

THE MAXIMUM QUANTUM EFFICIENCY OF CO₂ ASSIMILATION DECLINES WITH DEPTH IN
THE CANOPIES OF THE C₄ CROPS *MISCANTHUS X GIGANTEUS* AND *ZEA MAYS*

BY

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THESIS

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ABSTRACT

Leakage of CO₂ from bundle sheath cells of C₄ plants (ϕ) could limit their productivity. ϕ is thought to increase at low light in the C₄ crop *Miscanthus x giganteus* (*M. x giganteus*) (Kromdijk et al., 2008). This should reduce photosynthetic light use efficiency (PLUE) in the lower canopy. *Zea mays* (*Z. mays*), a close relative, may share this problem. Measurements were taken on healthy upper and lower canopy leaves of *M. x giganteus* and *Z. mays*, and isolated aging leaves of *M. x giganteus*. The maximum quantum yield of CO₂ assimilation ($\Phi_{CO_2, max}$) was derived from the initial linear slope of the responses of leaf CO₂ uptake (A) to photon flux (Q) corrected for leaf fractional light absorptance (α) measured in an integrating sphere. In addition, dark-adapted maximum quantum yield of photosystem II (PSII; $\Phi_{PSII, max}$), operating yields of CO₂ assimilation (Φ_{CO_2}), and PSII photochemistry (Φ_{PSII}) were determined. These allowed calculation and analysis of electron transport rate (J) and the proportion of maximal PSII capability used during light exposure (qP). In both species, A is greater in the upper canopy at all light levels and $\Phi_{CO_2, max}$ in the lower canopy leaves is significantly less despite minor variations in $\Phi_{PSII, max}$ and an increase in α in *M. x giganteus*. The inverse of the slope of A^* to J at low light is greater in the lower canopy, showing that more electrons are needed to assimilate CO₂ here, which could result from alternative electron sinks such as increased ϕ . When leaves are artificially maintained in high light, even at 60 days there is no decrease in $\Phi_{CO_2, max}$, showing that this loss is not the result of aging, but attributable to a developmental response to the altered light quality and quantity as they transition to the lower canopy. While this appears to be a maladaptation in both crops, the impact on overall carbon gain at the canopy level is shown to be small.

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TABLE OF CONTENTS

ABBREVIATIONS	1
I. INTRODUCTION.....	3
II. MATERIALS AND METHODS	7
III. RESULTS.....	15
IV. DISCUSSION.....	19
V. CONCLUSION.....	30
TABLES AND FIGURES.....	32
REFERENCES	40

ABBREVIATIONS

α : Leaf fractional light absorptance

φ : Leakage of CO₂ from bundle-sheath cells

Φ_{CO_2} : Operating quantum yield of CO₂ assimilation

$\Phi_{CO_2, \max}$: Maximum quantum yield of CO₂ assimilation

Φ_{PSII} : Operating quantum yield of PSII photochemistry (also F_v'/F_m')

$\Phi_{PSII, \max}$: Maximum dark-adapted quantum yield of PSII photochemistry (also dark-adapted F_v/F_m)

A : Net rate of CO₂ uptake

A^* : Gross rate of CO₂ uptake

A_{sat} : light-saturated rate of CO₂ uptake

ATP: adenosine triphosphate

BS: bundle sheath

CA: Carbonic anhydrase

CEF: cyclic electron flux

EBI: Energy biosciences institute

f : ratio of quanta absorbed by PSII per quanta absorbed by PSI

F_m : maximum PSII fluorescence on saturating pulse of illumination of a dark-adapted leaf (relative units).

F_m' : as for F_m but for leaves operating under illuminated conditions.

F_t : PSII fluorescence prior to saturating pulse of illumination of a light-adapted leaf

F_v : variable PSII fluorescence on saturating pulse of illumination of a dark-adapted leaf (relative units).

F_v' : as for F_v but for leaves operating under illuminated conditions.

