The older plant gets the sun: Age-related changes in Miscanthus × giganteus phenology

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Abstract
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Keywords
aging, bioenergy, competitor-stress tolerance-ruderal (CSR) theory, growth dilution, morphological development, nitrogen dilution, nonlinear models, perennial grass, staggered-start design

Disciplines
Agriculture | Agronomy and Crop Sciences | Climate | Soil Science

Comments
This article is published as Tejera, Mauricio D., Fernando E. Miguez, and Emily A. Heaton. "The older plant gets the sun: age-related changes in Miscanthus× giganteus phenology." GCB Bioenergy (2020). doi: 10.1111/gcbb.12745.

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RESEARCH REVIEW

The older plant gets the sun: Age-related changes in Miscanthus × giganteus phenology

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Funding information
National Institute of Food and Agriculture, Grant/Award Number: 1008969; Iowa Energy Center; Department of Energy, Office of Science, Grant/Award Number: DE SC0018420; Leopold Center for Sustainable Agriculture (U.S.); Iowa State University, Department of Agronomy

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Age-related changes are usually overlooked in perennial grass research; when they are considered it is usually as a change in plant size (e.g., biomass). Whether other physiological or developmental aspects change as stands age, and how those aspects may impact long-term stand dynamics, remains unclear. Conventional experimental designs study a single stand over multiple growing seasons and thereby confound age-related changes with growing season conditions. Here we used a staggered-start experimental design with three repeated planting years over two growing seasons to isolate growing season effects. We studied changes in Miscanthus × giganteus phenology during its yield-building stage (first 3 years) and estimated age, growing season and nitrogen (N) effects on development using nonlinear regression parameters. Stand age clearly changed plant growth; faster developmental rates were usually seen in 1-year-old stands (young), but because 2- and 3-year-old stands (mature) emerged 3 months earlier than newly planted stands they produced ~30% more stems with 30%–60% more leaves. Nitrogen fertilization modulated some age-related phenological changes. Fertilized 2-year-old stands reached similar stem densities as unfertilized 3-year-old stands and had fewer number of senesced leaves like 1-year-old stands. In addition, N fertilization had no effect on young M. × giganteus, but extended mature stands’ growing season more than 2 weeks by hastening emergence and delaying senescence. It also delayed flowering regardless of stand age. Our results suggest that, along with changes in size, M. × giganteus stands showed shifts in developmental strategies: young stands emerged later and developed faster, while mature stands grew for longer but more slowly. In temperate regions, where hard frost events are likely to interrupt development in late autumn, rapid early development is critical to plant survival. Nonlinear regression parameter differences proved effective in identifying phenological shifts.

KEYWORDS
aging, bioenergy, competitor-stress tolerance-ruderal (CSR) theory, growth dilution, morphological development, nitrogen dilution, nonlinear models, perennial grass, staggered-start design
Perennial energy grasses are a fundamental component of the emerging bioeconomy (Robertson et al., 2017). While many of their desired features (e.g., low-input requirements, high yields, reduced soil disturbance) are related to their perennial lifecycle, an essential aspect of their perenniality, age, is often overlooked. Age effects in perennial grasses are usually characterized in terms of changes in end-of-season biomass over growing seasons. Based on these changes, perennial grass lifecycle presents two main life stages: a yield-building stage (1–3 years), when end-of-season biomass gradually increases over growing seasons, and an adult stage (3+ years) when end-of-season biomass production plateaus and gradually decreases over growing seasons, associated with stand decline (e.g., Alexopoulou et al., 2015; Arnoult & Brancourt-Hulmel, 2015; Arundale et al., 2014). While age-related developmental or physiological changes, with implications in stress tolerance and resource allocation, have been successfully observed in other perennial plants, they remain poorly understood in bioenergy grasses.

Age effects could also be characterized in terms of changes in in-season development. During the growing season, perennial grasses grow a new crop of above-ground organs and undergo an annual developmental cycle. This annual development is divided into clearly defined growth stages grouped in three main phases: emergence phase, which encompasses shoot emergence from newly planted rhizomes or seeds, or from overwintering rhizomes in the case of already established stands; a vegetative phase, when stems rapidly produce leaves and elongate internodes, and a reproductive phase, when stems flower and set seed (e.g., Moore et al., 1991; Tejera & Heaton, 2017). This annual cycle ends when above-ground organs have totally senesced, which may occur as a natural developmental process or be triggered by environmental conditions. Age effects, now, can be ascribed in terms of changes in the duration and progression of each developmental phase.

Research on perennial cereals (Jaikumar, Snapp, & Sharkey, 2016), temperate (Vitasse, 2013) and evergreen trees (Bond, 2000), and forage grasses (Dayrell et al., 2018) all associate age-related changes in development with shifts in life strategies. During the yield-building stage, perennials can resemble r-strategists as they prioritize growth and resource acquisition. They emerge earlier in the season even at the risk of early frost events, develop faster to capture more resources. As they reach the adult stage, they shift toward a K-strategy where they emerge later to avoid late hard frosts in spring and start senescence earlier so internal nutrient recycling is not interrupted by early autumn frosts. As a result, adult perennials become more conservative and tend to favor stress tolerance (Boersma, Dohleman, Miguez, & Heaton, 2015; Bond, 2000; Jaikumar, Snapp, & Sharkey, 2013). These differences may enhance species fitness as plants face different challenges to survival across life stages. If this hypothesis holds for perennial grasses, the observed yield dynamic could be a consequence of a life-time program which prioritizes growth during the yield-building phase, when stands show faster growth rates and reach higher end-season biomass, and stress tolerance during the adult stage when end-season biomass tends to decline.

On the other hand, observed age effects could simply be an artifact of plant size, especially when comparing across plants in yield-building and adult phases. Dilution of nutrient pools, particularly nitrogen (N), in older and larger individuals could cause limitations that present themselves as age effects. Given the tight correlation between age and size, it is hard to study these effects separately. To reduce confounding effects, N fertilization treatments could be included in age studies to help separate the interactive effect of N on age-related changes.

