turhollow, 2010). *Panicum virgatum* is broadly adapted to a wide range of habitats from Central America to southern Canada (Parrish & Fike, 2005).

In the United States, it is a codominant species in climax tall-grass and mesic prairie communities (Rice & Parenti, 1978). The mixed community nature of its ecology suggests it may be poorly adapted to a monoculture production situation, although appropriate management may overcome this limitation, as with many other crops. A 4-year trial by Casler & Boe (2003) concluded that properly managed stands should maintain productivity, but that harvest before complete senescence can have severe long-term effects on stand persistence. Although the effect of stand age was not explicitly tested, Fike *et al.* (2006a) observed no tendency for decline in yield over a 10-year study of *P. virgatum* at eight locations across the southeastern United States managed with N-fertilization for biomass production. Similarly, a stand with only low-nitrogen input in Alabama showed no loss of yield over 13 years (Mclaughlin & Kszos, 2005). This study is the first to explicitly test the effect of stand age on *P. virgatum* yield, while accounting for interannual weather impacts.

When examining the effect of stand age on trials that were established in the same year, including weather variables it is necessary to isolate and independently test effects of stand age. Both *M. x giganteus* and *P. virgatum* growth has been shown to be dependent on both temperature and water availability (Heaton *et al.*, 2004b; Mclaughlin & Kszos, 2005; Parrish & Fike, 2005; Fike *et al.*, 2006a; Miguez *et al.*, 2008, 2009; Hastings *et al.*, 2009; Zub & Brancourt-Hulmel, 2010). The Palmer Hydrological Drought Index (PHDI) provides an integrated measure of water availability reflecting soil moisture and potential evapotranspiration throughout the entire year (Palmer, 1968; Heim, 2002), and is therefore more closely related to crop growth than precipitation alone. Similarly, annual, rather than seasonal, averages of PHDI are more appropriate as they will include soil moisture recharge due to precipitation outside of the growing season, which is significant for spring regrowth (Mclaughlin & Kszos, 2005; Fike *et al.*, 2006b). This measure ranges from −6 for the driest conditions to +6 for the wettest, with 0 as optimal for crop growth in a given climate zone, and is reported for most crop producing areas of the United States (Palmer, 1968). The PHDI was therefore used in this study to account for interannual variation in moisture availability. The threshold for leaf elongation to occur in *M. x giganteus* is about 10 °C, leaf elongation rate increasing linearly with temperature above this threshold (Clifton-Brown & Jones, 1997; Farage *et al.*, 2006). Regrowth is observed to begin at a similar date in the two species (Heaton *et al.*, 2008). Total yearly accumulation of growing degree-days with a base 10 °C (GDD10) will therefore provide a useful measure for the effects of interannual variation in temperature on yields of both *M. x giganteus* (Zub & Brancourt-Hulmel, 2010) and *P. virgatum* (Parrish & Fike, 2005). Heaton *et al.* (2008) and Dohleman (2009) established, maintained, and monitored seven research locations comparing the productivity of *M. x giganteus* and *P. virgatum* (*Cave-in-Rock*) along a transect spanning five degrees of latitude in Illinois and covering a range of soil types and significant variation in precipitation and temperature. Each used the same replicated plot design. These research locations were the first replicated field plot trials of establishment and production of *M. x giganteus* in the United States and the first ever side-by-side field comparisons of *M. x giganteus* and *P. virgatum*. As noted earlier, yields rose over the first 3 years following planting. At 3 years and beyond, yields are generally considered to have reached a ceiling in these crops. For years 3–5 at the three locations established in 2002 in Illinois, *M. x giganteus* yielded 29.6 ± 1.8 Mg ha⁻¹, about 2.7 times greater than *P. virgatum* (10.4 ± 1.0 Mg ha⁻¹; Heaton *et al.*, 2008; Dohleman 2009). These locations now represent the oldest comparative replicated field trials that have been continuously monitored within the United States, and they provide a unique opportunity for examining yields beyond the fifth growing season.