J : Electron transport rate

k : Number of electrons required through
LEF per CO₂ assimilated

LAI: leaf area index

LEF: linear electron flux

NADPH: nicotinamide adenine dinucleotide
phosphate

PAR: Photosynthetically active radiation

PEP: phosphoenolpyruvate

PEPc: phosphoenolpyruvate carboxylase

PLUE: photosynthetic light-use efficiency

PPDK: phosphoenol pyruvate dikinase

PSI: Photosystem I

PSII: Photosystem II

Q : incident photosynthetic photon flux
density

Q_{abs} : absorbed photosynthetic photon flux
density

qP : proportion of maximal PSII capability
used during light exposure

R_d : dark respiration

Rubisco: ribulose-1, 5-bisphosphate
carboxylase/oxygenase

RuBP: ribulose-1, 5-bisphosphate

I. INTRODUCTION

The C₄ perennial grass *Miscanthus x giganteus* (*M. x giganteus*) (Greef et Deu. 1993) has been shown to be very productive in the cool climate of eastern England, achieving a harvested yield of 20 t ha⁻¹ (Beale & Long, 1995). In the US corn belt, it was 59% more productive than *Zea mays* (*Z. mays*), achieving a harvested yield of over 30 t ha⁻¹ (Heaton et al., 2008; Dohleman & Long, 2009). Its efficiency of conversion of intercepted light energy into biomass has been shown to be remarkably high, at about two thirds of the theoretical maximum (Beale & Long, 1995; Heaton et al. 2008). This has been attributed to its high photosynthetic efficiency, even under chilling conditions (Beale et al. 1996; Wang et al. 2008, Dohleman et al. 2009).

Nearly all plants equipped with C₄ photosynthesis have leaves characterized by the Kranz, or wreath-like anatomy, with two approximately concentric rings of photosynthetic cells around each vascular bundle. The outer layer, or mesophyll (MES), is in contact with the leaf air space. In these cells carbonic anhydrase (CA) accelerates conversion of dissolved CO₂ (diffusing from the air space) into bicarbonate (HCO₃⁻) (Von Caemmerer and Furbank, 2003; Tazoe et al., 2008; Ubierna et al., 2011; Meinzer and Zhu, 1998). It is then assimilated by carboxylation of phosphoenolpyruvate (PEP) to form a C₄ dicarboxylate, oxaloacetate. This reaction is catalyzed by PEP carboxylase in the mesophyll cytoplasm. C₄ species are categorized into 3 broad groups, and all species of the grass tribe Andropogonae, which includes *Zea* and *Miscanthus* spp. are classified as NADP-Malic enzyme (NADP-ME) type. In these plants oxaloacetate from the MES is reduced to malate, which then diffuses to the inner ring of photosynthetic tissue, the bundle sheath (BS). Rubisco is localized in these cells, isolated from the leaf air space. CO₂ is generated in the BS by decarboxylation of malate to pyruvate by NADP-dependent malic

enzyme in the chloroplast, causing a concentration of CO_2 at Rubisco. The pyruvate diffuses back to the mesophyll where it is phosphorylated back to PEP in the chloroplast by phosphoenolpyruvate dikinase (PPDK) (Von Caemmerer and Furbank, 2003; Tazoe et al., 2008; Ubierna et al., 2011; Meinzer and Zhu, 1998). The rate of PEPc carboxylation, V_p , is assumed to be in equilibrium with the rate of C_4 acid decarboxylation.

A plant's potential productivity depends largely on its photosynthetic efficiency (Zhu et al, 2010): the effectiveness of C_4 photosynthesis depends on the efficiency of the mechanism which concentrates CO_2 into the relatively gas-tight BS cells of leaves. This "pumping" mechanism is the basis of C_4 photosynthesis and is referred to as the C_4 dicarboxylate cycle: it increases $[\text{CO}_2]$ adjacent to Rubisco, considerably boosting photosynthesis by causing a competitive inhibition of RuBP oxygenation, which would otherwise lead to photorespiration. Carbon assimilation inside the BS is referred to as the C_3 cycle or the photosynthetic carbon reduction (PCR) cycle and is homologous to photosynthesis in C_3 plants (von Caemmerer & Furbank, 2003).