Nitrogen fertilization generally delays flowering in perennial grasses; however, responses are highly variable across ecosystems and species, and quite unpredictable (Nord & Lynch, 2009). For example in natural communities, a 105 kg N/ha addition delayed or had no effect on flowering and fruiting time of different perennial grasses in a temperate steppe in northern China (Xia & Wan, 2013) and an alpine meadow on the Tibetan Plateau (Xi et al., 2015). The response is also variable across energy grasses: a 112 kg N/ha fertilization hastened switchgrass development by 3 weeks and made stands flower earlier in Texas, USA, but had no effect the following year (Sanderson & Reed, 2000). A similar year by N response interaction was found in Iowa, USA, where the N effect on morphological development of four different grasses was only evident in the second year (Waramit, Moore, & Heaton, 2014). Also, a 140 kg N/ha fertilization rate increased, reduced or had no effect on the number of individuals that reached the reproductive phase depending on the species (Waramit et al., 2014). Together, these studies support the hypothesis that N has an influence on phenology, however, as they are only based on perennial grasses in the adult phase of their lifecycle, they are insufficient to test the dilution effect hypothesis. A side-by-side comparison of the N response of stands at different life stages would help parse out this variable response and better understand whether age-related changes follow changes in life strategies or if they are the result of nutrient limitation.

We studied age-related changes in Miscanthus × giganteus (Greef et Deu.; Hodkinson & Renvoize, 2001) phenology. This clonally propagated energy crop is commercially adopted in temperate areas of the United States and Europe and its N demands have been widely studied, yet the yield response of M. × giganteus to N remains unclear (LeBauer et al., 2017). Research on perennial grasses’ yield dynamics are usually based on repeated observations of a single stand over subsequent years. While convenient and widely used, this method confounds growing season and stand age effects and ignores variability generated during the...
establishment of the stand. Previous research with this approach showed that establishment conditions, such as time between planting and fertilizing, and growing seasons, such as the length of it, are a strong driver of *M. × giganteus* yield response to N fertilization (Tejera et al., 2019). Since bioenergy crops are relatively new and lack long-term empirical data, accurate mechanistic modeling is essential to valuing and predicting their performance for the bioeconomy (Cosentino et al., 2018). Accurate modeling depends on accurate parameterization; thus, it is important to elucidate and model the relationships between age, phenology, N and yield (Dietze, Lebauer, & Kooper, 2013; Surendran Nair et al., 2012).

We used a staggered-start experimental design, where stands are repeatedly planted over subsequent years, to separate stand age effects from environmental effects (Casler, 1999; Loughin, Roediger, Milliken, & Schmidt, 2007; Tejera et al., 2019). First, we characterized *M. × giganteus* age-related changes in phenology and hypothesized that (a) *M. × giganteus* would produce more plant structures (i.e., stems, leaves, nodes) and reach more advanced developmental stages in a given growing season as it aged. Then we tested whether juvenile stands would prioritize resource acquisition and growth, and mature stands would favor nutrient recycling and winter survival. We hypothesized that (b) juvenile stands would have faster developmental rates and start development sooner and (c) mature stands would start senescence sooner in the growing season. Finally, we studied N fertilization effects on *M. × giganteus* phenology to assess whether N was limiting in mature stands and age-related changes were an artifact of size. We hypothesized that (d) fertilized juvenile stands would have similar dynamics as mature unfertilized stands.

## 2 MATERIALS AND METHODS

### 2.1 Experimental design

We studied *M. × giganteus* phenological responses to stand age and N fertilization during the yield-building phase and beginning of adult phase using a staggered-start experimental design. The experimental design was a split-plot with four blocks. Planting years (2015, 2016, and 2017) were the main-plot and N fertilization rates (0, 112, 224, 336, and 448 kg/ha) the subplot. Main plots were 24 m by 60 m, with five split-plot strips of 24 m by 12 m. This design allowed side-by-side comparison of *M. × giganteus* phenology across various N rates and stand ages over multiple growing seasons. Because stand ages were measured during growing seasons in different years, we were able to estimate the effect of the growing season on *M. × giganteus* development as well as its interaction with stand age and N fertilization. The study was carried out at the Iowa State University Sorenson Farm in central Iowa (42.0132N, −93.7430W), USA. The soil type was Typic Endoaquolls, average N soil content before planting was 57 kg N/ha. As plots within blocks were contiguous, we collected our samples from an area located ~1.5 m from the edges of the plot to avoid potential N diffusion between plots, or edge effects. *M. × giganteus* (Greef et Deu.) clone “Freedom” (sourced from Repreve Renewables, now AGgrow Tech) was planted in 0.30 m rows at a density of ~11 rhizomes/m² using a mechanized plot planter. The Freedom clone is genetically similar, if not identical to, both the “Illinois” clone widely used in the US and the “EMI-1” clone widely used in Europe (Glowacka et al., 2015). Planting occurred in May and N treatments were applied a few weeks prior to planting in all three study years (Table 1). Herbicides were applied for weed control and stands were mechanically harvested to ~10 cm stubble height each winter. See Tejera et al. (2019) for details of plot management.

### 2.2 Weather data

Historic and current weather data (Table 1) were obtained from the Iowa State University Soil Moisture Network (Iowa Environmental Mesonet, 2001). The weather station was 2.7 km from the experimental site. We defined the beginning

| Growing season | Planting date | Nitrogen fertilization date | Monthly average temperature (°C) | Seasonal GDD<sup>a</sup> (°C) | Seasonal precipitation (mm) | Seasonal incident PAR (MJ/m²) | Last spring frost<sup>b</sup> | First autumnal frost |
|----------------|---------------|----------------------------|---------------------------------|-----------------------------|----------------------------|-----------------------------|---------------------|---------------------|
| 2015           | 4 May         | 30 Apr.                    | 17.5                            | 2,540                       | 840                        | 1,863                       | 27 Mar.             | 20 Nov.             |
| 2016           | 6 May         | 26 Apr.                    | 18.6                            | 2,779                       | 713                        | 1,884                       | 3 Mar.              | 19 Nov.             |
| 2017           | 16 May        | 15 May                     | 18.9                            | 2,561                       | 580                        | 1,812                       | 15 Mar.             | 28 Oct.             |
| 1986–2013      | —             | —                          | 16                              | 2,423                       | 656                        | 1,768                       | —                   | —                   |

<sup>a</sup>Growing degree days (GDD) were calculated as mean daily temperature minus 6°C as baseline temperature (Farrell et al., 2006).

