

More Productive Than Maize in the Midwest: How Does *Miscanthus* Do It?¹[W][OA]

Frank G. Dohleman and Stephen P. Long*

Department of Plant Biology (F.G.D., S.P.L.), Department of Crop Sciences (S.P.L.), and Institute for Genomic Biology (S.P.L.), University of Illinois, Urbana, Illinois 61801

In the first side-by-side large-scale trials of these two C_4 crops in the U.S. Corn Belt, *Miscanthus* (*Miscanthus* \times *giganteus*) was 59% more productive than grain maize (*Zea mays*). Total productivity is the product of the total solar radiation incident per unit land area and the efficiencies of light interception (ϵ_i) and its conversion into aboveground biomass (ϵ_{ca}). Averaged over two growing seasons, ϵ_{ca} did not differ, but ϵ_i was 61% higher for *Miscanthus*, which developed a leaf canopy earlier and maintained it later. The diurnal course of photosynthesis was measured on sunlit and shaded leaves of each species on 26 dates. The daily integral of leaf-level photosynthetic CO_2 uptake differed slightly when integrated across two growing seasons but was up to 60% higher in maize in mid-summer. The average leaf area of *Miscanthus* was double that of maize, with the result that calculated canopy photosynthesis was 44% higher in *Miscanthus*, corresponding closely to the biomass differences. To determine the basis of differences in mid-season leaf photosynthesis, light and CO_2 responses were analyzed to determine in vivo biochemical limitations. Maize had a higher maximum velocity of phosphoenolpyruvate carboxylation, velocity of phosphoenolpyruvate regeneration, light saturated rate of photosynthesis, and higher maximum quantum efficiency of CO_2 assimilation. These biochemical differences, however, were more than offset by the larger leaf area and its longer duration in *Miscanthus*. The results indicate that the full potential of C_4 photosynthetic productivity is not achieved by modern temperate maize cultivars.

Maize (*Zea mays*) is the most important C_4 grain crop in the world in terms of global annual tons produced (Food and Agricultural Organization, 2009). However, much of this production is in the temperate zone, where C_4 photosynthesis and development are limited by the low temperatures of spring and autumn. Record maize yields are achieved on the deep fertile soils of the Midwestern U.S. Corn Belt, but could they be higher? It has been widely observed that C_4 plants, while highly productive under warm sunlit conditions are adversely affected by chilling conditions ($<14^\circ C$; Long et al., 1994). *Miscanthus* (*Miscanthus* \times *giganteus*; Hodgkinson and Renvoize, 2001), which is closely related to the three major C_4 crops, sugarcane (*Saccharum officinarum*), sorghum (*Sorghum bicolor*), and maize, has been found to be very productive even at $52^\circ N$ latitude in Britain, where summer heat is insufficient for a maize crop to be grown through to grain production (Beale and Long, 1995). More recently,

Miscanthus has been shown to be exceptionally productive in small plot trials (0.01 ha) in the Midwestern United States, even in comparison with the perennial cold-tolerant prairie C_4 grass switchgrass (*Panicum virgatum*; Heaton et al., 2008).

However, there have been no side-by-side field comparisons of grain maize and *Miscanthus* in large plot trials and, therefore, no field analysis of why the productivity of these two species may differ. Previous controlled environment studies have shown that *Miscanthus* can develop leaves at lower temperatures than maize, is less prone to photoinhibition at chilling temperatures, and on transfer to lower temperatures it shows acclimation at the molecular level that allows photosynthesis to continue (Farage et al., 2006; Wang et al., 2008). Controlled environment studies have also suggested that *Miscanthus* has a lower temperature optimum for light-saturated photosynthesis (Naidu and Long, 2004). It might therefore be expected that *Miscanthus* could develop leaves earlier than maize and maintain them later, but this cold tolerance could come at the expense of high rates during mid-summer. In the field, significant amounts of crop carbon assimilation occurs under non-light saturating conditions, i.e. in the shade of other leaves, on overcast days, and at dusk and dawn on all days. Beale et al. (1996) showed surprisingly high light-limited rates of photosynthesis for *Miscanthus* in the field in England, as measured by the initial slope of the response of CO_2 uptake (A) to incident photon flux and termed the maximum quantum yield of CO_2 uptake ($\Phi_{CO_2,max}$). More recently, Kromdijk et al. (2008) have shown

¹ This work was supported by the Illinois Council on Food and Agriculture Research (C-FAR) and the Dudley Smith Initiative. The Illinois Agriculture Experiment Station and University of Illinois provided land and facilities for these trials.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Stephen P. Long (slong@illinois.edu).

* Corresponding author; e-mail slong@illinois.edu.

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www.plantphysiol.org/cgi/doi/10.1104/pp.109.139162

significant and increasing CO_2 leakage from the bundle sheath with depth into *Miscanthus* canopies, which would lower $\Phi_{\text{CO}_2, \text{max}}$. Even in the absence of leaf photosynthetic differences, production could differ simply because *Miscanthus* may have leaves present earlier and later in the season, allowing it to take advantage of warm weather, outside of the normal maize growing season. Most importantly, if *Miscanthus* does prove to be more productive than maize, in side-by-side trials in the field, then understanding why will indicate how maize and other temperate C_4 crops might be improved to achieve yet higher yields in temperate climates.

Previously, using small plot trials (0.01 ha), we showed that *Miscanthus* was more than twice as productive as another cold-tolerant C_4 perennial grass, switchgrass. Both crops were similarly efficient in intercepting sunlight, but *Miscanthus* showed much higher rates of photosynthesis and was more efficient in its use of water and nitrogen (Heaton et al., 2008; Dohleman et al., 2009). Boehmel et al. (2008) in side-by-side trials in Germany similarly found a significant yield advantage of *Miscanthus* relative to switchgrass.

There have been attempts to project the effects of widespread use of maize grain and cellulosic feedstocks for biofuel production (Farrell et al., 2006; Heaton et al., 2008; Searchinger et al., 2008); however, to date, there have been no direct comparisons of grain maize, the most productive annual row crop in the world (Food and Agricultural Organization, 2009), and *Miscanthus*, the most productive perennial grass species in temperate climates (Beale and Long, 1995; Boehmel et al., 2008; Heaton et al., 2008), to validate whether the assertions made from these projections are correct.

This study presents the first large-plot replicated field trials of *Miscanthus* and grain maize reported in the peer-reviewed literature to determine their comparative annual biomass productivity, leaf area, leaf area duration, and leaf and canopy photosynthesis. Crops were grown using recommended agricultural practices for each species, with the annual use of fertilization for the maize plots and no fertilization of the *Miscanthus* plots.

The total productivity of a plant stand per unit land area is determined by the product of the total amount of solar radiation incident per unit land area and the efficiencies of light interception (ϵ_i) and conversion into biomass (ϵ_c ; Monteith, 1977). These efficiencies were measured so that differences in production could be partitioned between ϵ_i and ϵ_c . The annual progressions of leaf area were followed to further analyze the basis of variation in ϵ_i , while the diurnal courses of leaf photosynthesis in the upper and lower canopy were measured along with analysis of in vivo light and CO_2 responses with steady-state biochemical models to determine the basis of variation in ϵ_c .

This study tested directly the hypothesis that *Miscanthus* is even more productive than modern lines of grain maize bred for high productivity in the Midwest

and examined whether differences can be attributed to more efficient light capture or more efficient conversion of captured light into biomass through photosynthetic differences.

RESULTS

Climate Conditions

While the 2007 growing season was about 1°C warmer and 24% drier than the 30-year average, 2008 was about 1°C cooler and 34% wetter. Growing season solar radiation receipt was about 6% above average in 2007 and about 6% less in 2008 (Supplemental Fig. S1; Table I). The 2 years provided a useful contrast of a warmer and drier versus a wetter and cooler growing season, covering the span of growing conditions likely to be encountered across most years in the Corn Belt of the Midwestern United States.

Biomass Growth and Productivity

On all dates, standing dry biomass per unit land area (W_b) for *Miscanthus* was higher, reaching a peak of 30.3 t ha^{-1} in 2007 and of 29.5 t ha^{-1} in 2008. This was significantly higher ($P < 0.0001$) than the peak of 19.2 in 2007 and of 18.4 t ha^{-1} in 2008 for maize (Fig. 1). W_b then declined in both crops, with *Miscanthus* dropping sharply to 13.8 t ha^{-1} and maize to 18.1 t ha^{-1} in 2007, 11.4 t ha^{-1} of which was grain. In 2008, the decline in *Miscanthus* was less pronounced to 22.0 t ha^{-1} , while W_b for maize declined to 15.7 t ha^{-1} , of which 8.7 t ha^{-1} was grain (Fig. 1; Table II).

Light Interception and Green Leaf Area Index

In both growing seasons, *Miscanthus* produced leaves and began intercepting radiation much earlier in the growing season than maize and continued intercepting radiation much later (Fig. 2A). *Miscan-*

Table I. Macroclimate data for maize and *Miscanthus* plots in 2007 and 2008

Mean annual and April–October climate data collected daily from the monitoring stations of the Illinois Climate Network located at Urbana, IL. The 30-year averages are from 1979 to 2008.