This study therefore asked two questions. (i) Over the now 8–10 year duration of these trials, how do the yields of *Miscanthus × giganteus* and *Panicum virgatum* compare with each other and across locations? (ii) Are yields of *Miscanthus × giganteus* and *Panicum virgatum* sustained over 8–10 years without fertilization or do they decline?

### Materials and methods

#### Crop establishment

Seven field trials comparing the productivity of *M. x giganteus* (*Illinois*) and *P. virgatum* (*Cave-in-Rock*) were established in 2002 (3 locations) or 2004 (4 additional locations; Table 1; Fig. S1). The trials span 5° of latitude and a range of soil types across Illinois in the Midwestern United States (Table 1). Three field trials were established in 2002 in Dekalb, Urbana, and Dixon Springs, IL, and were described in detail by Heaton *et al.* (2008). Four additional field trials, of the same experimental design and with the same plant materials as the trials established in 2002, were established in 2004 in Havana, Browns-town, Fairfield, and Orr, IL (Table 1). In brief, each field trial
was planted in the same completely randomized design of four 10 × 10 m plots each of *M. × giganteus* and *Panicum virgatum* (*n* = 4 for each species at each location; 56 plots total). Plants of *M. × giganteus*, propagated from a single clone, were grown in a greenhouse in 10 cm pots and transplanted at a density of 1 plant m⁻². *Panicum virgatum* ‘Cave-in-Rock’ seed was sown into a fine tilth at the rate of at least 13 kg pure live seed ha⁻¹. Both procedures follow protocols for prior separate trials of these crops (Heaton *et al.*, 2008). To ensure even stand development, irrigation was employed, but only during the establishment year. Weed control was implemented as required by a combination of manual cultivation, pre-emergent, and postemergent herbicide applications throughout the duration of this study (Table S1). These locations were maintained and harvested annually after completion of senescence.

### Growing conditions

Soil descriptions, cropping history, and previous plot maintenance are available in Heaton *et al.* (2008), Dohleman (2009) and Table 1. Details of plot maintenance from 2009–2011 are provided in Supplementary Table 1. Weather data were obtained from NOAA’s Midwestern Regional Climate Center’s Applied Climate System (http://mrcc.isws.illinois.edu) and are reported from the weather station nearest to each field having weather records for ≥ 91% of the days in a given year (Fig. 1). Growing Degree days₁₀ were calculated as follows:

\[
GDD_{10} = \sum_{i=1}^{n} \left( \frac{T_{\text{max},i} + T_{\text{min},i}}{2} - 10 \right) \]

where, \( T_{\text{max},i} \) is maximum temperature on day \( i \) and \( T_{\text{min},i} \) the minimum, and where \( GDD_{10} \) is zero on days where \( (T_{\text{max},i} + T_{\text{min},i})/2 \leq 10 \) °C.

### Biomass sampling

Biomass was sampled for 8 or 10 consecutive years, depending on site, to calculate yield. Heaton *et al.* (2008) have previously

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**Table 1** Description of locations where side-by-side trials of *Miscanthus × giganteus* and *Panicum virgatum* ‘Cave-in-Rock’ were planted in 2002 and 2004. Soil descriptions were from USDA-NRCS (2012). Annual temperature and precipitation averages are 30 year averages (Angel, 2007)