<sup>b</sup>Days with air temperatures below 0°C for >12 hr. Hourly weather data were not available to estimate historic frost events.
of the growing season as the planting date for new stands (Table 1), and the date of last spring hard frost for established stands. The first hard autumnal frost marked the end of the growing season. A hard frost was defined as when the air temperature stayed below 0°C for more than 12 hr (Kaiser & Sacks, 2015). Thermal time was measured in growing degree days (GDD, °C day) as:

\[
GDD = \left[ \frac{T_{\text{max}} + T_{\text{min}}}{2} \right] - T_b,
\]

where \(T_{\text{max}}\) and \(T_{\text{min}}\) are daily high and low temperatures, and \(T_b\) is a base growth temperature of 6°C (Farrell, Clifton-Brown, Lewandowski, & Jones, 2006). Use of GDD to track development allowed us to avoid confounding factors associated with management and planting dates. One-year-old stands inevitably started their growing season later as they were mechanically planted, and this required drier soils typical of later spring in Iowa. Our criteria to start GDD accumulation at planting for newly planted stands and after the last hard frost of spring for already-planted stands allowed comparison of each relative to permissible growth conditions.

2.3 | A unified morphological scale

Out of the 10 principal growth stages (PGS) described by Tejera and Heaton (2017) for \(M. \times\) giganteus, we focused on five (Figure 1): emergence (PGS 0), leaf development (PGS 1), stem elongation (PGS 3), flowering (PGS 5), and senescence (PGS 9). Within emergence, we specifically used growth stage 09: emergence of rolled leaves through the soil surface (hereafter referred to as emergence) which allowed us to count the number of emerged stems.

2.4 | Data collection

Phenology was measured during the 2016 and 2017 growing seasons from early spring, when rolled leaves started to emerge, until late fall when stands were fully senesced or hard frosts killed upper canopy leaves. Emergence was assessed weekly from planting to July and then biweekly until stands reached constant values in August–September. At each sampling date, we determined stem density as the average number of stems from ten 0.25 m² quadrat measurements at random places along three long-wise transects. Leaf development, stem elongation, inflorescence emergence, and senescence were assessed bi-weekly. At each sampling date, we randomly collected 10 stems from each plot and counted the number of green leaves, nodes, and senesced leaves on each. Flowering progression was hard to quantify with this sampling procedure. Therefore, on 29 September 2017 we characterized flowering progression in over 30 stems per plot. In 1-year-old stands, senescence was sporadic and could not be confidently assessed following our method, so the last sampling date values were considered the final end-of-season number of senesced leaves.

2.5 | Data analysis

To test our hypotheses, we constructed statistical models of growth stage progression with thermal time. We then compared model predictions and parameters to address how stand age and N changed \(M. \times\) giganteus phenology.

2.5.1 | Regression models for each growth stage

All stages showed a nonlinear response to accumulated GDD and were best described by either an asymptotic exponential function or a logistic function (Figure 2). Emergence in 2- and 3-year-old stands was best represented by asymptotic exponential regression, according to the equation:

\[
GS_0 = \text{Asym} \left(1 - e^{-\text{Rate} \frac{\text{GDD}}{\text{Asym}}} \right),
\]

where emergence, as the number stems (growth stage 0; \(GS_0\)) over a growing season is a function of the asymptotic number of stems (\(\text{Asym}\)) and the initial slope of stem progression.

**FIGURE 1** Schematic developmental cycle of *Miscanthus × giganteus* principal growth stages (PGS) according to a BBCH-based *Miscanthus* spp. morphological scale (Tejera & Heaton, 2017). Complete senescence (100% senesced leaves) may not be achieved if conditions do not get cool enough, or if a hard frost freezes tissues and precludes internal nutrient recycling (dashed box line).
Appearance at the beginning of the growing season (Rate; Figure 2a). The asymptote refers to the projected maximum number of stems that would emerge in an infinitely long growing season. Usually, emergence is interrupted by environmental stressors (e.g., killing frosts) and stands do not reach $Asym$. In some cases, the data collected until emergence is interrupted may suffice to estimate the projected asymptotic value of the stand. $Rate$ represents the number of new stems that emerge per GDD early in the growing season, during the period of linear increase (Figure 2a), then as the season progresses the rate of emergence decreases until it plateaus to zero.

$\text{Rate}_{50} = \Delta y / \Delta x$

$\Delta x$

$\Delta y$

$Asym$ (100%)

$90\%$ $t_{90}$ (End)

$10\%$ $t_{10}$ (Start)

$50\%$ $t_{50}$

GDD (°C day)

Autumnal hard frost

 Emergence in 1-year-old stands, and leaf development, stem elongation, and senescence for all stand ages were best represented by a logistic model, following the equation:

$$GS_i = \frac{Asym}{1 - e\left(-\frac{GDD_{i} - t_{50}}{scal}\right)},$$

(3)

where the progression of emergence, leaf development, stem elongation, or senescence ($GS_i$; with $i = 0, 1, 3$, and 9, respectively) is a function of $Asym$, the thermal time it takes to reach 50% of the asymptotic value ($t_{50}$), and the $scal$ parameter, related to the steepness of the progression (Figure 2b). In this case, $Asym$ refers to the projected value of stems in 1-year-old stands, leaves, nodes, or senesced leaves (depending on the developmental stage modeled) in an infinite-long growing season. Mathematically, $scal$ values represent the GDD required to go from 50% to ~73% of the asymptote, this implies that larger $scal$ values generate flattened curves as the stand requires more GDD to attain ~73% of the asymptote. Note that we used Equation (2) to model emergence of 1-year-old stands, and Equation (3) for 2- and 3-year-old stands.

2.5.2 | Assessing age, N fertilization, and interaction effects on growth stage progression

We used nonlinear mixed models to model the effects of age, N, and growing season on growth stage development. Model selection was guided by information criteria (AIC and BIC), likelihood ratio tests, and the adequacy of diagnostic plots (e.g., residuals v. fitted, QQ plot, predicted values). Block, and main-plot and subplot were considered as random. Our model-building strategy was to start with a model with random effects for all parameters and then examine the fitted object to decide which random effects could be dropped from the model (Pinheiro & Bates, 2000). Then, we included fixed effect terms (i.e., stand age, N, and growing season) and assessed their effect on the model parameters. We used the R statistical software (R Core Team, 2017) nlme R package (Pinheiro & Bates, 2000) for model construction and testing. Model parameters were compared using the emmeans package (Lenth, Singmann, Love, Buerkner, & Herve, 2018).