Year	Solar Radiation	Mean Annual Temperature	Precipitation
	MJ m^{-2}	$^\circ\text{C}$	mm
Annual			
2007	5,696	12.1	862.1
2008	5,512	10.2	1,335.5
30-year average	5,368 ^a	11.1	1,022.6
Growing season			
2007	4,341	19.8	508.3
2008	4,199.2	17.8	895.9
30-year average	4,083 ^a	18.4	667.3

^aTwenty years of solar radiation data were available from 1989 to 2008.

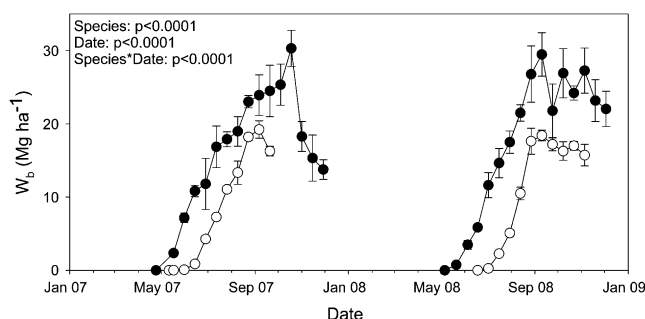


Figure 1. Accumulated aboveground dry biomass (W_b) of Miscanthus (black circles) and maize (white circles) over the 2007 and 2008 growing seasons. The intercept ($W_b = 0$) indicates the date of emergence for each crop in each year. Values represent arithmetic means \pm 1 SE ($n = 4$).

thus emerged and began forming leaves on May 6, 2007 and May 2, 2008. By contrast, maize emerged on May 11, 2007 and was delayed until June 18, 2008 due to wet field conditions that delayed sowing of the crop. Although the emergence dates were similar in 2007, Miscanthus ϵ_i rose much faster. For example, by the second sampling date on May 22, Miscanthus was intercepting 78% of the incoming radiation compared to 8% in maize. In Miscanthus, ϵ_i exceeded 0.8 by May 29, while it did not exceed 0.8 in maize until July 6. The growing season length for Miscanthus was on average 59% longer, at 203 d in 2007 and 195 d in 2008 compared to 125 d in 2007 and 126 d in 2008 for maize.

In the middle of each growing season, maize intercepted nearly as much light as Miscanthus, but in the 2008 growing season, a mid-season storm led to lodging in maize that caused a drop in light interception (Fig. 2A). Miscanthus had a closed canopy ($\epsilon_i > 0.9$) from June 27 to October 4, 2007 and from July 16 to October 9, 2008, while the maize canopy was closed from July 24 to August 8, 2007 and was only closed on August 14 in 2008. As a result, of the 2,693 MJ m⁻² of total photosynthetically active radiation (PAR) in 2007, 1,491 MJ m⁻² were intercepted by the Miscanthus canopy but only 957 MJ m⁻² by the maize canopy, an annual ϵ_i of 0.55 and 0.36, respectively (Table II). In 2008, of the 2,607 MJ m⁻² of total PAR, 1,318 MJ m⁻² were intercepted by the Miscanthus canopy and 752 MJ m⁻² by the maize canopy, an annual ϵ_i of 0.51 and

0.29, respectively. Therefore, Miscanthus intercepted just over half (53%) of the total PAR across the entire 2-year period compared to just under one-third (33%) for maize.

Light interception paralleled differences in green leaf area index (GLAI) with species and time (Fig. 2B). Miscanthus had a GLAI that was 259% of that of maize ($P < 0.0001$) when integrated and averaged over the two growing seasons. There were green leaves on the Miscanthus crop for 168 d in 2007 and for 152 d in 2008, compared to 98 and 99 d, respectively, in maize. GLAI for Miscanthus reached a peak of 6.1 on July 12, 2007 and of 5.9 on July 30, 2008. The peak GLAI for maize was 3.3 on July 12, 2007 and 4.3 on August 13, 2008.

Conversion Efficiency and Diurnal Patterns of A , g_s , and c_i

Based on the peak biomass, ϵ_i , and PAR values presented here and assuming an energy content of 18 MJ kg⁻¹ for plant biomass (Beale and Long, 1995), the conversion efficiency of solar radiation to aboveground biomass (ϵ_{ca}) for Miscanthus averaged 0.042 or 4.2% over both growing seasons, while maize averaged an ϵ_{ca} of 0.043 or 4.3% ($P = 0.8108$; Table II). This lack of difference in ϵ_{ca} between species is clear when biomass accumulation is plotted against cumulative intercepted PAR, since the slope (ϵ_{ca}) of the best-fit lines for the two species are virtually identical and not significantly different ($P = 0.6892$; Fig. 3).

Of the 26 measurement dates over two seasons in which photosynthesis was measured on upper-canopy Miscanthus leaves, maize had green leaves on 16 of those dates. Miscanthus had green leaves ≥ 1 m below the top of the canopy on 20 of the measurement dates, and maize had green leaves ≥ 1 m below the top of the canopy on 12 of the measurement dates (Supplemental Figs. S2 and S3).

Averaged across each day and the respective growing seasons, for sunlit leaves photosynthetic photon flux density (PPFD) was 923.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and leaf temperature 25.9°C for Miscanthus and 992.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 28.6°C for maize. This difference is due to the presence of Miscanthus leaves during cooler and lower insolation periods of spring and through much

Table II. Annual interception and conversion efficiencies of maize and Miscanthus

Total annual incident PAR, interception efficiency (ϵ_i), total annual PAR_i, annual peak biomass yield (W_b), energy content of biomass (k), and conversion efficiency for aboveground biomass (ϵ_{ca}) for Miscanthus and maize canopies over the 2007 and 2008 growing seasons near Urbana, IL.

Year	Species	PAR	ϵ_i	PAR _i	W_b t ha ⁻¹	k	ϵ_{ca}
		MJ m ⁻²		MJ m ⁻²	kg m ⁻²	MJ/kg	
2007	Miscanthus	2,693	0.55	1,491	30.3 (3.03)	18	0.039
2007	Maize	2,693	0.36	957	19.2 (1.92)	18	0.038
2008	Miscanthus	2,607	0.51	1,318	29.5 (2.95)	18	0.045
2008	Maize	2,607	0.29	752	18.4 (1.84)	18	0.048

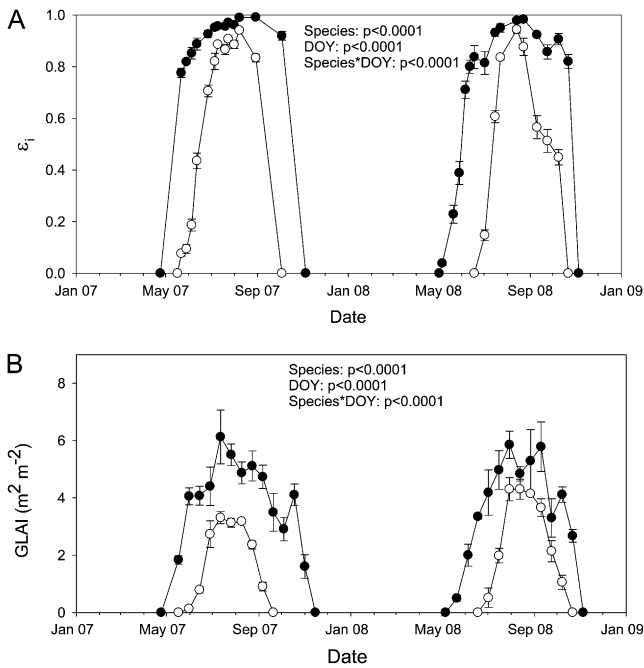


Figure 2. Radiation interception efficiency (ϵ_i) and GLAI of Miscanthus (black circles) and maize (white circles) over the 2007 and 2008 growing seasons. X-intercepts represent the dates of crop emergence and completion of senescence for each species in each year. Significant effects of species, date (DOY, day of year), and the species by date interaction are indicated by the P values.

of the autumn. For shade leaves of Miscanthus, when present at 1 m depth into the canopy, the mean PPFD was $153.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 26.5°C for Miscanthus compared to $305.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 27.5°C for maize. The much lower value in Miscanthus was partly due to the longer season but also due to a much denser canopy, impeding PPFD penetration to this depth.

Of the 16 dates on which there were upper canopy and therefore sunlit leaves for both species, sunlit

leaves of maize had higher A than Miscanthus ($P < 0.05$) on 11 dates, there was no significant difference between species ($P > 0.05$) on two measurement dates, and Miscanthus had a higher A than maize ($P < 0.05$) on three measurement dates (Supplemental Figs. S2 and S3). All cases in which Miscanthus A was greater than or equal to maize, the maize crop was either early or late in its growing season, indicating immature photosynthetic apparatus or the onset of senescence, or it could indicate chilling impairment. Averaged across the daylight hours of all measurement dates, A of Miscanthus sunlit leaves was $17.2 \mu\text{mol m}^{-2} \text{s}^{-1}$, 70% that of maize sunlit leaves that averaged $24.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($P = 0.0005$). The differences in A between species were greatest near midday, when light levels and temperatures were highest, with maize and Miscanthus having an average midday A of $34.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $26.8 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Fig. 5A), and midday peak values of $57.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ for maize and $38.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ for Miscanthus (Supplemental Figs. S2 and S3). The average A for shaded maize leaves was $8.65 \mu\text{mol m}^{-2} \text{s}^{-1}$ compared to $4.20 \mu\text{mol m}^{-2} \text{s}^{-1}$ for shaded Miscanthus leaves ($P < 0.0001$), again with the highest differences at midday. On 11 of the 12 measurement dates in which both species had green leaves 1 m below the top of the canopy, maize leaves had a higher photosynthesis rate ($P < 0.05$), with the exception of the penultimate measurement date in 2008 in which there was no difference between species (Supplemental Figs. S2 and S3).