The net energetic cost of the C_4 cycle is 2 mol ATP/ mol CO_2 , required for each PEP regeneration in the MES. As a result, if any of the CO_2 molecules pumped into the BS leak back to the MES, extra energy must be used by the plant to recycle the CO_2 back to the BS. This additional energetic cost increases the quantum requirement per mol CO_2 assimilated (i.e. amount of quanta of light needed to fix one mol CO_2), thus decreasing the quantum yield of CO_2 assimilation (ΦCO_2). This will have little or no impact by definition under light saturating conditions. However, under low light conditions a loss of efficiency will lower CO_2 uptake and

be reflected in a decreased maximum quantum yield, defined as the initial slope of the response of A to absorbed Q (i.e. Q_{abs}), $\Phi CO_{2,max}$ (von Caemmerer & Furbank, 2003).

In NADP-ME species, the MES-BS interface is typically a very gas-tight “membrane” due to the presence of a suberin layer in the BS cell wall adjacent to the MES; any leakage of CO_2 is therefore expected to occur via the plasmodesmata used for metabolite transport between these cells. C_4 photosynthesis is thus an energetic compromise between retaining CO_2 , releasing O_2 , and allowing metabolite transport and diffusion between the BS and MES. However, uncertainties relative to the magnitude and variability of φ , the physiological efficiency of light harvesting and the prominence of energy sinks other than carbon assimilation, all of which affect ΦCO_2 , prohibit the use of ΦCO_2 as a means of obtaining numerical estimates of φ (Meinzer and Zhu, 1998; Von Caemmerer and Furbank, 2003; Tazoe et al., 2008; Von Caemmerer and Furbank, 1999; Tazoe et al., 2008; Ubierna et al., 2011; Hatch et al., 1995; Farquhar, 1983; Farquhar et al., 1989). This method is still valid, however, to determine an increasing or decreasing trend. Specifically, leakiness (φ) is defined by Farquhar, 1983 as the ratio of the CO_2 leak rate out of the BS/ V_p .

Kromdijk et al. (2007) used leaf and canopy-level field measurements of gas exchange and isotopic carbon discrimination to estimate φ in *M. x giganteus* growing in Ireland, reporting that it increased significantly under low light, causing considerable loss of productivity. If φ increases with depth and decreasing light in the canopy, requiring progressively more energy to fix one CO_2 , then this should result in a decreased $\Phi CO_{2,max}$. We tested this prediction by using combined leaf gas-exchange and fluorescence measurements, coupled with fractional light

absorptance measurement with an integrating sphere to determine exact quantum yields in field grown *M. x giganteus* crops. Because *M. x giganteus* is closely related to *Z. mays*, similar measurements were also taken on field-grown *Z. mays* crops to determine if a similar decline is observed in this important crop.

II. MATERIALS AND METHODS

Site description

Plants were sampled from mature replicated stands of *M. x giganteus* and *Z. mays* on the University of Illinois Agricultural Research Station near Champaign, IL, USA (40°02'N, 88°14'W, 228m above sea level), as described previously (Dohleman and Long, 2009), in the summer and autumn of 2011 and 2012. Soils at this site are deep Drummer/Flanagan series (a fine silty, mixed, mesic Typic Endoaquoll) with high organic matter typical of the central Illinois Corn Belt. As in previous studies the “Illinois” clone of *M. x giganteus* was used, and was unfertilized (Dohleman and Long, 2009). The crop was planted in 2005, and the above-ground shoots harvested each winter. *Z. mays* cv. Dekalb DK61-69 was planted in April 2011 and in June 2012 in rotation with soybean (*Glycine max*): it was rainfed and received standard fertilization of 180 kg[N] ha⁻¹, just prior to planting in line with regional production practice. For photosynthetic analysis, stems of each species were cut at the base before dawn, the cut ends immersed in water and immediately recut to avoid any air blockage in the xylem. Since the objective was to determine the potential of leaves at different heights in the canopy, this procedure avoided impacts of transient phenomena through the day, such as photoinhibition or low water potentials

To isolate the effect of age on lower canopy *M. x giganteus* leaves, eight plants were grown in 23 L pots in a greenhouse and regularly watered and fertilized until they were 5 months old. Leaves were tagged on emergence of the ligule and as other leaves formed above, they were artificially held to the side to avoid any shading of the tagged leaves.