2.5.3 | Characterizing growth stage progression

In addition to the model parameters, we used generic indices to characterize growth stage progression and compare changes caused by age, N fertilization, and growing season (Figure 2). The first generic index was the “start” of the
stage, which we set to be the accumulated GDD value corresponding to 10% progression through the stage (10% of the asymptotic value; Figure 2). The next generic index was the progression rate (i.e., slope) when stands had reached 50% of the asymptote value (i.e., derivative of the function at \( t_{50} \); \( Rate_{50} \); Figure 2) to compare stage progression across stand ages with different nonlinear models. “End” was the accumulated GDD value corresponding to 90% progression through the stage (90% of the asymptotic value). End-of-season, “final” values for stems and leaves were determined as the value projected by the regression at the first autumnal hard frost date. Senescence was sporadic in 1-year-old stands and therefore could not be modeled, so the last sampling date values were considered as the final end-of-season number of senesced leaves. To compare Start, End, \( Rate_{50} \), and final value, we constructed bootstrapped distributions of the indices after 1,500+ successful iterations and compared 95% confidence intervals among treatments. We used the same approach to estimate 2- and 3-year-old emergence \( t_{50} \), as it is not a model parameter of the asymptotic exponential equations. In addition, we used \( t \) tests for direct comparisons between bootstrap estimates across stand ages. When N fertilization did not have a significant effect on model parameters or generic index, values are presented as the average over N rates.

3 | RESULTS

Stand age during the first 3 years of growth influenced \( M. \times giganteus \) development more than any other factor assessed. Older stands produced stems with more leaves and nodes than 1-year-old stands, mainly due to earlier emergence. The stand age effect was even larger than the cumulative effects of N fertilization, as fertilized stands were fertilized yearly. Nitrogen fertilization interacted with stand age to influence \( M. \times giganteus \) emergence (\( P_{Age*Nrate} = 0.0937 \)), senescence (\( P_{Age*Nrate} < 0.001 \)), leaf development (\( P_{Age*Nrate} < 0.001 \)) and stem elongation (\( P_{Age*Nrate} < 0.001 \)). Fertilized 2-year-old stands reached similar stem densities as unfertilized 3-year-old stands and had fewer senesced leaves, similar to 1-year-old stands. Differences between N rates on leaf development and stem elongation were minimal. Growing season conditions also interacted with stand age dynamics, influencing 1-year-old stands the most (\( P_{Age*Nrate} = 0.003 \)).

3.1 | Older \( M. \times giganteus \) stands produce more plant structures than 1-year-old stands within a season

Overall, \( M. \times giganteus \) consistently produced more stems as it aged, but inter-annual variability was high. One- and two-year-old stands produced 30% more stems in 2016 than in 2017 (\( P_{1year} = 0.001 \); \( P_{2year} < 0.001 \); Table 2). Two-year-old stands had ~20% more stems (asymptote and final value) than 1-year-old stands when averaged over N rates and across growing seasons (Table 2; Appendix S1, see Supplemental Data with this article). The stand age effect was larger in 3-year-old stands: they had 45%–50% more stems than 1-year-old stands (\( p < .0001 \)) and ~25% more stems than 2-year-old stands (\( p = .002 \); Table 2); however, there were no differences with the 2-year-old stand grown in 2016 (\( p = .589 \)).

Two- and three-year-old stands consistently produced stems with more leaves. Two-year-old stands had 33% and 90% more leaves than 1-year-old stands in 2016 and 2017, respectively (\( P_{2016} < 0.0001 \); \( P_{2017} < 0.0001 \); Table 3; Appendix S1). Three-year-old stands had stems with twice as many leaves than did 1-year-old stands (\( p < .0001 \)) and with ~5% more leaves than 2-year-old stands (\( p < .0001 \)). Similar trends were found for the asymptotic value (Table 3). Stem elongation showed different stand age effects across growing seasons (\( P_{Age*GrowSeason} < 0.001 \)). In 2017, stems in older stands exposed almost three times more nodes than 1-year-old stems (\( P_{3year-1year} < 0.001 \); \( P_{2year-1year} < 0.001 \)), but differences were minimal in 2016. In 2016, 1-year-old stems had a much higher asymptotic value than stems in 2-year-old stands, but their development was interrupted by a hard frost and their final value was almost half of it (Table 3). Two-year-old stands reached a similar number of leaves and nodes in both years, but 1-year-old stem elongation showed higher inter-annual variability: stands planted in 2017 had ~50% fewer nodes than those planted in 2016 (\( p < .001 \); Table 3).

3.2 | Faster developmental rates did not lead to more advanced stages at the end of the growing season

We used \( Rate_{50} \), the emergence rate when stands reached 50% of their asymptotic value (Figure 2), to compare emergence progression across stand ages. In addition to producing more stems as described in the previous paragraph, older stands also had faster \( Rate_{50} \) when averaged over N rates. For example, in 2016, when 2-year-old stands had 15% more stems than 1-year-old stands, their \( Rate_{50} \) was almost four times faster (\( Rate_{50.1year} = 0.070 \), \( Rate_{50.2year} = 0.349 \); Appendix S1). Similarly, in 2017, the 3-year-old stand stem emergence \( Rate_{50} \) was 50% faster than the 2-year-old \( Rate_{50} \), and three times faster than 1-year-old stand \( Rate_{50} \) (\( Rate_{50.1year} = 0.054 \), \( Rate_{50.2year} = 0.100 \), \( Rate_{50.3year} = 0.162 \)).

Stands produced more leaves and exposed more nodes as they aged, but their dynamics differed across growing
TABLE 2  Age, nitrogen (N) fertilization and growing season effects on Miscanthus × giganteus emergence model parameters and end-of-season value (*Final Value*). Emergence in 1-year-old stands better fit a logistic growth model with *Asym* (i.e., long-term maximum number of stems in the stand in infinite-long growing season), *t<sub>50</sub>* (growing degree days required to reach 50% of the asymptotic value), and *scal* (i.e., required GDD to go from 50% of the asymptote to ~73%, larger *scal* values generate flattened curves) parameters. Emergence in 2- and 3-year-old stands better fit an asymptotic exponential growth model with *Asym* (same as in logistic growth model) and *Rate* (i.e., rate of stem appearance per growing degree day during early during the linear increase of the model).