The daily total photosynthetic CO_2 uptake per unit leaf area (A') followed a similar pattern to A , with Miscanthus sunlit leaves having an average A' of $0.74 \text{ mol m}^{-2} \text{d}^{-1}$ and an average of $1.04 \text{ mol m}^{-2} \text{d}^{-1}$ for maize. The average A' for Miscanthus and maize shaded leaves was 0.15 and $0.31 \text{ mol m}^{-2} \text{d}^{-1}$, respectively (Fig. 4A). The 2-year average seasonal integrals of leaf-level photosynthesis on a mass of carbon basis (A_m'') of sunlit leaves is 15.4 and $12.7 \text{ t C ha}^{-1} \text{ year}^{-1}$

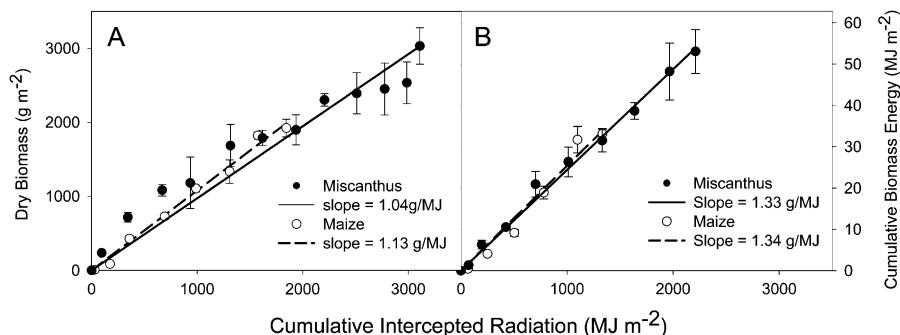
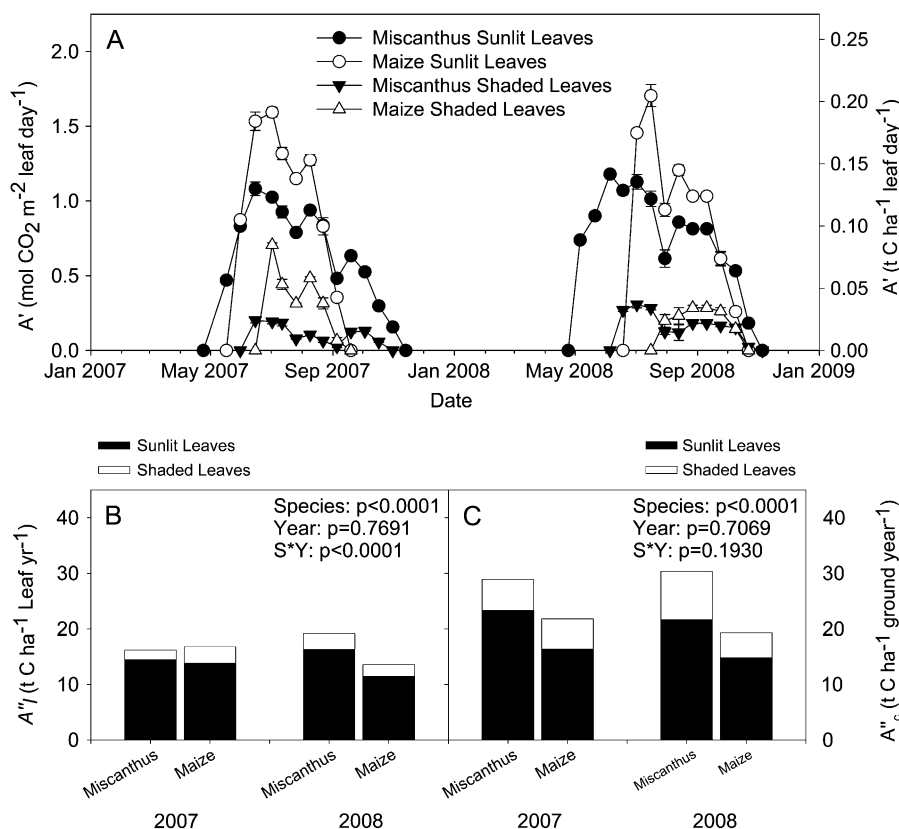


Figure 3. The accumulated dry biomass (mean ± 1 SE) of Miscanthus (black circles) and maize (white circles) plotted against cumulative intercepted total solar radiation in 2007 (A) and 2008 (B). Lines represent the mean best fit linear slope of least squares regression for each species ($n = 4$). Two-way ANOVA shows that there is no significant difference in slope between species ($P = 0.6892$); however, there is a marginally significant effect of year ($P = 0.0706$), but no effect of the species by year interaction ($P = 0.7277$).

Figure 4. Annual course of the daily integrals of measured CO_2 uptake per unit leaf area (A' ; A), total annual leaf-level CO_2 uptake (A'_m) by a single sunlit and single shaded canopy layer (B), and total annual canopy-level CO_2 uptake (A''_c ; C) for Miscanthus and maize based on measured leaf level photosynthetic rates, and calculated sunlit and shaded GLAI over two growing seasons in the Midwestern United States. Significant effects of species, time, and the species by time interaction are indicated by the P values. S*Y, Species \times year.



for Miscanthus and maize, respectively, and of shaded leaves is $2.3 \text{ t C ha}^{-1} \text{ year}^{-1}$ and $2.5 \text{ t C ha}^{-1} \text{ year}^{-1}$ for Miscanthus and maize, respectively (Fig. 4B). When summed over both canopy layers, there is a significant difference in A_m'' between species ($P < 0.0001$), no difference between years ($P = 0.7691$), and there is a significant interaction between species and year ($P < 0.0001$). Pairwise comparisons reveal that there was no difference in A_m'' between species in 2007 ($P = 0.2734$); however, Miscanthus was 25% and significantly higher in 2008 ($P < 0.0001$). Miscanthus has a much larger GLAI, so how does this affect total annual carbon uptake per unit ground area?

When total sunlit and shaded GLAI is taken into account, the average seasonal totals of canopy-level photosynthesis could be calculated. The average seasonal integrals of canopy-level photosynthesis on a mass of carbon basis A_{cm}'' for Miscanthus was $29.6 \text{ t C ha}^{-1} \text{ year}^{-1}$, 44% greater than the $20.6 \text{ t C ha}^{-1} \text{ year}^{-1}$ for maize ($P < 0.0001$; Fig. 4C). There was no difference between years or interaction between year and species ($P = 0.7069$ and 0.1930 , respectively). Of the $29.6 \text{ total t C ha}^{-1} \text{ year}^{-1}$ assimilated by the Miscanthus canopy, $20.8 \text{ t C ha}^{-1} \text{ year}^{-1}$ was assimilated on the dates when maize also had green leaves, showing that nearly all of the 44% difference in canopy carbon assimilation between species occurred due to the longer growing season in Miscanthus.

Averaged across both years at midday on dates where both species had green leaves, leaf stomatal

conductance to water vapor (g_s) of Miscanthus sunlit leaves was $0.194 \text{ mol m}^{-2} \text{ s}^{-1}$, compared to an average of $0.226 \text{ mol m}^{-2} \text{ s}^{-1}$ for maize ($P = 0.0437$; Fig. 5B). Of the 16 dates on which Miscanthus and maize both had green sunlit leaves, maize had significantly higher ($P < 0.05$) or marginally significantly ($P < 0.10$) higher g_s on six of the measurement dates, there was no significant difference between species on eight measurement dates, and Miscanthus had higher g_s ($P < 0.05$) on two of the measurement dates (Supplemental Figs. S4 and S5). The linear regression of all individual points of A versus g_s where $A > 0$ shows that maize has an 11.6% higher slope (i.e. intrinsic leaf water use efficiency) than Miscanthus (Fig. 6; $P = 0.0267$).

When averaged over all days on which there were green leaves for both species, the mean midday ratio of intercellular $[\text{CO}_2]$ to ambient air $[\text{CO}_2]$ (c_i/c_a) was significantly higher at 0.35 for Miscanthus compared to 0.29 for maize (Fig. 5C; $P = 0.0064$). Of the 16 dates on which Miscanthus and maize both had green sunlit leaves, maize had a significantly lower ($P < 0.05$) c_i/c_a across the entirety of two of the measurement dates, and there was no significant difference between species on the remaining 14 measurement dates when taken over the entire day (Supplemental Figs. S6 and S7).