<table>
<thead>
<tr>
<th>Location</th>
<th>Lat, long</th>
<th>Soil taxonomic classification, description, and land capability class (LCC)</th>
<th>Average annual temperature (°C)</th>
<th>Average annual precipitation (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Planted in 2002</strong></td>
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<tr>
<td>Dekalb</td>
<td>88.85, 41.85</td>
<td>Fine-silty, mixed, superactive, mesic Typic Endoaquolls; formed from loess or silty material and the underlying till. Poorly drained. Slope 0%, 2w</td>
<td>9</td>
<td>95</td>
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<td>Northern Illinois Agronomy Research Center</td>
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<td>Shabbona, IL</td>
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<td>Urbana</td>
<td>88.23, 40.08</td>
<td>Fine-silty, mixed, mesic Typic Endoaquoll; very deep and formed from loess and silt parent material deposited on the till and outwash plains. Somewhat poorly drained. Slope 0%, 1</td>
<td>11</td>
<td>104</td>
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<tr>
<td>Crop Science Research and Education Center</td>
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<td>Urbana, IL</td>
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<tr>
<td>Dixon Springs</td>
<td>88.67, 37.45</td>
<td>Fine-silty, mixed, active, mesic Oxyaquic Fragiudalfs; formed in loess and underlying weathered stone. Moderately permeable above the fragipan and very slowly permeable in the fragipan. Slope 0–2%, 2e</td>
<td>15</td>
<td>123</td>
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<td>Dixon Springs Agricultural Research Center</td>
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<td>Simpson, IL</td>
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<td><strong>Planted in 2004</strong></td>
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<tr>
<td>Havana</td>
<td>89.94, 40.30</td>
<td>Sandy, mixed, mesic Entic Hapludollls; very deep, excessively drained soils formed in sandy outwash that has been reworked by wind. Slope 1–7%, 4s</td>
<td>9</td>
<td>96</td>
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<td>Central Illinois Irrigated Growers Association</td>
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<td>Havana, IL</td>
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<tr>
<td>Orr</td>
<td>90.82, 39.81</td>
<td>Fine-silty, mixed, mesic Oxyaquic Hapludolls; very deep, moderately well drained, moderately permeable soils formed in loess. Slope 5–10%, 3e</td>
<td>9</td>
<td>96</td>
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<td>Orr Research and Education Center</td>
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<td>Perry, IL</td>
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<td>Brownstown</td>
<td>88.96, 38.95</td>
<td>Fine, smectitic, mesic Mollic Albaqualfs; very deep, poorly drained, slowly or very slowly permeable soils on till plains. Slope 0–2%, 3w</td>
<td>13</td>
<td>93</td>
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<td>Brownstown Research and Education Center</td>
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<tr>
<td>Fairfield</td>
<td>88.39, 38.38</td>
<td>Fine, smectitic, mesic Typic Albaqualfs; very deep, slowly or very slowly permeable, poorly drained soils on broad till plains. Slope 0–2%, 3w</td>
<td>13</td>
<td>112</td>
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<td>Wayne County Extension Office</td>
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reported on the 2003–2006 growing season for Dekalb, Urbana, and Dixon Springs. Dohleman et al. (2012) reported the average 3 year (2006–2008) above-ground yield for the Urbana location. These previously published data are included in the present analysis to examine long-term trends. Trials were harvested at each location after all plants in the plots had senesced, typically in December for *M. × giganteus*. End-of-season *P. virgatum* yields were calculated from biomass sampling following the first hard freeze and *M. × giganteus* yields are calculated from harvest following leaf drop (Table S2). Biomass sampling followed the methods described by Heaton et al. (2008); briefly, two randomly selected 0.19 m² quadrats per plot were cut by hand to a 5 cm stubble height and dried at 74 °C to constant mass. The outer one meter of each plot was treated as guard row, and samples were taken from the inner 64 m². To facilitate a separate chemical treatment study, starting in 2007 or 2008, depending on location, each plot was divided into four subplots. One sub-plot of 25 m² within each replicate plot remained untreated, and this study was restricted to these untreated subplots.

**Statistical analysis**

Prior to subdivision of the plots, two subsamples per plot were taken and the mean of these values was treated as a single sample to avoid pseudo-replication. After the study locations were converted to a split-plot study, one subsample per subplot was taken. Therefore, under all circumstances in this study, plot (P) was identified as the experimental unit and all statistical analyses were conducted on either the plot mean (prior to subdivision) or on the single subplot measurement (*n* = 4).

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Fig. 1 Cumulative annual growing degree days using a base temperature of 10 °C (GDD₁₀) (●) and Palmer Hydrological Drought Index (PHDI) (▼) from seven monitoring stations of the Midwestern Regional Climate Center (http://mrcc.isws.illinois.edu) near field trials at Dekalb, Urbana, Dixon Springs, Havana, Orr, Brownstown, and Fairfield. Dashed line indicates 30-year averages of GDD₁₀.
A mixed model repeated-measures ANOVA was used to examine differences in yield (y) between species and locations across the entire study period. Analysis was performed using restricted maximum likelihood (REML; Proc Mixed, SAS version 9.2, SAS Institute, Cary, NC, USA). Species (S) and Location (L) were treated as fixed categorical variables, and Year (Y) was specified as a repeated-measures term with plot within location as the experimental unit. Year × L was included as a random effect to account for differences in weather across years and locations, in addition to other location dependent effects.