| N rate (kg/ha) | 1-year old | 2-year old | 3-year old |
|---------------|------------|------------|------------|
|               | Average<sup>i</sup> | 0 | 112 | 224 | 336 | 448 | 0 | 112 | 224 | 336 | 448 |
| *Emergence (stem/m²)* | | | | | | | | | | | |
| 2016          | | | | | | | | | | |
| *Asym* (stem/m²) | 72.1<sup>ii</sup> | 77.6 bc | 80 abc | 82 ab | 83 ab | 85.3 a | | | | | |
| *Rate* (stem m<sup>−2</sup>GDD<sup>−1</sup>) | — | 0.58 b | 0.64 ab | 0.97 ab | 0.75 a | 0.710 a | | | | | |
| *scal* (GDD<sup>−1</sup>) | 276 | — | — | — | — | — | | | | | |
| *t<sub>50</sub>* (GDD) | 1,418 a | 95.2 b | 88.6 b | 86.3 b | 77.3 b | 84.6 b | | | | | |
| *Final Value* (stem/m²) | 70.4 c | 77.3 b | 79.7 ab | 81.4 ab | 82.7 ab | 85.0 a | | | | | |
| 2017          | | | | | | | | | | |
| *Asym* (stem/m²) | 54 e | 60.8 de | 63.2 cd | 64.9 cd | 66.3 cd | 68.6 bc | 75.7 bc | 78.1 ab | 79.8 ab | 81 ab | 83.6 a |
| *Rate* (stem m<sup>−2</sup>GDD<sup>−1</sup>) | — | 0.111 d | 0.17 cd | 0.20 bcd | 0.28 abc | 0.24 bc | 0.237 b | 0.29 ab | 0.32 ab | 0.41 a | 0.36 a |
| *scal* (GDD<sup>−1</sup>) | 276 | — | — | — | — | — | | | | | |
| *t<sub>50</sub>* (GDD) | 1,320 a | 394 b | 269 bc | 236 bc | 167 cd | 205 bcd | 225 bc | 186 cd | 173 cd | 139 d | 160 cd |
| *Final Value* (stem/m²) | 54.2 e | 59.7 de | 63.1 d | 65.0 cd | 66.4 cd | 68.7 cd | 75.4 bc | 77.8 ab | 79.5 ab | 80.9 ab | 83.1 a |

<sup>i</sup>Nitrogen fertilization did not have a significant effect (*p* < .05) on emergence of 1-year-old stands and values are presented as the average of all five N rates.

<sup>ii</sup>Bold font within columns indicates a significantly higher value of a parameters between growing seasons (*p* < .05).

<sup>iii</sup>Different letters within rows indicate parameters differed significantly between stand ages or N rates (*p* < .05).

<sup>iv</sup>Different letters within rows indicate parameters differed significantly between stand ages or N rates (*p* < .05).
seasons. In 2016, leaf development progressed at similar rates in 1- and 2-year-old stands \((p = .796)\), but 2-year-old stands reached 50% of the asymptote \((t_{50})\) much sooner (Table 3). In 2016, stem elongation in 1-year-old stands had a twofold larger asymptotic value; however, they required more than twice as many GDD to reach \(t_{50}\) (Table 3). At the end of the growing season, 2-year-old stands had more exposed nodes per stem than 1-year-old stands \((p < .001)\). In 2017, older stands had a much slower progression rate (i.e., larger \(scal\)) than 1-year-old stands.

### TABLE 3

|                        | 1-year old Average | 2-year old Average | 3-year old Average |
|------------------------|--------------------|--------------------|--------------------|
| **Leaf development**   |                    |                    |                    |
| 2016                   |                    |                    |                    |
| Asym (leaves)          | 15.9a              | 16.4               | —                  |
| \(scal\) (GDD\(^{-1}\)) | 464                | 422                | —                  |
| \(t_{50}\) (GDD)       | 1,965              | 1,094              | —                  |
| Final Value (leaves)   | 12.2b\(^i\)        | 15.9a              | —                  |
| 2017                   |                    |                    |                    |
| Asym (leaves)          | 8.78b              | 18.2a              | 18.6a              |
| \(scal\) (GDD\(^{-1}\)) | 143                | 437                | 403                |
| \(t_{50}\) (GDD)       | 1,138b             | 1,264a             | 1,173b             |
| Final Value (leaves)   | 8.67c              | 16.6b              | 17.4a              |
| **Stem elongation**    |                    |                    |                    |
| 2016                   |                    |                    |                    |
| Asym (nodes)           | 20.6               | 11.8               | —                  |
| \(scal\) (GDD\(^{-1}\)) | 443                | 347                | —                  |
| \(t_{50}\) (GDD)       | 2,495a             | 1,110b             | —                  |
| Final Value (nodes)    | 10.3b              | 11.6a              | —                  |
| 2017                   |                    |                    |                    |
| Asym (nodes)           | 4.97b              | 14.6a              | 14.4a              |
| \(scal\) (GDD\(^{-1}\)) | 142b               | 380a               | 341a               |
| \(t_{50}\) (GDD)       | 1,218b             | 1,410a             | 1,203b             |
| Final Value (nodes)    | 4.84b              | 13.3a              | 13.8a              |

\(^{a}\)Nitrogen fertilization did not have a significant effect \((p > .05)\) on leaf development or stem elongation, values are presented as the average of all five N rates.

\(^{i}\)Bold font within columns indicates a significantly higher value of a parameters between growing seasons \((p < .05)\).

\(^{ii}\)Different letters within rows indicate parameters differed significantly between stand ages \((p < .05)\).

#### 3.3 Older stands started development almost 3 months sooner than 1-year-old stands

Averaged over N rates, 2- and 3-year-old stands completed emergence at least 1,500 GDD, equivalent to approximately 90 Julian days, sooner than 1-year-old stands (Figure 3; Appendix S1), which still had stems emerging in late summer. In 2016, 3-year-old stands had already completed (i.e., achieved 90% of the asymptotic value) emergence by the time 1-year-old stands started to emerge. Similarly, older stands started leaf development and stem elongation 2–4 months sooner than 1-year-old stands regardless of growing season conditions (Figure 4). By the time 1-year-old stands had started to produce leaves or expose nodes, older stands had reached the \(t_{50}\) point (Table 2).

#### 3.4 Older stands began senescence sooner in the growing season and had higher senescence percentages at the end of the growing season

One-year-old stands started senescence so late in the growing season that their progression through this stage could not be properly studied. Their number of senesced leaves at the last sampling date was almost half of that observed in unfertilized older stands regardless of the growing season (Table 4), while 2- and 3-year-old stands reached similar asymptotic values \((p = .438; \text{Table } 4)\). Stand age effects on \(M. \times \text{giganteus}\) senescence also varied across growing seasons (Figure 5). In 2017, unfertilized 2- and 3-year-old stands started to senesce around 1,500 GDD, while the 2-year-old started around 850 GDD in 2016. Regardless of growing season, both stand ages completed senescence around 3,000 GDD (Figure 5; Appendix S1).