Mean midday levels of whole-chain electron transport rate (J_{PSII}) were $156 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for Miscanthus and significantly higher at $179 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in maize, averaged across the dates when both species had green leaves (Fig. 5D; $P = 0.0379$). Midday maximum effi-

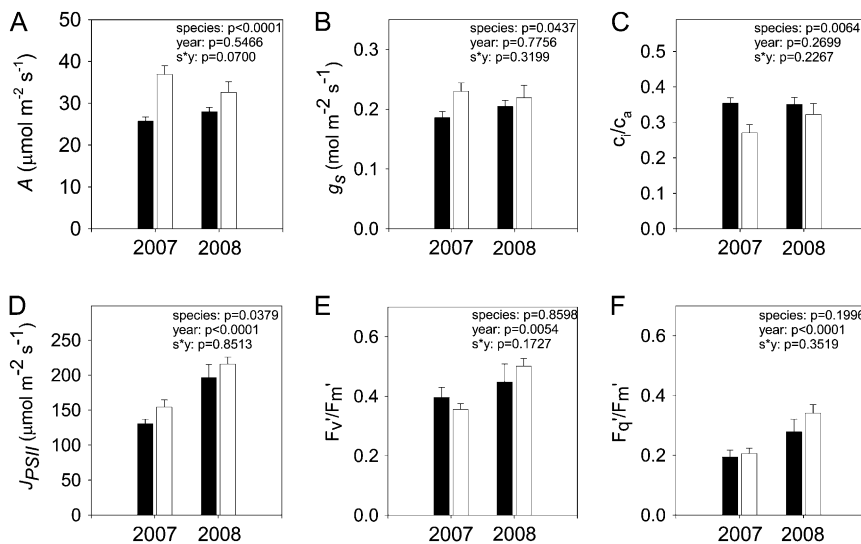


Figure 5. Mean midday leaf-level photosynthetic rate (A; A), stomatal conductance (g_s ; B), ratio of intercellular $[CO_2]$ to atmospheric $[CO_2]$ (c_i/c_a ; C), whole-chain electron transport rate (J_{PSII} ; D), maximum efficiency of PSII (F_v'/F_m' ; E), and operating efficiency of PSII (F_q'/F_m' ; F) for field-grown sunlit Miscanthus (black bars) and maize (white bars) leaves averaged over dates where both crops were measured in the 2007 and 2008 growing seasons. Bars represent arithmetic means \pm 1 SE, and P values represent the mixed-model ANOVA where species, year, and species \times year (S \times Y) were the main effects.

ciency of PSII (F_v'/F_m' ; Fig. 5E) averaged 0.41 for both Miscanthus and maize and was not different between species ($P = 0.8598$), and midday operating efficiency of PSII (F_q'/F_m') averaged 0.23 for Miscanthus and 0.26 for maize, but the difference was not significant (Fig. 5F; $P = 0.1996$). A/J_{PSII} was 24% higher in maize ($P < 0.0001$) averaged across all measurements where $A > 0$ across both growing seasons (Supplemental Fig. S8). Commensurate with this finding, $F_q'/F_m' / \Phi_{CO_2}$ for the same measurement points was 14% higher in Miscanthus (Supplemental Fig. S9; $P < 0.0001$).

A/PPFD and A/c_i Responses

Analysis of the response of A to PPFD of upper-canopy maize leaves sampled in mid-summer showed a significantly higher rate of light saturated rate of photosynthesis (A_{sat}), maximum quantum yield of CO_2 assimilation ($\Phi_{CO_2, max}$), and dark respiration rate (R_d) when compared to Miscanthus. By contrast, the convexity of the response function (θ) was significantly greater in Miscanthus (Fig. 7A; Table III).

Analysis of the responses of A to c_i for sunlit leaves sampled in mid-summer showed that both the maximum velocity of phosphoenolpyruvate (PEP) carbox-

ylation (V_{pmax}) and maximum rate of PEP regeneration (V_{pr}) were significantly higher ($P < 0.05$) in maize, the difference being most pronounced in V_{pmax} (Fig. 7B; Table III). V_{pmax} and V_{pr} of both species dropped on the August 26 measurement date, possibly due to an abnormally dry August in that year (Supplemental Fig. S1); however, maize values were still significantly higher than those in Miscanthus. Leaf [N] on an area basis was 19% higher for maize than Miscanthus; however, the much higher V_{pr} of maize causes a photosynthetic nitrogen use efficiency (PNUE) that is 41% higher for maize (Table III). Figure 7 shows the response of a representative leaf from the $A/PPFD$ and A/c_i response functions, but the means and SEs for all leaves are shown in Table III ($n = 4$).

DISCUSSION

This study tested directly the hypothesis that Miscanthus is even more productive than modern lines of maize bred for high productivity in the Corn Belt of the Midwestern United States. It further examined whether differences may be attributed to more efficient light capture or more efficient conversion of captured light into biomass through photosynthetic

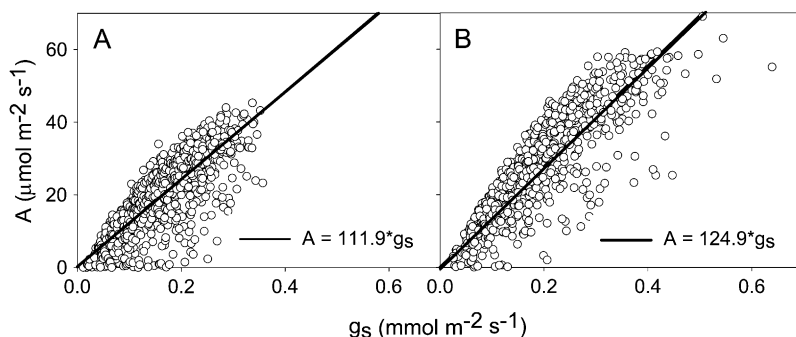


Figure 6. Best fit linear regression analysis of A versus g_s for Miscanthus (black circles) and maize (white circles) over the 2007 and 2008 growing seasons. Points represent individual subsamples from diurnal measurements with lines representing best-fit linear regression with all respiration ($A < 0$) values removed and forced through the origin. The difference in slope between species is statistically significant ($P = 0.0267$; $n = 4$).

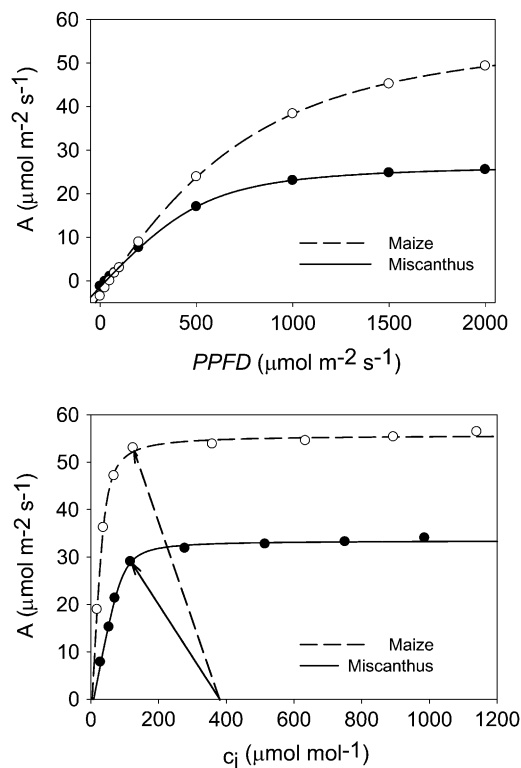


Figure 7. Representative responses of leaf CO₂ uptake (*A*) to incident photon flux (*Q*; *n* = 4; A) and intracellular CO₂ concentration (*c_i*; B) for field-grown leaves of Miscanthus (black circles) and maize (white circles). Arrows represent supply function for each representative curve. Fitted values for all measurements are given in Table II.

differences. The maximum biomass achieved by Miscanthus was 30.3 t ha⁻¹ and 29.4 t ha⁻¹ in 2007 and 2008, respectively, compared to 19.2 t ha⁻¹ and 18.4 t ha⁻¹ for maize, on average 59% greater. The climate was slightly warmer and drier than the long-term average in 2007 and slightly cooler and wetter than average in 2008, suggesting that the differences shown here are representative of those that would be ex-

pected across years at this location. Maize grain yield in 2007 was 11.4 t ha⁻¹ and very close to the county average of 11.8 t ha⁻¹ (U.S. Department of Agriculture National Agricultural Statistics Service, 2009), suggesting that the crop here is the representative of this area in which some of the highest world yields of maize are achieved. The mean peak productivity of Miscanthus over the two growing seasons in the trials described here was only 61% of the peak shown in small plot (0.01 ha) trials at the same location in 2004 to 2006 (Heaton et al., 2008). The trials used in this study were the first large plot replicated trials in the United States and were planted at a lower density than the previously studied small plots. The lower yield may in part reflect this, but also the fact that by contrast to maize there is very little agronomic experience with Miscanthus. The 59% higher maximum biomass, while large, may therefore underestimate the potential difference between these two crops.

Was the higher biomass yield of Miscanthus due to a higher ϵ_i or higher ϵ_c ? The answer is clear in Figure 3. The rate of accumulation of biomass, and energy in biomass, when plotted against cumulative intercepted PAR is linear and virtually identical between the two species; i.e. ϵ_c was not different between the two species. The difference is that Miscanthus, with its longer growing season and greater GLAI, intercepts more of the available radiation over the year (Fig. 2A). In both years, Miscanthus emerged earlier, senesced later, and intercepted more radiation throughout the growing season than maize. While Miscanthus light interception was similar across years, maize light interception was shifted toward the end of the growing season in 2008, with the crop not emerging until 2 d prior to the summer solstice. Most striking is that in mid-May 2007 when maize was just emerging, ϵ_i was already 0.80. It took maize another 6 weeks to reach this level. Similarly, Miscanthus maintained an $\epsilon_i > 0.90$ for a month longer than maize in the autumn. This difference was even more pronounced in the cooler 2008 growing season (Fig. 2A). Interception efficiency (ϵ_i) is traditionally calculated from the total incident

Table III. Parameter calculations for *A/Q* and *A/c_i* responses of maize and Miscanthus

Means ± 1 SE for gas exchange parameters of the leaves from four plots of Miscanthus and maize plants grown in the field and measured in the laboratory on July 28 and August 26, 2008. *P* values represent significant differences ($\alpha < 0.05$, *n* = 4) between species for each parameter on each measurement date. DOY, Day of year.