Model I:

\[ y = S + L + S \times L + L(P) + Y \times L \]

Tukey’s least significant difference (LSD) tested pairwise comparisons of yields of *M. x giganteus* and *P. virgatum*, between locations pooled across all years, and at each location in each year individually.

Because interannual variation in weather has a large effect on yield, it was necessary to isolate age effects from weather effects. Therefore, a second multivariate regression model was constructed to correct observed effects for weather-induced variation in yield (y) for each location. Mean annual PFDI (Palmer, 1968), annual cumulative degree days above 10 °C (GDD10), and stand age (SA) were continuous fixed effects, and species (S) and location (L) were discrete fixed effects using the following model and same dataset as Model I:

Model II:

\[
\begin{align*}
    \text{PHDI} \times \text{PHDI}^2 + \text{GDD}_{10} + \text{GDD}_{10}^2 + S + S^2 + S^3 + S \\
    + L + \text{GDD}_{10} \times S + \text{GDD}_{10}^2 \times S + S \times S + S^2 \times S + S^3 \times S \\
    + S \times L + S^2 \times L + S^3 \times L + \text{PHDI} \times S + \text{PHDI}^2 \times S + S \\
    \times L + L(P)
\end{align*}
\]

Observations of each plot within a location were treated as repeated measures by year with a first-order autoregressive covariance structure grouped by species. Given that this dataset was limited to 8–10 growing seasons over seven locations established at two time points, there were insufficient degrees of freedom available to run all possible interactions. Therefore, terms were selected based on known biological relevance. Palmer Hydrological Drought Index by L was not included because it already accounts for differences in soil types and water holding capacities. For both PHDI and GDD10, incorporating a quadratic effect of these terms provided a statistically better fit, as determined by a log-likelihood ratio test, to the observed data than a linear term alone or with the addition of a cubic term. It is known that perennial grasses exhibit a ‘yield building’ phase as they approach maturity; therefore, a simple linear term was not sufficient to capture changes in yield over time. The yield in year of planting and the yield in the second year following planting were omitted, as it is considered that 3 years are required to achieve mature yields (Heaton et al., 2008). A quadratic SA term was included to allow for further yield building, continuing after the second year, as well as decline, but this led to nonsensical (negative) predictions of yield. Therefore, a cubic SA term was included to allow for differing rates of stand building and decline; that is, yield may not actually decline with stand age, or the decline may be slower than the initial increase seen in the first few years. When a quartic SA term was included, yield predictions were once again nonsensical (e.g., negative). To verify statistical assumptions of independence, normality, and homogeneity of variance, residuals were examined post hoc. Furthermore, a plot of observed values vs. predicted values was constructed to examine the goodness of fit to the model (Fig. S2).

To graphically present the effect of stand age, predictions of yield were calculated for all stand age, location, and species combinations given the mean PHDI and GDD10 for each location over the period of the study. That is, for each location, PHDI and GDD10 was set to a constant equal to the mean observed PHDI and GDD10 and y was calculated for each species, stand age, and location. Using the mean values of weather observed at a location, it was possible to remove the stochastic effect of weather between each year and look explicitly at the effect of stand age.

### Results

The period of this study encapsulated a wide variety of weather conditions across all of the locations (Fig. 1). In general, mean annual cumulative GDD10 approximated the measured 30-year historical averages at all locations except Urbana, which experienced consistently higher temperatures throughout the 10 years of this study. Conditions in the latter part of this study (2007–2011) were generally wetter across all locations and cooler in 2007 and 2008 than in the early years (2004–2006).