Dark-adapted measurements

On each plant, the lowest green leaf and the highest fully developed leaf, as indicated by ligule emergence, were selected for measurement. Leaf CO₂ and water vapor exchange were measured in cuvettes with controlled temperature, humidity and photon flux within a portable open path gas-exchange system incorporating infra-red CO₂ and water vapor analyzers (LI-COR 6400; LI-COR, Inc., Lincoln, NE USA). Chlorophyll pulse amplitude modulated fluorescence was measured simultaneously with a fluorometer incorporated into the cuvette lid (LI-6400-40; LI-COR, Inc.). 30 seconds after clamping onto the leaf, measurements were made of F_o and F_m by exposing them to one flash of saturating light (Baker, 2008; Maxwell and Johnson, 2000). This allowed determination of the maximum quantum yield of PSII photochemistry:

$$\Phi_{PSII, max} = F_v/F_m \quad (1)$$

where $F_v = (F_m - F_o)$.

Light-adapted measurements

Once dark-adapted measurements were completed, the leaf cuvette was set to expose the leaves to 30 °C, 400 $\mu\text{mol mol}^{-1}$ CO₂, 50-70% relative humidity, and 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light.

Irradiance was provided by integrated red (635 nm wavelength) and blue (465 nm wavelength) light-emitting diodes (LED) such that 10% of the light was blue, and the remainder red. Leaves were allowed to acclimate (60-90 minutes) until the net rate of leaf CO₂ uptake (A) reached a steady state and A , F_o' and F_m' were recorded to determine the operating quantum yield of CO₂ assimilation (Φ_{CO_2}), the operating quantum yield of PSII photochemistry (Φ_{PSII}), the proportion of maximal PSII capability used during light exposure (qP) and electron transport

rate through whole-chain linear electron flux (J) (Long & Bernacchi, 2003; Baker, 2008; Maxwell and Johnson, 2000):

$$\Phi CO_2 = A^*/(Q_{abs}) \quad (2)$$

Where $A^* = A - R_d$, representing gross CO_2 uptake (i.e. net CO_2 uptake, A , corrected for dark respiration, R_d); and $Q_{abs} = Q * \alpha$ representing absorbed photosynthetic photon flux density (i.e. incident photosynthetic photon flux density or light flux, Q , corrected for leaf fractional light absorptance of photosynthetically active light, α).

$$\Phi PSII = F_v'/F_t \quad (3)$$

Where $F_v' = (F_m' - F_t)$, F_m' is maximal leaf fluorescence during the saturating pulse and F_t the light-adapted fluorescence prior to the pulse.

$$qP = \frac{\Phi PSII}{\Phi PSII, max} \quad (4)$$

$$J = \Phi PSII * Q_{abs} * f \quad (5)$$

Where f is the proportion of excitons partitioned to PSII rather than PSI (dimensionless; 0 to 1). f was assumed to be 0.4, accounting for increased cyclic electron flow (CEF) around PSI to provide the additional ATP required for C_4 photosynthesis (Von Caemmerer and Furbank., 1999).

These measurements were made at 14 progressively lower photon fluxes, 6 of these between $Q=40$ and $140 \mu mol m^{-2} s^{-1}$, comprising the linear portion of the response of A to Q and necessary for a precise estimate of apparent quantum yield. Leaves were allowed to

acclimate to each step reduction in Q , as assessed by a resumption of a steady-state A , typically requiring 5-10 minutes. After the light curves were completed, leaf reflectance and transmittance was measured using an integrating Taylor sphere and attached illuminator and measuring optics (Li- 1800-12; LI-COR, Lincoln, Neb., USA). The signal was processed through a fiber optic spectrometer (USB2000; Ocean optics, Dunedin, Fl., USA) and analyzed with spectrometer operating software (Spectrasuite; Ocean optics, Dunedin, Fl., USA). Reflectance and transmittance were measured for each leaf by first taking a reading of a white reference (barium sulfate; LI-COR, Lincoln, Neb., USA) and a dark reading, used to calibrate the sample reading. Reflectance at a given wavelength λ was calculated as

$$reflectance_{\lambda} = \frac{S_{\lambda} - D_{\lambda}}{R_{\lambda} - D_{\lambda}} * 100\% \quad (6)$$

Transmittance at a given wavelength λ was calculated as

$$transmittance_{\lambda} = \frac{S_{\lambda} - D_{\lambda}}{R_{\lambda} - D_{\lambda}} * 100\% \quad (7)$$

Where S_{λ} is the sample reading, D_{λ} the dark reading, and R_{λ} the white reference reading.