Parameter	DOY	Miscanthus	Maize	<i>P</i> Value
<i>A</i> _{sat} (μmol m ⁻² s ⁻¹)	208	28.6 ± 1.4	49.3 ± 2.1	<0.0001
Φ _{CO2,max}	208	0.047 ± 0.001	0.068 ± 0.001	<0.0001
θ	208	0.84 ± 0.042	0.67 ± 0.040	0.0070
<i>R</i> _d (μmol m ⁻² s ⁻¹)	208	1.37 ± 0.07	3.39 ± 0.18	<0.0001
<i>V</i> _{pmax} (μmol m ⁻² s ⁻¹)	208	44.9 ± 7.4	176.2 ± 29.2	<0.0001
<i>V</i> _{pr} (μmol m ⁻² s ⁻¹)	208	31.0 ± 0.7	52.9 ± 3.2	<0.0001
[N] (mmol [N] m ⁻²)	208	81.4 ± 3.4	96.8 ± 8.0	0.1276
PNUE (μmol mol ⁻¹ s ⁻¹)	208	386 ± 23	544 ± 23	0.0025
<i>V</i> _{pmax} (μmol m ⁻² s ⁻¹)	238	19.3 ± 1.7	83.2 ± 16.5	0.0037
<i>V</i> _{pr} (μmol m ⁻² s ⁻¹)	238	24.4 ± 1.2	33.7 ± 2.0	0.0007

PAR over the growing season (Monteith, 1977; Beale and Long, 1995; Heaton et al., 2008; Murchie et al., 2009). However, when comparing species with substantially different growing season lengths, this calculation will be misleading as to the total efficiency with which the annual photosynthetically active solar energy resource of $2,650 \text{ MJ m}^{-2}$ was used. Annual ϵ_i was therefore calculated as the sum of the daily products of ϵ_i and PAR over the entire year. When averaged over both years, Miscanthus ϵ_i was 0.53 compared to 0.33 for maize, a 61% difference between species, while the traditional calculation, i.e. limited to the growing season, yielded an average over the 2 years ϵ_i of 0.81 and 0.65 for Miscanthus and maize, respectively, only a 25% difference. The resulting amount of intercepted PAR for each species is the same in either calculation; however, the use of annual ϵ_i reflects more closely the differences in crop function. The growing season ϵ_i for Miscanthus of 0.81 is remarkably similar to the 0.80 recorded in small plot trials at the same site in 2004 to 2006 and to the 0.83 for stands, putatively of the same germplasm, growing in Southern England (Heaton et al., 2008). Throughout the year, total intercepted radiation (PAR_i) was $1,405 \text{ MJ m}^{-2}$ for Miscanthus and 855 MJ m^{-2} for maize (Table II). Since biomass production is proportional to the product of ϵ_i and ϵ_{ca} , and since peak biomass was 59% higher in Miscanthus, this could be accounted for entirely by ϵ_i , which was 61% higher. So was there no difference leaf and canopy photosynthesis?

While ϵ_{ca} is similar between species, they use different strategies to achieve their respective ϵ_{ca} . The leaf-level A' for maize was substantially higher than for Miscanthus during the middle portion of each growing season. However, the ability of this Miscanthus germplasm to produce and maintain photosynthetically active leaves at much lower temperatures (Farage et al., 2006; Wang et al., 2008) allows for substantial carbon gain during early and late portions of the growing season (Fig. 4A; Supplemental Figs. S1 and S2). Not only did Miscanthus show substantial assimilation at times of the year when no green maize leaves were produced or present, but it also showed significantly higher values at the beginning and end of the maize growing season, which could reflect the poorer low-temperature tolerance of maize (Fryer et al., 1995). A' values shown here for maize and Miscanthus are similar to those shown in separate studies at nearby locations (Leakey et al., 2004; Dohleman et al., 2009).

While Miscanthus is noted for its exceptional cold tolerance, this study shows clearly that maize has a substantial advantage in leaf-level photosynthesis over Miscanthus under the mid-summer growing conditions that are found in the Midwestern United States. The finding is consistent with prior controlled environment studies where maize was able to maintain higher A_{sat} when grown at 25°C; however, when grown at 14°C, A_{sat} measured at 25°C for A_{sat} for Miscanthus was substantially higher than maize (Naidu and Long, 2004).

In this study under field-grown conditions, Miscanthus was able maintain a substantial amount of photosynthetically active leaf area prior to maize emergence in the spring and subsequent to maize senescence in the autumn, as evident in Figure 4A. Using these two distinct strategies for carbon assimilation, maize and Miscanthus achieved similar total annual carbon gains in 2007 at the leaf level, although Miscanthus was about 25% higher in 2008 (Fig. 4B). On a canopy level, sunlit leaves account for 77.5% of the total carbon assimilation for Miscanthus and 75.6% of the total carbon assimilation for maize.

Recent work has shown significant leakiness of CO_2 from bundle sheath cells back to mesophyll cells in Miscanthus stands under light-limiting conditions, causing an energetic cost to the plant and therefore a decrease in ϵ_{ca} (Kromdijk et al., 2008). Kromdijk et al. (2008) conducted their study in the much cooler growing conditions of Ireland, where average biomass yields were only about half those found here (Clifton-Brown et al., 2007). Therefore, it is uncertain the extent to which this efficiency loss applies to the warmer location of this study.

On all dates in both years, GLAI was greater in Miscanthus; therefore, total canopy-level carbon assimilation is 44% higher in Miscanthus (Fig. 4C; $P < 0.0001$). This 44% higher total C assimilation fails to account in full for the 59% higher in peak aboveground biomass of Miscanthus. Two factors could account for this discrepancy: higher respiratory losses and/or a greater diversion of assimilate into belowground organs in maize. For Miscanthus stands growing in Southern England, about 40% of biomass was partitioned below ground compared to 14% to 26% for maize (Beale and Long, 1995; Bonifas and Lindquist, 2006). In Miscanthus, this accumulation occurred over a 3-year period, while maize must renew its investment in roots annually. This suggests that more biomass could be diverted into belowground organs in maize on an annual basis. Substantial energy is also likely expended in translocation of resources to, and construction of, the grain in maize, a cost avoided by Miscanthus, which invests <2% of accumulated biomass into reproductive structures (Heaton et al., 2008).

These findings suggest that there is potential for improvement in both maize and Miscanthus photosynthesis. If maize were able to produce photosynthetically active leaves under colder temperatures, it could take advantage of the radiation in the early spring and autumn under these growing conditions as Miscanthus does. It is not clear whether a lower photosynthetic capacity at high temperatures is a penalty of improved low temperature tolerance. Across terrestrial plants, species capable of high photosynthetic rates at high temperatures typically have low rates at low temperature and vice versa. The cool temperate C_4 grass *Spartina angelica* showed a temperature optimum of A_{sat} of about 10°C less than C_4 grasses of tropical origin, but in contrast continued

photosynthesis below 14°C (Long et al., 1975). However, both C_3 and C_4 species vary in their ability to acclimate their photosynthetic capacity to temperature (Sage and Kubien, 2007). Therefore, it would be important to screen *Miscanthus* germplasm to determine whether lines may be identified that can match the photosynthetic rates of maize in midsummer, while maintaining capacity to assimilate CO_2 at lower temperatures.

A cost of higher A in midsummer in maize is a higher g_s (Fig. 5B; Supplemental Figs. S3 and S4); however, the regression of A to g_s shows an intrinsic leaf water use efficiency (A/g_s) that is 12%, and significantly, higher than in *Miscanthus* (Fig. 6; $P = 0.0267$). Coupled with its longer growing season and a higher GLAI throughout (Fig. 2B), this suggests that *Miscanthus* may use a great deal more water than maize to achieve its 61% higher biomass yield. Both direct measurements of soil moisture in the same plots across growing seasons (G. McIsaac, unpublished data) and canopy evapotranspiration in the 2007 growing season (G. Hickman, unpublished data) showed that *Miscanthus* uses more water over the full growing season. The higher intrinsic water use efficiency of maize is also consistent with its significantly lower midday c_i/c_a during the summers of both 2007 and 2008 (Fig. 5C).

While PNUE was significantly higher in maize at the leaf level, at the whole crop level, *Miscanthus* is clearly more efficient. The high yields obtained here were without any nitrogen fertilization for *Miscanthus*, compared to an annual input of 168 kg [N] ha^{-1} for maize. Nitrogen fertilizer is over half the energy input used in producing a maize crop. Assuming the same inputs in producing *Miscanthus* and maize crops, as calculated by Boehmel et al. (2008), the average annual input for *Miscanthus* would have been 6 GJ ha^{-1} compared to 24 GJ ha^{-1} for maize. This corrects for the higher N fertilization rate used here, by comparison to Boehmel et al. (2008). Therefore, one-quarter of the energy input is required to obtain 60% more biomass.