*Miscanthus x giganteus* and *P. virgatum* were successfully established in 2004 at four additional locations and trials established in 2002 continued to produce good yields. All yields reported here are based on the above-ground dry biomass harvested at the completion of senescence; for brevity this measure is hereinafter referred to as yield. When Model I is employed to examine the overall effects of species and location, pooled across all seven locations and all years (2002–2011), yield was significantly higher in *M. x giganteus* (23.4 ± 1.2 Mg ha⁻¹ yr⁻¹) than in *P. virgatum* (10.0 ± 0.9 Mg ha⁻¹ yr⁻¹, *P* < 0.001; Tables 2 and 3). When each year is examined independently, *P. virgatum* always yielded less than *M. x giganteus*, but the difference was not always statistically significant in every year (Table S3). There was a significant interaction effect of location by species (*P* < 0.05; Model II, Table 2). That is, the magnitude of difference between *M. x giganteus* and *P. virgatum* yields varied by location. Notably, this difference was highest at Dixon Springs where there was a threefold advantage of *M. x giganteus* over *P. virgatum* yields and least at Brownstown and Havana where it was just 1.7-fold (Table 3). When compared across locations, and pooled across all years, yield of *M. x giganteus* was highest at Urbana, Fairfield, Dixon...
Springs, and Orr, while *P. virgatum* was highest at Urbana, Fairfield, Orr, and Havana (Table 3).

When the observed data are pooled across locations both crops show an increase in yield from year three to five, followed by a decline which appears to arrest at year 8 (Fig. 2a). However, this pattern could be a feature of the crops or could result from interannual variation in weather. To test for and to account for the effects of weather, the multivariate regression model which incorporated the effect of weather on yield was applied to isolate and independently test the effect of stand age (Model II). Observed vs. predicted yields from this model for both species showed a near 1:1 agreement (Fig. S1). Figure 2b applies this model to show the yields for *M. × giganteus* and *P. virgatum* pooled across all locations predicted if soil moisture (PHDI = 0) and temperature (GDD10) were the same in each year. This confirms the trend of the observed data.

The response of both *M. × giganteus* and *P. virgatum* to increasing stand age was curvilinear and best fit by a cubic relationship; however, the magnitude of the response differed between the species (Fig. 2). There was an initial period of yield increase (SA × S, *P* < 0.05) that reached a maximum or ‘ceiling’ yield, the greater increase in *M. × giganteus* during this period is evident in Fig. 2. Following this ceiling, yield declined (SA2 × S, P < 0.05) and leveled (SA3 × S, P < 0.1; Fig. 2). This response to stand age was not location dependent, all locations showed a similar pattern of changes in yield with increasing age, when corrected for weather (SA × L, SA2 × L, and SA3 × L, P > 0.10; Table 2).

When the weather effects were tested via Model II, there is a significant effect of both PHDI and GDD10 (P < 0.05, Table 2). *M. × giganteus* and *P. virgatum* had similar patterns of response to both GDD10 and PHDI (GDD10 × S, GDD102 × S, PHDI × S, PHDI2 × S, *P* > 0.1, Table 2) although the response of GDD10 was location dependent (GDD10 × L, GDD102 × L, *P* < 0.05, Table 2).

Figures 3 and 4 show the observed yields at all locations across all years with a cubic fit (solid line) showing the trend of yield building, stability, and then reduction at many of the locations, but not all. However, weather is interannually variable and is confounded with this simple fit of observed values; when yield is corrected for weather variation by predicting

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Table 2 Mixed model analysis of variance for fixed effects and Wald tests for covariance parameter estimates of random effects associated with end-of-season above-ground biomass yield of *Miscanthus × giganteus* and *Panicum virgatum* ‘Cave-in-Rock’ across the three locations planted in 2002 for the 3rd–10th growing seasons and four locations planted in 2004 (Table 1) for the 3rd–9th growing seasons for Models I and II (see Methods). Values in bold are significant at *P* < 0.1.