#### 3.5 Nitrogen fertilization partially compensated for age-related changes

Nitrogen fertilization had no effect on \(M. \times \text{giganteus}\) 1-year-old stands \((p = .991)\) but affected 2- and 3-year-old stand emergence \((p < .001)\) and senescence \((p < .001)\). Fertilized 2- and 3-year-old stands had 10%–15% more stems and up to 2.5-fold faster emergence rates than unfertilized stands regardless of the growing season (Table 2). Given the faster rate, fertilized 2- and 3-year-old stands
reached emergence $t_{50}$ 4 and 2 weeks sooner than unfertilized plots, respectively (Table 2). In 2017, the 25% higher stem density in fertilized 2-year-old stands accounted for the stand age effect between 2- and 3-year-old stands and resulted in fertilized 2-year-old stands with similar stem densities as unfertilized 3-year-old stands ($p = .988$). Nitrogen fertilization also affected stem elongation ($p < .001$) but the fertilized stands showed only one more exposed node at the end of the growing season.

Nitrogen fertilization's effect on senescence was more evident in 2017 (Table 4) for 2-year-old stands where the...
## Table 4

Age, nitrogen fertilization, and growing season effects on *Miscanthus × giganteus* senescence model parameters and end-of-season values (*Final Value*). Number of senesced leaves (SL) better fit a logistic growth model for with Asym (i.e., long-term maximum number of stems in the stand in infinite-long growing season), $t_{50}$ (growing degree days required to reach 50% of the asymptotic value), and $scal$ (i.e., required GDD to go from 50% of the asymptote to ~73%, larger $scal$ values generate flattened curves) parameters. One-year-old stands started senescence so late in the growing season that their progression through this stage could not be modeled.

| N rate (kg/ha) | 1-year old | 2-year old | 3-year old |
|---------------|------------|------------|------------|
|               | Average$^1$ | 0  | 112 | 224 | 336 | 448 | 0  | 112 | 224 | 336 | 448 |
| **Senescence**|            |    |     |     |     |    |    |     |     |     |    |
| 2016          |            |    |     |     |     |    |    |    |     |     |    |
| Asym (SL)     | —          | 8.86 | 10.1 | 9.01 | 10  | 9.93 | —  | —  | —  | —  | —  |
| $scal$ (GDD$^{-1}$) | —      | 396  | —    | —    | —   | —   | —  | —  | —  | —  | —  |
| $t_{50}$ (GDD) | —          | 1.777 | 1.928 | 1.853 | 1.896 | 1.943 | —  | —  | —  | —  | —  |
| Final Value (SL) | 4.47 b$^3$ | 8.72 a | 9.88 a | 8.86 a | **9.83 a** | **9.74 a** | —  | —  | —  | —  | —  |
| 2017          |            |    |     |     |     |    |    |    |     |     |    |
| Asym (SL)     | —          | **18.1$^{11}$ a** | 13.5 ab | **14.5 ab** | 12.7 ab | 11.6 b | 15.6 ab | 14.5 ab | 15.4 ab | 13.7 ab | 15.3 ab |
| $scal$ (GDD$^{-1}$) | —      | 396  | 396  | 396  | 396  | 396  | 396  | 396  | 396  | 396  | 396  |
| $t_{50}$ (GDD) | —          | **2,465 a** | **2,367 ab** | **2,468 a** | **2,335 ab** | **2,246 ab** | 2,221 ab | 2,154 b | 2,168 b | 2,100 b | 2,188 b |
| Final Value (SL) | 7.46 b | **15.2 a** | 11.8 b | **12.2 a** | 11.2 b | 10.5 b | 14.1 ab | 13.4 ab | 14.1 ab | 12.7 ab | 14.0 ab |

$^1$Nitrogen fertilization did not have a significant effect ($p < .05$) on emergence of 1-year-old stands and values are presented as the average of all five N rates.

$^2$Different letters within rows indicate parameters differed significantly between stand ages or N rates ($p < .05$).

$^3$Bold font within columns indicates a significantly higher value of a parameters between the growing seasons ($p < .05$).
number of senesced leaves in fertilized stands was similar to the number observed in 1-year-old stands ($p = .217$). When assessed in 2017, N fertilization affected flowering in 2- and 3-year-old stands ($P_{\text{Age} \times \text{Nrate}} = 0.361$). Inflorescence emergence in unfertilized stems was, regardless of stand age, two times higher than fertilized plots (Table 5; Appendix S2 for photographic evidence in 2016).

4 | DISCUSSION

We studied $M. \times$ giganteus phenology during the yield-building phase of the crop (first three growing seasons) under five different N fertilization rates. Our results showed that stand age is the main driver of $M. \times$ giganteus phenology within this life phase. Two- and three-year-old stands behaved similarly, suggesting that in our study $M. \times$ giganteus could have been entering the adult phase after the second year of growth. Our results showed that $M. \times$ giganteus stands changed life strategies as they age. One-year-old stands prioritize growth and resource acquisition, mainly evidenced by faster growth rates, while adult stands (2- and 3-year old) showed a more conservative use of resources and shifts towards stress tolerance, mainly evidenced by earlier senescence. Nitrogen dilution could be a possible cause behind these changes as N fertilization compensated for some age-related phenological changes. Fertilized 2-year-old stands reached similar stem densities as unfertilized 3-year-old stands and had fewer senesced leaves similar to 1-year-old stands.

4.1 | Older stands develop further, but it is their earlier start, not their faster rate, that gets them there

Our results support previous research and our first hypothesis: that $M. \times$ giganteus produces more plant structures and reaches more advanced developmental stages as it ages, at least during the establishment phase studied here. Two- and three-year-old stand stem density was $\sim 70$ stems/m$^2$, which was on average 15% higher than in 1-year-old stands (Table 2). These values are within the range of previously reported $M. \times$ giganteus stem densities in Europe (e.g., Christian, Riche, & Yates, 2008; Clifton-Brown et al., 2001; Kalinina et al., 2017) and the United States (e.g., Lee, Wycislo, Guo, Lee, & Voigt, 2017; Maughan et al., 2012).

**TABLE 5** $Miscanthus \times$ giganteus inflorescence emergence as of 29 September 2017 for five nitrogen fertilization rates (kg/ha) averaged over 2- and 3-year-old stands

| $\text{kg N/ha}$ | 0   | 112 | 224 | 336 | 448 |
|-----------------|-----|-----|-----|-----|-----|
| $\text{Accumulated GDD}$ | 850 | 1,125 | 1,500 | 2,350 | 2,850 | 3,350 |
| $\text{Accumulated GDD}$ | 5.77 | ± 0.65 | 2.18 | ± 0.64 | 2.36 | ± 0.67 | 2.04 | ± 0.67 | 2.17 | ± 0.67 |

$^{a}$Values according to Tejera and Heaton (2017) $Miscanthus$ spp. morphological scale; 0 represents no inflorescence and 10, 100% inflorescence exposure.