Across the growing seasons, A/J_{PSII} in maize was 24% greater than in *Miscanthus* (Supplemental Fig. S8; $P < 0.0001$). This suggests that a significantly higher proportion of whole chain electron transport is used in CO_2 assimilation in maize; however, F_v'/F_m' did not differ, suggesting no differences in diversion of absorbed light energy into nonphotochemical sinks.

What biochemical limitations underlie the higher A in maize during the summer months (Supplemental Figs. S1 and S2)? Both A/c_i and A/Q curves were constructed and analyzed to address this question. Values of all leaf photosynthetic parameters (Table III) in maize were similar to those determined previously in field-grown maize at an adjacent site (Leakey et al., 2006) and for field-grown *Miscanthus* in England (Beale et al., 1996). When compared in controlled environments, values for all parameters for *Miscanthus* and maize grown at 25°C were similar to those

reported here for field-grown *Miscanthus*, except that V_{pr} and V_{pmax} were significantly higher in maize (Naidu and Long, 2004). A_{sat} , V_{pr} and V_{pmax} were all substantially higher in the field-grown maize. V_{pr} is metabolically controlled by the activity of pyruvate Pi dikinase and Rubisco and V_{pmax} by the activity of PEP carboxylase (Furbank et al., 1997). Collectively, these enzymes account for a large proportion of leaf nitrogen. The higher activities inferred from in vivo analysis (Table III) might therefore be related to the fact that cultivation of both crops followed standard practice; that is, maize was grown with the addition of 168 kg [N] ha^{-1} , while *Miscanthus* was grown without any addition of nitrogen. Maize leaf [N] on an area basis was 19% higher than in *Miscanthus*, suggesting larger amounts of key photosynthetic enzymes in maize; however, even with this increase in leaf N, the PNUE of maize is 41% higher (Table III), suggesting a much greater biochemical efficiency of CO_2 assimilation. The PNUE values shown here for maize are substantially higher than the range of 200 to 320 $\mu mol\ mol^{-1}\ s^{-1}$ in naturally occurring C_4 species (Taub and Lerdau, 2000). A significantly higher V_{pmax} and V_{pr} in maize was also found in controlled environment studies, where equal amounts of nitrogen were applied to both species, supporting the idea of a genetic component to the difference between species (Naidu and Long, 2004).

Analysis of A/Q response functions revealed that $\Phi_{CO_2,max}$ was also significantly higher in maize, implying a lower maximum efficiency of transduction of absorbed light energy into CO_2 assimilation, due to a higher level of alternative photochemical and/or non-photochemical sinks. Higher A_{sat} and $\Phi_{CO_2,max}$ in maize may be at the expense of increased mitochondrial respiration, since R_d is significantly higher in maize. The lower A_{sat} and $\Phi_{CO_2,max}$ for upper-canopy leaves of *Miscanthus* must however be offset by other factors since ϵ_c was almost identical for the two crops across the entire growing season (Fig. 3; Table II). This may result both from the lower R_d (Table III) and much greater GLAI of *Miscanthus* (Fig. 2B).

Grain maize yields in central Illinois are some of the highest in the world. Yet in this first side-by-side comparison to such a crop, it was shown that the related C_4 grass, *Miscanthus*, can achieve a 61% higher biomass yield. This was not due to higher mid-summer leaf photosynthetic rates in *Miscanthus*, but due to its ability to produce and maintain photosynthetically competent leaves earlier and later than maize. Importantly, the results show that if a similar capacity could be engineered into maize then a 60% biomass increase, and if partitioning efficiency of biomass into grain remained constant, a 60% yield increase, even above the high yields already obtained in the Midwestern United States, may be possible. Furthermore, if higher leaf-level photosynthesis could be engineered into *Miscanthus*, then there should be potential for yield increase in that species as well. This study highlights the need for an integrated approach to gain an understanding of how molecular differences in photosyn-

thesis might be exploited to achieve large gains in crop productivity in field conditions.

MATERIALS AND METHODS

Field Site and Cultivation

This study was conducted in 0.2 ha (61 m × 31 m) plots of *Miscanthus* (*Miscanthus* × *giganteus*) and of *Zea mays* within a completely randomized experimental design ($n = 4$) at the University of Illinois Agricultural Research Station near Champaign, IL (40°02'N, 88°14'W, 228 m above sea level). Soils are deep Drummer/Flanagan series (fine silty, mixed, mesic Typic Endoaquoll) with high organic matter typical of the central Illinois Corn Belt. Daily meteorological data, including total solar radiation, temperature, and precipitation, were collected within 4 km of the trial location by the long-term monitoring station of the Illinois Climate Network (Angel, 2008).

Miscanthus rhizomes were propagated, as described in establishing other trials (Heaton et al., 2008), and planted in rows of 1.22 m with plants spaced at 1.22 m within a row in 2005. As a rhizomatous perennial, *Miscanthus* produces an annual crop of shoots from perennial underground rhizomes; shoots are harvested dead in the winter. As in the case of these plots, it takes 3 years for the rhizomes to expand sufficiently so that the shoots can fill the space between the original plantings. Typically the annual dry matter yield of shoots is low in year one, increases in year two, and reaches a peak in year three, which may be maintained for several subsequent years (Clifton-Brown et al., 2001); therefore, when this comparison began in 2007, the *Miscanthus* stand was assumed to have reached this peak. Maize cv 34H35 (Pioneer Hi-Bred International) was planted at a 0.762 m row spacing, a density of approximately 80,000 plants ha⁻¹, on May 8, 2007 and emerged on May 11, 2007. The same area had been planted to soybean (*Glycine max*) in 2006. Due to an exceptionally wet spring, maize (cv Dekalb 61-69; Monsanto Co.) was not planted in 2008 until June 12 and emerged on June 17. The change in cultivar was necessitated by the late spring and by the need to minimize the risk of pest damage by following one maize crop with a second. Both cultivars of maize were recommended as highly productive for central Illinois. In both years, 168 kg [N] ha⁻¹ was applied prior to the planting of the maize crop, following standard recommendations for obtaining high yields in this region. No fertilizer was added to the *Miscanthus* plots in either year.

Biomass Sampling and Leaf Area Index

Standing shoot biomass per unit land area on a dry weight basis (W_b) was determined biweekly from subsamples taken throughout the 2007 and 2008 growing seasons. For *Miscanthus*, two subsamples of 10 randomly selected tillers were taken from randomly selected plants within each plot, and the total tiller number of that plant was also recorded. Samples were oven-dried at 75°C to constant weight. W_b was then obtained from the product of the estimated whole-plant mass and the planting density. For maize, two subsamples per plot of 1 m within randomly selected rows were removed and dried as before to determine dry sample mass. W_b was the ratio of dry sample mass and sample area for the respective crops. No samples of either species were taken within 3 m of the edge of a plot to avoid any border effects (Roberts et al., 1993). The final subsamples were taken immediately prior to the end-of-season whole plot harvest. The final harvested yield of the total area for each species fell within the 95% confidence limits of W_b estimated from these subsamples, providing validation of the subsampling method.

GLAI was determined destructively at each biomass sampling point by excising green leaves from the freshly cut shoot subsamples used for biomass determination. These leaves were passed through a planimetric leaf area meter (LAI-3100; LI-COR) prior to drying. The area meter was calibrated against paper standards of known area. Leaves with a laminar area that was <50% green tissue were not used (Morgan et al., 2005). GLAI was then scaled up, as described above in the calculation of W_b , from the biweekly subsampling.

Light Interception

Canopy interception of PAR (400–700 nm) was determined biweekly, in parallel with determination of W_b and GLAI, above. PPFD was measured above (I_a) and below the canopy (I_b) in three randomly selected areas in each

plot between 10:00 and 14:00 on clear-sky days with a line quantum sensor (AccuPAR LP-80; Decagon Devices), which was 0.87 m in length to obtain a spatial average in the heterogeneous light environment of the canopy. A single measurement consisted of five point observations of I_a simultaneous with five observations of I_b across a 1-m transect below the canopy. Efficiency of PAR interception (ϵ_i) is given by the mean $(1 - I_b/I_a)$ for each species (Nobel et al., 1993). To determine PAR_i on a daily basis from the data collected here, a least-squares quadratic equation was fit to the progression of ϵ_i across each year to estimate daily radiation interception for each plot in each year (PROC REG SAS v9.1; SAS Institute). Daily incident PAR was calculated using total incoming radiation (Angel, 2008) and assuming that 47.3% of that radiation was PAR (Papaioannou et al., 1993). Summing the daily PAR_i across each year gave the annual PAR_i. The ratio of PAR_i to total annual PAR was used to determine annual ϵ_i . An ϵ_i of 0 was assumed on all dates prior to crop emergence and subsequent to the completion crop senescence.