Table 3 Model I estimates of yield and SE (Mg ha⁻¹) for *Miscanthus × giganteus* and *Panicum virgatum* ‘Cave-in-Rock’ pooled across all locations and years. Letters indicate Tukey’s least significant difference (LSD) means separation groups at *P* = 0.05 separately for each species (A, B, C for *M. × giganteus* and Y, Z for *P. virgatum*).
expected yields for normalized weather across all years at each locations the explicit effect of stand age is seen (dashed line; Figs 3 and 4). This confirms a yield decline for both species at most sites.

Discussion

Although relatively high yields of M. × giganteus were recorded across seven locations and 8–10 years,
averaging $23.4 \pm 1.2 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, yields clearly declined at stand age 6 years and beyond, as did \textit{P. virgatum}, averaging $10.0 \pm 0.9 \text{ Mg ha}^{-1} \text{ yr}^{-1}$. This appears the first clear evidence of yield decline in \textit{M. \times giganteus}, especially in stands of this age. Such a strong decline has not been observed in European studies, and as outlined earlier, could result from higher earlier yields in Illinois exhausting limiting soil nutrients sooner (Heaton \textit{et al.}, 2008; Dohleman \textit{et al.}, 2012). However, the pattern is strongly affected by both location and interannual variation in weather (Table 2).

\textit{Miscanthus \times giganteus} consistently produced yields more than twice those of \textit{P. virgatum} (Fig. 2; Table 2). This is consistent with the higher yields of \textit{M. \times giganteus} relative to those of \textit{P. virgatum} reported by Heaton \textit{et al.} (2008) for the first 3 years of trials at the three locations planted in 2002. These continue into the long term (10 years) and are repeated here at a wider range of locations. These locations include both a range of soil qualities from land capability class (LCC) 1, soils that are considered ideal for crop production, to class 4 soils with little organic matter, poor water-holding capacity, and low fertility (Table 1). The \textit{M. \times giganteus} yields reported in this study ($23.4 \pm 1.2 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) are 21% lower overall than those reported by Heaton \textit{et al.} (2008) for the 2002 established trials over 2004–2006 ($29.6 \pm 1.8 \text{ Mg ha}^{-1} \text{ yr}^{-1}$). This is in part driven by lower yields on the four additional sites chosen in 2004 (Table 1), since if only the three sites established in 2002 are considered then the average yield for years 3–10 across these sites was $25.8 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, declines only 13%. Yields observed for \textit{P. virgatum} ($10.0 \pm 0.9 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) were similar, trending only slightly lower than those reported by Heaton \textit{et al.} (2008) ($10.4 \pm 1.0 \text{ Mg ha}^{-1} \text{ yr}^{-1}$). This difference in end-of-season yield in \textit{M. \times giganteus}, but not in \textit{P. virgatum}, is consistent with the suggestion that the higher initial yields could be depleting limiting nutrients, more rapidly, driving a sharper decline with stand age, and therefore a more pronounced ‘peak’ yield, of \textit{M. \times giganteus} relative to \textit{P. virgatum} (Figs 3–4).

For both \textit{M. \times giganteus} and \textit{P. virgatum}, yields were higher in southern compared with northern locations (Table 2) consistent with the higher temperatures (GDD$_{30}$) and soil moisture (PHDI). Yields at Browns-town, however, were a notable exception to this trend and could be a result of slow establishment due to hot and dry conditions in the early years compounded by the heavy soil (LCC 3w) which likely impeded development of roots and rhizomes, and access to water deeper in the profile.