$^{b}$Different letters indicate significant difference ($p < .05$).
Older *M. × giganteus* stands also produced 15%–50% more leaves and nodes over the study period. The final number of leaves and exposed nodes was also within range of those previously reported (Clifton-Brown, Lewandowski, Bangerth, & Jones, 2002; Kaack, Schwarz, & Brander, 2003). These differences in development led to changes in end-of-season biomass: averaged over years, 2-year-old stands yielded almost three times more biomass than 1-year-old stands, and 3-year-old stand yield was almost six times higher than 1-year-old stands (Tejera et al., 2019).

Contrary to our second hypothesis—juvenile stands would have faster developmental rates and start development sooner—we did not find a consistent response. Developmental rates were variable across growth stages and growing seasons and did not correspond with more advanced stages at the end of the growing season (Tables 2–4). Data from 2017, when all stand ages could be tested, suggest a clear change in life strategies. One-year-old stands had notably faster leaf development and stem elongation rates, favoring growth and resource acquisition. Young stands of other perennial plants also favored resource acquisition, either by increased CO₂ assimilation during unfavorable conditions (Boersma et al., 2015; Jaikumar et al., 2016) or increased light interception (Dayrell et al., 2018). Earlier emergence has also been associated with young stands (e.g., Vitasse, 2013); however, in our study adult stands emerge earlier than 1-year-old stands. In our experiment, earlier stage initiation was the main factor leading 2- and 3-year-old stands to reach more advanced developmental stages at the end of the growing season. Across stages, older stands started developmental stages 700+ GDD, equivalent to ~10 weeks, sooner than the newly planted 1-year-old stands (Figure 6). As 1-year-old stands were mechanically planted, planting and emergence will inevitably occur later in the year. We controlled for this by starting GDD count when 1-year-old stands were planted, rather than after the last frost. Despite this, it still took 1-year-old stands a considerably larger amount of GDD to emerge (700+ GDD). In temperate regions where hard frost events are frequent in late autumn, development is likely to be interrupted. Therefore, stands with faster developmental rates still may not reach high end-season-values if development starts late in the season. In 2016, for example, a hard frost event interrupted development and since 1-year-old stands were ~1,000 GDD (~2 months) behind older stands, all stands ages ended with similar values, even though 1-year-old stands had faster developmental rates.

Two- and three-year-old stands emerged 3 months sooner than 1-year-old stands and while this was partly caused by a later planting date, older stands also had a lower GDD requirement (Figure 6). This earlier start of development phenology of older perennial grasses is opposite to trends observed in forest trees, where seedlings started development earlier than adults. Vitasse (2013) proved the earlier start of young individuals between conspecific forest trees were caused by ontogenetic differences rather than by the micro-environmental differences experienced by young individuals in the understory. While these hypotheses have not been considered in perennial grass phenology, we could draw some comparisons. Research on perennial grasses usually compares stands of different ages grown side-by-side under the same growing conditions or follows the same stand across growing seasons. For the first case, stand ages grow under the same environmental conditions; therefore, environmental conditions cannot be driving phenological differences among stand ages. For the latter, growing conditions may be different across seasons and may change the magnitude of the stand age effect, but rarely reverse it (Tejera et al., 2019).

We propose that the earlier phenology of older perennial grasses could also be generated by below-ground plant reserves. Rhizome biomass and nutrient content can increase up to ~50% each year during establishment (Christian, Poulton, 2016).
4.2 Phenology of older stands shows a switch towards stress tolerance strategies

Our results supported our third hypothesis—that older stands will start senescence sooner in the growing season—however, our experiment confounded age-related changes and light environments across canopies. Early in the growing season, senescence remobilizes nutrients from shaded leaves in the lower canopy to sink organs (e.g., rhizomes, fully sun-lit leaves in the upper canopy; Guiboileau, Sormani, Meyer, & Masclaux-Daubresse, 2010). Towards the end of the growing season, senescence also results in the remobilization of nutrients from the canopy into rhizomes for storage. If our hypothesis holds true, older stands should start the latter movement sooner than 1-year-old stands to favor stress tolerance and winter-survival. However, older M. × giganteus also had more leaves and a closed canopy sooner. Thus, their earlier senescence could be driven by the first mechanism, where the earlier senescence is stimulated by light, not age. Our experiment was not able to differentiate between these two mechanisms, so we were not able to distinguish age-related changes from changes in resource acquisition strategies. That said, the reduced senescence observed in 1-year-old stands, even with an already closed canopy, may suggest possible age-related mechanisms. In a different study, adult stands of M. × giganteus also showed more conservative strategies as they tend to reduce CO₂ assimilation sooner in the year to avoid hard frosts that may interrupt internal nutrient. Similarly, adult stands of perennial cereals reduced CO₂ assimilation during summer to conserve water during the dry period (Jaikumar et al., 2016).

4.3 Under nitrogen fertilization older M. × giganteus stands increased stem density and held fewer senesced leaves

Our results supported our fourth hypothesis—fertilized M. × giganteus juvenile stands would have similar dynamics as mature unfertilized M. × giganteus stands. We found N modulated age-related responses in emergence and senescence, suggesting the observed changes could also be caused by N limitation as older stands are notably larger. Unfertilized 3-year-old stands emerged twice as fast as their 2-year-old counterparts in 2017; N fertilization hastened emergence 2.5-fold, allowing the 2-year-old stands to achieve emergence rates even higher than 3-year-old stands (Table 2). Emergence did not show clear changes in life strategies among stand ages, possibly given the constraints of mechanical planting. The fact that N fertilization of 2-year-old stands notably reduced the difference with 3-year-old stands suggest a nutrient limitation in 2-year-old stands and highlights the importance of internal reserves in adult stands driving phenology. Unfertilized 2-year-old stands had double the number of senesced leaves than 1-year-old stands, but fertilized 2-year-old stands had 30% fewer senesced leaves than their unfertilized counterparts. This roughly cuts the stand age effect in half and makes the number of senesced leaves statistically similar between fertilized 2-year-old and all 1-year-old stands. The hastened senescence in 2-year-old stands could be a consequence of N limitation in larger plants, which was also supported by the notable reduction in senesced leaves after N fertilization. However, self-shading in these larger stands could also trigger senescence confounding the underlying mechanisms.