In Situ Leaf Gas Exchange

On 26 d, distributed across the 2007 and 2008 growing seasons, leaf CO₂ and water vapor exchange were measured at approximately 2-h intervals from predawn to postdusk. Upper-canopy sunlit leaves of three separate randomly selected plants in each plot ($n = 4$) were measured on all dates (sunlit leaves). In addition, when canopies had reached sufficient height, a parallel sample of three leaves was measured at 1 m below the canopy top (shaded leaves). In cases where both species were present and at least one species had green leaves at 1 m below the canopy top, two gas exchange systems, calibrated against the same standards, were used. Each measurement cycle took 45 to 75 min to complete.

Leaf CO₂ and water vapor exchange were measured in cuvettes with controlled temperature and photon flux within a portable open path gas-exchange system incorporating infrared CO₂ and water vapor analyzers (LI-COR 6400; LI-COR). Modulated chlorophyll fluorescence was measured simultaneously with a fluorometer incorporated into the cuvette lid (LI-6400-40; LI-COR). Dew was often present on leaves in the early morning and occasionally postdusk. In these cases, leaves were blotted immediately prior to enclosure into the leaf cuvette, and g_s was not calculated, since residual surface moisture could increase apparent water fluxes from the leaf.

Immediately prior to the start of a measurement cycle, the red-blue LED light source in the cuvette head was set to the incident PPFD, as recorded at that point in time with a line quantum sensor above the plant canopy (LP-80; Decagon Devices). In the case of lower-canopy leaves, PPFD was measured at 1 m below the top of each crop's canopy five times across a spatial transect prior to the measurement cycle. Importantly, the sensor is a line quantum sensor that is able to give an average spatial reading of light levels within a canopy since point estimates can be quite heterogeneous. Similarly, the measurement temperature of the gas exchange cuvette block was set to the open air temperature recorded at the start of the measurement cycle using the leaf thermocouple junction of the LI-6400. Humidity in the cuvette was that of the outside air, except during periods when relative humidity was so high that condensation could occur. During these periods, which were typically around dawn and dusk, the air was partially dried but the leaf vapor pressure deficit was never allowed to exceed 1.0 kPa. The light and temperature conditions within the chamber were held constant for the duration of each measurement cycle, regardless of short-term fluctuations in ambient light and air temperature. Reference [CO₂] in the cuvette was set to the external air concentration of 380 μmol mol⁻¹. Measurements were recorded once CO₂ uptake and stomatal conductance stabilized after enclosure within the cuvette, typically within 45 to 60 s. Calculations of A and g_s followed the equations of von Caemmerer and Farquhar (1981). Operating PSII efficiency (F_q'/F_m'), maximum efficiency of PSII (F_v'/F_m'), and the proportion of open PSII centers (F_q'/F_v') were calculated following Genty et al. (1989) and using the nomenclature of Baker and Oxborough (2004). The rate of whole-chain electron transport through PSII (J_{PSII}) was given by the product of F_q'/F_m' and absorbed PPFD. A posteriori analysis of data revealed that fluorescence measurements from one of the gas exchange systems did not produce a saturating pulse over the duration of the experiment; therefore, all fluorescence values presented were recorded from the other gas exchange system.

Total daily leaf CO₂ uptake (A') was calculated from the instantaneous measurements made for each day of the growing season by summing the trapezoidal area described under each pair of adjacent measurement cycles, over each day (SAS Institute). Calculations for A' were only made for the time

of day when $A \geq 0$; i.e. when net photosynthesis was positive. Canopy-level A' (A'_c) was estimated from the following equation:

$$A'_c = (A'_{\text{sun}} * \text{LAI}_{\text{sun}}) + (A'_{\text{shade}} * \text{LAI}_{\text{shade}})$$

where A'_c is the daily canopy level CO_2 uptake ($\mu\text{mol m}^{-2} \text{d}^{-1}$), A'_{sun} is the daily CO_2 uptake by sunlit leaves ($\mu\text{mol m}^{-2} \text{d}^{-1}$), LAI_{sun} is the sunlit GLAI ($\text{m}^2 \text{m}^{-2}$), A'_{shade} is the daily CO_2 uptake by shaded leaves ($\mu\text{mol m}^{-2} \text{d}^{-1}$), and $\text{LAI}_{\text{shade}}$ is the shaded GLAI ($\text{m}^2 \text{m}^{-2}$).

A'_{sun} and A'_{shade} were determined from leaf-level photosynthesis measurements. LAI_{sun} and $\text{LAI}_{\text{shade}}$ at any given point in time were determined from the incident photon flux above the canopy, sun angle, and overlying GLAI using the equations of Forseth and Norman (1993), as implemented in the software for the mechanistic plant production model, WIMOVAC (www.life.uiuc.edu/plantbio/wimovac; Humphries and Long, 1995). Seasonal integrated rates of canopy-level CO_2 uptake (A'_c) were determined by estimating canopy-level A' on each measurement date, then integrating over the growing season as before.

A/c_i and A/PPFD Responses

Predawn on July 28 and August 26 of 2008, two individual plants per plot of each species were cut at their base and then immediately cut under water and kept immersed. Plants were then transported back to the laboratory and kept in the dark until approximately 20 min prior to measurement, then illuminated to allow leaves to adapt to light conditions. The objective of this analysis was to determine differences between species in photosynthetic capacity of leaves; therefore, measurement in the controlled conditions of the laboratory were necessary to avoid short-term biochemical and water stress limitations that can occur under more variable field conditions (Leakey et al., 2006). The gas exchange system described above was used in the laboratory to measure A/c_i curves at a PPFD of $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a block temperature setting of 25°C . Following the theory of von Caemmerer (2000), PEP carboxylase efficiency (V_{pmax}) is given by the initial slope (dA/dc_i) of the response of A to c_i , in this case the average of dA/dc_i values at $c_i < 50 \mu\text{mol mol}^{-1}$. V_{pmax} , the maximum rate of regeneration of PEP, was given by the c_i -saturated value of A , in this case the average of all values of A above the inflection in the response of A to c_i . On July 28, A/PPFD was measured at a $[\text{CO}_2]$ of $380 \mu\text{mol mol}^{-1}$ and fitted with a nonrectangular hyperbola as described previously to determine both the maximum efficiency of light-limited photosynthetic CO_2 uptake ($\Phi_{\text{CO}_2, \text{max}}$), rate of dark respiration (R_d), and convexity of the response function (θ ; Bernacchi et al., 2005). For the purposes of this study, the light-saturated rate of photosynthesis (A_{sat}) was assumed to be the mean A at a PPFD of $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Immediately following gas exchange measurements, leaf discs of a known area were cut, oven dried at 75°C to a constant mass, and weighed to determine specific leaf area. Samples were then ground to a fine powder using a stainless steel pulverizer (Kleco Pulverizer; Kinetic Laboratory Equipment Company) and stored under desiccation, and nitrogen level was determined using a combustive elemental analyzer (Costech Analytical Technologies), calibrated with an acetanilide standard. N_i was obtained from the quotient of N_m and specific leaf area multiplied by the atomic mass of nitrogen.

Statistical Analysis

To avoid pseudoreplication, in all cases, the individual plot was the experimental unit, with this value being the mean of the measures made on the two to three randomly subsampled plants selected at any given time point. The diurnal measurements were analyzed separately for each day of the year using a mixed-model repeated measures ANOVA (PROC MIXED, SAS v9.1; SAS Institute), with time of day, treatment, and time of day by treatment interaction as fixed effects. For all measures over the 2007 and 2008 growing seasons, a mixed-model ANOVA was used with species, day of year, and species by day of year interaction as fixed effects and year as a random effect. A priori determined pairwise comparisons between the species were performed for each measure. Where applicable, the best-fit covariance matrices were chosen for each variable using Akaike's information criterion to correct for inequality of variance between the sampling time periods (Keselman et al., 1998; Littell et al., 1998, 2000). This was most often a heterogeneous-autoregressive covariance matrix. F-values and statistical significance is reported at $\alpha = 0.05$. Arithmetic means are reported on graphs with 1 SE of the mean represented by the error bars.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Monthly average of temperature and monthly totals of solar radiation and precipitation for the study site over the period of study.

Supplemental Figure S2. Diurnal course of sunlit and shaded leaf photosynthesis for both species for 13 d across the 2007 growing season.

Supplemental Figure S3. Diurnal course of sunlit and shaded leaf photosynthesis for both species for 13 d across the 2008 growing season.

Supplemental Figure S4. Diurnal course of sunlit and shaded leaf stomatal conductance for both species for 13 d across the 2007 growing season.

Supplemental Figure S5. Diurnal course of sunlit and shaded leaf stomatal conductance for both species for 13 d across the 2008 growing season.

Supplemental Figure S6. Diurnal course of sunlit and shaded leaf intercellular CO_2 concentration for both species for 13 d across the 2007 growing season.

Supplemental Figure S7. Diurnal course of sunlit and shaded leaf intercellular CO_2 concentration for both species for 13 d across the 2008 growing season.

Supplemental Figure S8. Relationship of leaf CO_2 uptake rate (A) to electron transport rate (J_{PSII}) through PSII for each species.

Supplemental Figure S9. Relationship of the operating efficiency (F_q' / F_m') of PSII to quantum yield of CO_2 uptake (Φ_{CO_2}) for each species.

Supplemental Appendix S1. List of abbreviations of terms and their units.