\textit{Miscanthus \times giganteus} yields were more variable across locations than yields of \textit{P. virgatum} (Table 2). The greater variability with stand age in yield of \textit{M. \times giganteus} than in \textit{P. virgatum} is not a result of response to weather conditions (GDD$_{10}$ and PHDI) as there was no interaction between species and these weather variables (Table 2). Yields for both species had a quadratic relationship with PHDI ($P = 0.0341$) indicating an optimum, suggesting that excessive water slowed development through water logging, as well as likely being correlated with less sunlight. The optimum indicated by this relationship was an average annual PHDI of $-0.04$ and close to the assumed optimum of 0 for the Palmer Index. The yield response to GDD$_{10}$ was also quadratic and location dependent (GDD$_{10}^2 \times L$, $P = 0.0227$) and therefore a different optimum GDD$_{10}$ would appear to apply to each location. As the same genotype was used at each site, this has to be the result of an environmental interaction. The large range of observed average yields across the seven locations in \textit{M. \times giganteus} (14.7–31.14 \text{ Mg ha}^{-1} \text{ yr}^{-1}) and \textit{P. virgatum} (7.45–13.71 \text{ Mg ha}^{-1} \text{ yr}^{-1}) is most likely the result of soil conditions as seen by the significant location effect and species by location interaction (L, $P = 0.0051$, $S \times L$, $P < 0.0001$, Table 2, Model II). As noted earlier, however, \textit{M. \times giganteus} showed its highest yields at the more southerly sites. Average yields at Urbana and the two most southerly sites, Dixon Springs and Fairfield, exceeded 30 \text{ Mg ha}^{-1} \text{ yr}^{-1} even though the latter was land-capability class 3w. This confirms that high yields can be consistently achieved on some, but not all challenging soils (Tables 1 and 2).

This study shows a period of yield building followed by a period of rapid decline as indicated by the significant quadratic response of yield to stand age. In both species yield was not maintained, but rather declined progressively, within the timeframe of this study. This decrease in yield is earlier than the 15–20 year prediction of interannual stability of yields for \textit{M. \times giganteus} based on European trials (Lewandowski \textit{et al.}, 2000; Bullard, 2001) and the 10–20 year predictions for \textit{P. virgatum} (Hopkins \textit{et al.}, 1995). This earlier decrease may result from the higher yields of the earlier years, relative to previous studies, which would result in more nutrient off-take. In the case of \textit{M. \times giganteus} the lower atmospheric N deposition levels in the United States relative to the European trial locations could exacerbate soil N depletion (Holland \textit{et al.}, 2005). The data indicate that the decline, which began at around year 5, had slowed and yields were stabilized around year 8. This is statistically validated by the fact that incorporating a cubic term, which describes this stabilization into the statistical model, accounts for significantly more variation than a quadratic (Table 2).

These trials were grown without fertilization and perhaps nutrient addition could recover end-of-season yields to ‘ceiling’ values. Nonetheless, this study
confirms the results of Heaton et al. (2008) that \( M. \times giganteus \) yields significantly more biomass than \( P. virgatum \) across all the growing conditions observed in this study. Furthermore, it is seen that despite the sharper decline in yield of \( M. \times giganteus \) relative to \( P. virgatum \) across the entire timeframe observed in this study, higher yields were still achieved by \( M. \times giganteus \) and that viable yields are still obtained in the 8–10th growing seasons without fertilization. While 3 years is commonly suggested to be the time taken for both crops to reach ceiling annual yields following initial planting, it is clear that overall yields of both crops across these sites continued to increase into year 5 (Fig. 2; Table 2). Although \( P. virgatum \) clearly performed best on the highest LCC site, \( M. \times giganteus \) performed equally well on the two LCC 3w sites as on the LLC 1 site (Table 2). Even on the LCC 4s site \( M. \times giganteus \) achieved an average yield of 16 t ha\(^{-1}\).

The findings clearly call for research to discover how the higher earlier yields might be maintained into the longer term. Possible causes of decline are exhaustion of soil nutrients, especially nitrogen, soil compaction, and pest and disease pressure. As noted earlier soil nitrogen appears to be a particularly likely candidate, as it would explain why yield decline is more marked than in Europe where nutrient off-take is lower due to lower yields and atmospheric nitrogen deposition higher. Soil compaction is commonly cited as the cause of successive annual declines in yield of sugarcane (\( S. officinarum \)) shoots, where it progressively impedes root growth (Smith et al., 2005; Singh et al., 2012). \( S. officinarum \) and \( M. \times giganteus \) are closely related to the