Nitrogen fertilization presents a trade-off between resource capture and resource use efficiency. Under N fertilization plants may increase leaf area, show faster development and increase light interception, or increase N content per unit of leaf area and increase the conversion of the intercepted light into energy (Sinclair & Horie, 1989). Averaged over stand ages, fertilized M. × giganteus produced more stems with fewer senesced leaves that flowered
later in the growing season. Considering that leaf development remained constant across N rates, *M. × giganteus*’ N response strategy was to increase green leaf area and favor resource capture. The canopy of fertilized stands should have therefore intercepted more light, as they had more stems with the same amount of leaves and canopies that stayed green for longer in the growing season. Under unfertilized conditions, *M. × giganteus* maintained a consistent number of green leaves throughout the growing season, mainly through a faster rate of senescence that allowed N recycling to younger leaves. These responses are similar to other C₄ grasses, such as maize (*Zea mays* L.; Lemaire, van Oosterom, Jeuffroy, Gastal, & Massignam, 2008; Massignam, Chapman, Hammer, & Fukai, 2011). The way N fertilization almost halved the number of senesced leaves in 2-year-old plants may support the hypothesis that nutrient dilution limits productivity in older and larger plants. The minimal response to N fertilization in 3-year-old senescence, in turn, may suggest a change in strategy rather than nutrient dilution. If N would be diluted and limiting in these large stands, N fertilization should have reduced the number of senesced leaves in these stands. Growth dilution of nutrients is a general and fundamental response under elevated CO₂ in annual, especially C₃ crops, but mechanisms remain poorly understood (Uddling, Broberg, Feng, & Pleijel, 2018). Growth dilution has also been reported in pine trees (Finzi, DeLucia, & Schlesinger, 2004; Johnson, Ball, & Walker, 1997). Here we suggest that it may also occur in perennial grasses as they age, but may not be the only driver, as internal reserves or changes in life strategies also drive age-related changes.

One-year-old *M. × giganteus* did not flower in any of the growing seasons. Based on other studies, first year flowering is rather rare (Clifton-Brown & Lewandowski, 2002; Clifton-Brown et al., 2001; Cosentino, Patane, Sanzone, Copani, & Foti, 2007). Nitrogen fertilization delayed flowering in older *M. × giganteus* stands. A similar effect was found in a study in Italy where N fertilization delayed flowering by 4 days (Cosentino et al., 2007). Delayed flowering has the potential to extend vegetative growth and increase biomass production, however, given the tight coordination between flowering, senescence and dormancy it could also delay end-of-the-season nutrient recycling with potential negative consequences on over-winter survival and next season emergence (Schwartz & Amasino, 2013). Multiple studies show a steady decline in *M. × giganteus* above-ground nutrient concentration across the growing season (Beale & Long, 1997; Cosentino et al., 2007; Dohleman, Heaton, Arundale, & Long, 2012; Heaton, Dohleman, & Long, 2009) suggesting that flowering may not affect nutrient recycling. If flowering does not impact nutrient recycling, delayed flowering could be advantageous for perennial biomass production rather than a threat for over-winter survival.

The N effect on *M. × giganteus* phenology found in 2017, when fertilized 3-year-old stands produced more stems and 2-year-old stands had fewer senesced leaves, coincided with a positive N response in end-of-season biomass in 2017 (Tejera et al., 2019). Similarly, there was no biomass response in 2016 (Tejera et al., 2019) when the N effect on *M. × giganteus* phenology was reduced. While other factors such as rhizome quality, weather, and crop husbandry also affect *M. × giganteus* development and yield during the yield-building phase, the large effect of earlier emergence on *M. × giganteus* development may imply that biomass differences between N rates could be a consequence of fertilized stands being developmentally more advanced (e.g., with more leaves and nodes). More research, beyond the establishment period, is needed to determine the likelihood that N will change phenology and/or biomass in a given growing season. While early canopy development and late canopy senescence both significantly correlated with higher yield over a wide range of Miscanthus genotypes (Robson et al., 2013), light-limiting conditions in autumn could reduce growth rates and prevent proper acclimation to winter stress (Ergon, 2017). Moreover, cold temperatures and negative associations between freezing resistance and leaf growth could limit early growth in spring (Ergon, 2017). Considering the large variability in *M. × giganteus* yield response to N fertilization reported in the literature, Miscanthus improvement programs could use phenology to identify mechanisms underpinning different genotypic and phenotypic responses to N.

5 | CONCLUSION

Stand age was the main driver of *M. × giganteus* phenological development during the establishment phase (first three growing seasons). Our results show that phenological differences between mature and newly planted perennial grasses were driven by delayed emergence and potentially internal reserve status, rather than micro-climate or ontogenetic differences. Management practices that shorten the planting-to-emergence period may hasten development and increase yields. The use of a staggered-start experimental design enabled separation of previously confounded environmental and stand age effects. For the first time, we studied stand age and N interactive effects in different years to isolate growing season variability and account for N dilution in larger plants. Nitrogen fertilization did not change phenology in 1-year-old stands and mainly affected emergence and senescence in older (2- and 3-year-old) stands. Fertilized stands emerged earlier, had more stems, maintained more green leaves until the end of the growing season, and flowered later than unfertilized stands, indicating mechanisms by which N fertilizer can increase *M. × giganteus* biomass.
production. These responses were variable between growing seasons; however, and more research is needed on mature stands to understand the relationship between N, phenology, and biomass yields in mature stands.

ACKNOWLEDGEMENTS
We thank Nicholas Boersma, Valeria Cano-Camacho, and Collin De Graff for their help in designing the experiment, plot maintenance, data collection, and sample processing; Mike Ficus and general staff at the research farm for their support with on-farm management and data collection, AGGrow Tech (Greensboro, NC, USA) for providing rhizomes and specialized farming equipment; and Deborah Aller for critical reviews of this manuscript. This project was funded by USDA National Institute of Food and Agriculture Hatch grant 1008969; the Leopold Center for Sustainable Agriculture; the Iowa Energy Center; the Iowa State University Department of Agronomy; and U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research DE SC0018420 through the Center for Advanced Bioenergy and Bioproducts Innovation.

AUTHOR CONTRIBUTION
All authors contributed ideas; M.D.T. collected and analyzed the data and led the writing of the manuscript; F.E.M. made substantial contributions to data analysis; E.A.H. designed the experiment. All authors contributed to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT
The datasets generated during and/or analyzed during the current study are available in the Mendeley Data repository: http://dx.doi.org/10.17632/9rrfxshnm6.2 (Tejera & Heaton, 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Tejera MD, Miguez FE, Heaton EA. The older plant gets the sun: Age-related changes in Miscanthus × giganteus phenology. GCB Bioenergy. 2020;00:1–17. https://doi.org/10.1111/gcbb.12745