ACKNOWLEDGMENTS

We thank Emily Doherty, Rebecca Arundale, Joseph Crawford, Allison Luzader, Melissa Kocak, Rhea Kressman, Andrew Leakey, Emily Heaton, Fernando Miguez, Kevin Hollis, Jeremy Pillow, Tom Straight, Caroline Thrun, Drew Schlumpf, Rich Pyter, Robert Dunker, and Mike Kleiss for help in establishing these trials and/or for assisting with the measurements reported here.

Received March 28, 2009; accepted June 14, 2009; published June 17, 2009.

LITERATURE CITED

- Angel J (2009) The Water and Atmospheric Resources Monitoring Program. Illinois State Water Survey, University of Illinois at Urbana-Champaign. <http://www.isws.uiuc.edu> (February 6, 2009)
- Baker NR, Oxborough K (2004) Chlorophyll fluorescence as a probe of photosynthetic productivity. In G Papageorgiou, Govindjee, eds. *Chlorophyll Fluorescence: A Signature of Photosynthesis*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 66–82
- Beale CV, Bint DA, Long SP (1996) Leaf photosynthesis in the C4-grass *Miscanthus x giganteus*, growing in the cool temperate climate of southern England. *J Exp Bot* **47**: 267–273
- Beale CV, Long SP (1995) Can perennial C4 grasses attain high efficiencies of radiant energy conversion in cool climates? *Plant Cell Environ* **18**: 641–650
- Bernacchi CJ, Morgan PB, Ort DR, Long SP (2005) The growth of soybean under free air $[\text{CO}_2]$ enrichment (FACE) stimulates photosynthesis while decreasing in vivo Rubisco capacity. *Planta* **220**: 434–446
- Boehmel C, Lewandowski I, Claupein W (2008) Comparing annual and perennial energy cropping systems with different management intensities. *Agric Syst* **96**: 224–236
- Bonifas KD, Lindquist JL (2006) Predicting biomass partitioning to root versus shoot in corn and velvetleaf (*Abutilon theophrasti*). *Weed Sci* **54**: 133–137
- Clifton-Brown JC, Breuer J, Jones MB (2007) Carbon mitigation by the energy crop, *Miscanthus*. *Glob Change Biol* **13**: 2296–2307
- Clifton-Brown JC, Long SP, Jorgensen U (2001) *Miscanthus* productivity.

- In MB Jones, M Walsh, eds, *Miscanthus for Energy and Fiber*. James and James (Science Publishers) Ltd., London, pp 46–67
- Dohleman FG, Heaton EA, Leahey ADB, Long SP** (2009) Does greater leaf-level photosynthesis contribute to greater solar energy conversion efficiency in *Miscanthus* when compared to switchgrass? *Plant Cell Environ* (in press)
- Farage PK, Blowers D, Long SP, Baker NR** (2006) Low growth temperatures modify the efficiency of light use by photosystem II for CO₂ assimilation in leaves of two chilling-tolerant C₄ species, *Cyperus longus* L. and *Miscanthus x giganteus*. *Plant Cell Environ* **29**: 720–728
- Farrell AE, Plevin RJ, Turner BT, Jones AD, O'Hare M, Kammen DM** (2006) Ethanol can contribute to energy and environmental goals. *Science* **311**: 506–508
- Food and Agricultural Organization** (2009) FAOSTAT. <http://faostat.fao.org> (February 20, 2009)
- Forseth IN, Norman JM** (1993) Models of canopy carbon and water balance. In DO Hall, JMO Scurlock, HR Bolhar-Nordenkampe, SP Long, eds, *Photosynthesis and Production in a Changing Environment*. Chapman and Hall, London, pp 207–219
- Fryer MJ, Oxborough K, Martin B, Ort DR, Baker NR** (1995) Factors associated with depression of photosynthetic quantum efficiency in maize at low growth temperature. *Plant Physiol* **108**: 761–767
- Furbank RT, Chitty JA, Jenkins CLD, Taylor WC, Trevanion SJ, von Caemmerer S, Ashton AR** (1997) Genetic manipulation of key photosynthetic enzymes in the C₄ plant *Flaveria bidentis*. *Aust J Plant Physiol* **24**: 477–485
- Genty B, Briantais JM, Baker NR** (1989) The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* **99**: 87–92
- Heaton EA, Dohleman FG, Long SP** (2008) Meeting US biofuel goals with less land: the potential of *Miscanthus*. *Glob Change Biol* **14**: 2000–2014
- Hodkinson TR, Renvoize SA** (2001) Nomenclature of *Miscanthus x giganteus* (Poaceae). *Kew Bull* **56**: 759–760
- Humphries SW, Long SP** (1995) Wimovac: a software package for modeling the dynamics of plant leaf and canopy photosynthesis. *Comput Appl Biosci* **11**: 361–371
- Keselman HJ, Algina J, Kowalchuk RK, Wolfinger RD** (1998) A comparison of two approaches for selecting covariance structures in the analysis of repeated measures. *Comm Statist Simulation Comput* **27**: 591–604
- Kromdijk J, Schepers HE, Albanito F, Fitton N, Carroll F, Jones MB, Finnan J, Lanigan GJ, Griffiths H** (2008) Bundle sheath leakiness and light limitation during C₄ leaf and canopy CO₂ uptake. *Plant Physiol* **148**: 2144–2155
- Leahey ADB, Bernacchi CJ, Dohleman FG, Ort DR, Long SP** (2004) Will photosynthesis of maize (*Zea mays*) in the US Corn Belt increase in future [CO₂] rich atmospheres? An analysis of diurnal courses of CO₂ uptake under free-air concentration enrichment (FACE). *Glob Change Biol* **10**: 951–962
- Leahey ADB, Uribealarea M, Ainsworth EA, Naidu SL, Rogers A, Ort DR, Long SP** (2006) Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO₂ concentration in the absence of drought. *Plant Physiol* **140**: 779–790
- Littell RC, Henry PR, Ammerman CB** (1998) Statistical analysis of repeated measures data using SAS procedures. *J Anim Sci* **76**: 1219–1231
- Littell RC, Pendergast J, Natarajan R** (2000) Modelling covariance structure in the analysis of repeated measures data. *Stat Med* **19**: 1793–1819
- Long SP, Humphries S, Falkowski PG** (1994) Photoinhibition of photosynthesis in nature. *Annu Rev Plant Physiol Plant Mol Biol* **45**: 633–662
- Long SP, Incoll LD, Woolhouse HW** (1975) C₄ photosynthesis in plants from cool temperate regions, with particular reference to *Spartina townsendii*. *Nature* **257**: 622–624
- Monteith JL** (1977) Climate and the efficiency of crop production in Britain. *Phil Trans Roy Soc Lond* **281**: 277–294
- Morgan PB, Bollero GA, Nelson RL, Dohleman FG, Long SP** (2005) Smaller than predicted increase in aboveground net primary production and yield of field-grown soybean under fully open-air [CO₂] elevation. *Glob Change Biol* **11**: 1856–1865
- Murchie EH, Pinto M, Horton P** (2009) Agriculture and the new challenges for photosynthesis research. *New Phytol* **181**: 532–552
- Naidu SL, Long SP** (2004) Potential mechanisms of low-temperature tolerance of C-4 photosynthesis in *Miscanthus x giganteus*: an in vivo analysis. *Planta* **220**: 145–155
- Nobel PS, Forseth IN, Long SP** (1993) Canopy structure and light interception. In DO Hall, JMO Scurlock, HR Bolhar-Nordenkamp, RC Leegood, SP Long, eds, *Photosynthesis and Production in a Changing Environment*. Chapman and Hall, London, pp 79–90
- Papaioannou G, Papanikolaou N, Retalis D** (1993) Relationships of photosynthetically active radiation and shortwave irradiance. *Theor Appl Climatol* **48**: 23–27
- Roberts MJ, Long SP, Tieszen LL, Beadle CL** (1993) Measurement of plant biomass and net primary production of herbaceous vegetation. In DO Hall, JMO Scurlock, HR Bolhar-Nordenkamp, RC Leegood, SP Long, eds, *Photosynthesis and Production in a Changing Environment: A Field and Laboratory Manual*. Chapman and Hall, London, pp 1–21
- Sage RE, Kubien DS** (2007) The temperature response of C-3 and C-4 photosynthesis. *Plant Cell Environ* **30**: 1086–1106
- Searchinger T, Heimlich R, Houghton RA, Dong E, Elobeid A, Fabiosa J, Tokgoz S, Hayes D, Yu T** (2008) Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land use change. *Science* **319**: 1238–40
- Taub DR, Lerdau MT** (2000) Relationship between leaf nitrogen and photosynthetic rate for three NAD-ME and three NADP-ME C₄ grasses. *Am J Bot* **87**: 412–417
- U.S. Department of Agriculture National Agricultural Statistics Service** (2009) 2007 Champaign County Agricultural Statistics. <http://www.nass.usda.gov> (February 3, 2009)
- von Caemmerer S** (2000) *Biochemical Models of Leaf Photosynthesis*. CSIRO Publishing, Collingwood, Australia
- von Caemmerer S, Farquhar GD** (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of *Phaseolus vulgaris* cultivar Hawkesbury-Wonder leaves. *Planta* **153**: 376–87
- Wang D, Portis AR, Moose SP, Long SP** (2008) Cool C₄ photosynthesis: pyruvate P_i dikinase expression and activity corresponds to the exceptional cold tolerance of carbon assimilation in *Miscanthus x giganteus*. *Plant Physiol* **148**: 557–567