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**Fig. 4** As for Fig. 3, but showing the trials planted in 2004 at Havana, Orr, Brownstown, and Fairfield, IL, for the 2006–2011 growing seasons.
extent that fertile hybrids can be obtained from crosses between the *M. × giganteus* parent *M. sinensis* and *S. officinarum* (Chen et al., 1993). Therefore, a parallel decline might be expected in *M. × giganteus*. However, in this study the only annual operation was harvesting, which was by hand or small equipment, which would cause minimal compaction. Pests and diseases could also cause decline. No visible symptoms of infestations or diseases were recorded for these sites over this study period. However, root grazing nematodes and viruses that may cause minimal visible shoot symptoms have now been identified in both crops (Mekete et al., 2009, 2011; Agindotan et al., 2010a, b). These have the potential to accumulate to cause a decline. None of these three possible causes appear insurmountable, but will require multi-factorial experiments to test these competing hypotheses. This in turn will allow improved management to avoid the clear yield decline observed here to provide reliable feedstock supply to meet bioenergy demand.

The Renewable Fuel Standard (RFS) established by the Energy Independence and Security Act (EISA) of 2007 mandates the production of 133 billion liters of biofuel by 2022 (EPA, 2012). Heaton et al. (2008) predicted from the average yield observed across the three locations established in 2002 the amount of land that would be required to meet this 133 billion liter mandate. Based on years three through five at these sites they calculated that it would require 12 million hectares of *M. × giganteus* and 34 million hectares of *P. virgatum* to reach the mandate (Table 4). This study which presents the average yields observed across three locations from the third through tenth growing seasons along with those from four locations from the third through eighth growing seasons shows that the earlier predictions probably under-estimated the amount of land required in the long term. In fact, meeting this mandate with *M. × giganteus* and *P. virgatum* alone based on the longer term records reported in this study would require 15 and 35 Mha, respectively. As only 60 billion liters in the RFS is mandated for cellulosic ethanol, these area requirements reduce to 6.8 Mha and 15.8 Mha, respectively. Despite the lower yields reported in the longer term here, these areas still appear manageable for the United States. This is given a total land area for the 48 contiguous US states of 808 Mha of which 164 Mha is in cropland, 166 Mha in pasture, 16.0 Mha in the farmland Conservation Reserve program and another 13.0 Mha abandoned from agriculture in the last decade, alone (USDA-ERS, 2012).

**Acknowledgements**

We acknowledge the Energy Biosciences Institute, State of Illinois Council for Food and Agricultural Research (C-FAR) and the Illinois Experimental Station for supporting this research over the past 10 years. We thank Keith Chan, Joey Crawford, Ben Lewis, Ethan Kessler, Melissa Kocik, Rhea Kressman, Mike Hradek, John Jurveis, Neal Makela, Noel Platek, Ariel Raneri, and Emily Thomas, who helped in establishing and maintaining these trials.

**References**


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**Table 4** The land area that would be required to meet the 35 billion gallons (133 billion liters) United States. renewable fuel standard mandate using *Miscanthus × giganteus* and *Panicum virgatum* based on the mean yields reported by Heaton et al. (2008) and updated from this study. Calculation of the volume of ethanol produced per dry Mg of biomass, in this study, follows the methods of Heaton et al. (2008)

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Rayburn AL, Crawford JD, Rayburn CM, Jurik JA (2008) Genome size of three Miscanthus species. Plant Molecular Biology Reports, 27, 184–188.


Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Location of trial sites within Illinois and illustration of one of the replicated trials. (Photocredit: Andrew Leakey, University of Illinois).

**Figure S2.** Predicted yields vs. observed for *Miscanthus × giganteus* (green, ○) and *Panicum virgatum* (orange, +). The middle lines show the fitted linear relationship of predicted to observed values, and the banded area around each line is the 95% confidence limit on the mean.

**Table S1.** Management practices applied to the field trials by location.

**Table S2.** Table of sampling dates for *Miscanthus × giganteus* and *Panicum virgatum* for the 2004–2011 growing seasons.

**Table S3.** Tukey’s least squared difference pairwise comparison of *Miscanthus × giganteus* yield (Mg ha⁻¹) to that of *Panicum virgatum* cv. Cave-in-Rock end-of-season for each location and year. Values in bold represent where *M. × giganteus* yield was not significantly greater (*P* > 0.1) than that of *P. virgatum* at that location during that year.