Leaf fractional light absorptance, weighted for red and blue light, was calculated as:

$$\alpha = 1 - 0.1(reflectance_{465} + transmittance_{465}) - 0.9(reflectance_{635} + transmittance_{635}) \quad (8)$$

Where $reflectance_{465}$ and $reflectance_{635}$ are respectively the leaf reflectance of blue (i.e. 465 nm wavelength) and red (i.e. 635 nm wavelength) light, and $transmittance_{465}$ and $transmittance_{635}$ are respectively the leaf transmittance of blue (i.e. 465 nm wavelength) and

red (i.e. 635 nm wavelength) light. Weighting absorptance for red and blue light was necessary as only 10% of the provided light was blue in the cuvette measurements of A .

Photosynthetic efficiency

Gas-exchange data were calculated as in von Caemmerer and Farquhar (1981). $\Phi CO_{2, \max}$ was obtained from linear regression of A against Q for six light levels (40-140 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (proc reg, SAS v8.02; SAS Institute, Cary, NC, USA) corrected for leaf fractional light absorptance (Long et al., 1996). Lower light points were not used in case they were affected by increased respiration, i.e. the Kok effect. Higher light levels were not used as dA/dQ began to decline in these leaves above $Q=140 \mu\text{mol m}^{-2} \text{s}^{-1}$:

$$\Phi CO_{2, \max} = \frac{dA}{dQ_{abs}} \quad (9)$$

k describes the number of electron equivalents produced by LEF required to reduce one molecule of CO_2 (Baker, 2008; Maxwell and Johnson, 2000). This term was obtained from the inverse of a linear regression across the same low light points previously described as the response of A^* to J .

$$k = \frac{1}{dA^*/dJ} \quad (10)$$

To derive the parameters determining the non-rectangular hyperbola that describes the response of A to Q the following equation was fit. The parameters derived are the light saturated rate of photosynthetic CO_2 uptake (A_{sat}), the maximum apparent quantum yield or initial slope of the response ($\Phi CO_{2, \max}'$) and the convexity coefficient that describes the length of the transition from light-limited to light-saturated photosynthesis with respect to Q (θ).

$\Phi CO_{2, \max}'$ describes the same parameter as $\frac{dA}{dQ}$ and if corrected for α would describe the same parameter as $\Phi CO_{2, \max}$ (equation 9), but was considered a less accurate estimate of the initial slope of the response, because it is a compromise including estimates of A_{sat} and θ . The curve was fitted by iterative least-squares non-linear regression (proc NLIN, SAS v8.02; SAS Institute, Cary, NC, USA):

$$A = (Q * \Phi CO_{2, \max}' + A_{sat} - \sqrt{(\Phi CO_{2, \max}' * Q + A_{sat})^2 - 4 * \Phi * A_{sat} * \theta * Q}) / (2 * \theta) + R_d \quad (11)$$

Effect of leaf age on performance

To distinguish the effect of leaf age from leaf light history on lower canopy photosynthetic efficiency in *M. x giganteus*, measurements were taken on a single leaf of greenhouse-grown plants every 30 days during three months. On the first measurement day, the highest fully developed leaf was chosen, measured and marked, so that measurements could be taken from it 30 and 60 days later. All of the plant's tillers except for the one bearing this leaf were wrapped together and separated from the measured leaf, ensuring that it was not overshadowed over the course of the experiment. Therefore, the leaf was not exposed to the environmental conditions of the lower canopy (lower light levels, changing light quality, changing atmospheric $[O_2]$ and $[CO_2]$, changing temperature, etc.), and it was assumed that its only changing parameter over the course of the experiment was its increasing age. Measurements of A and α were taken over light levels ranging from 0-500 Q and used to calculate $\Phi CO_{2, \max}$ as previously described.

Light attenuation

The proportion of PAR intercepted by the canopy was obtained from Arundale and Wang: measurements were taken every month from June to September in these crop canopies by measuring Q above the mature crop canopy using an external sensor (Model LI-190, LI-COR Biosciences) connected to a line quantum sensor (Ceptometer, Model PAR-80, Decagon Devices, Inc.) which was used to measure the amount of PAR every 10 cm from the top to base of the crop canopy, between 10:00 and 14:00 on mostly sunny days when $Q \geq 1400 \mu\text{mol m}^{-2} \text{s}^{-1}$. Light attenuation was calculated by determining the decrease in Q at different depths in the canopy relative to Q measured simultaneously above the canopy.

Statistical analysis

Data from the field-grown plants (comparing upper and lower canopy leaves in *Z. mays* and *M. x giganteus*) were analyzed using Proc mixed (SAS Institute Inc., Cary, NC, USA). A randomized complete block mixed model ANOVA was first performed to analyze the fixed effect of canopy position and species as well as their fixed interaction, while blocking by the random main effect of year. The differences of least squares means were used to further analyze the interaction effect. Unstructured covariance was chosen to bypass the need for homogeneity of variance between canopy positions and species (i.e. variances were not assumed equal between canopy positions and species, at the cost of experimental error df). Proc univariate (SAS Institute Inc., Cary, NC, USA) was used to verify normality of data, with a 1% threshold probability of committing a type 1 error (chosen because of the large sample size, and the test's robustness to non-normal data). Over 50 plants were sampled in total.

Data from the greenhouse-grown plants, to test the photosynthetic efficiency of aging *M. x giganteus* leaves, were analyzed using Proc mixed (SAS Institute Inc., Cary, NC, USA). A repeated measures fixed model ANOVA was performed while blocking by day of measurement with Post Hoc Tukey HSD contrast statements to analyze the linear fixed effect of time on leaf photosynthetic performance. Proc univariate (SAS Institute Inc., Cary, NC, USA) was used to verify normality of data, with a 5% threshold probability of committing a type 1 error.

III. RESULTS

Light is primarily intercepted by the uppermost leaves of both species, such that at the depth corresponding to lower canopy leaf insertion (1.3m and 2m for *M. x giganteus* and *Z. mays*, respectively, as described in materials and methods) light levels are 5% of above-canopy levels in a *M. x giganteus* field and 10% of above-canopy levels in a *Z. mays* field. This is seen in Fig. 1, which shows light attenuation through the canopy of both species.

Measures of leaf fractional light absorptance (α) show a significant main effect of canopy position and a significant canopy position*species interaction, indicating that α is greater in the lower canopy of *M. x giganteus*, and marginally, but not significantly, greater in the lower canopy of *Z. mays* (Tables 1 and 2). In *M. x giganteus*, α was 0.85 ± 0.004 and 0.87 ± 0.0044 in the upper and lower canopy, respectively. In *Z. mays*, it was 0.91 ± 0.0049 and 0.9 ± 0.0051 in the upper and lower canopy, respectively (Table 2; Fig. 2a). The values are very similar and within 3% of each other suggesting this variation can only marginally affect photosynthetic performance.

PLUE is higher in the upper canopy under light-limiting conditions, as shown by a significantly higher initial slope of the A vs. Q response corrected for α , or $\Phi CO_{2, \max}$, of upper canopy leaves of both *Z. mays* and *M. x giganteus* (Table 1, Fig. 2b, 3b and 4). In *M. x giganteus*, $\Phi CO_{2, \max}$ was 0.056 ± 0.0028 and 0.041 ± 0.003 in the upper and lower canopy, respectively. In *Z. mays*, it was 0.055 ± 0.0022 and 0.045 ± 0.0023 in the upper and lower canopy, respectively (Fig. 2b, Table 2). These represented significant declines of 27% in *M. x giganteus* and 18% in *Z. mays*.

The response of photosynthesis to light is effectively linear from 40-140 Q. Residuals were obtained from the linear regression of low-light points (40-140 Q) previously used to obtain the initial linear slope of the A vs. Q curves measured on field-grown *Z. mays* and *M. x giganteus* plants (Fig. 3b). These residuals appear to be randomly distributed across the range of utilized light fluxes, showing no variation in deviation from linearity from 40-140 Q (Fig. 5).

Φ_{PSII} appears greater in the upper canopy of both species at all light levels (Fig. 3e and 3f). However, while there is no main effect of canopy position on dark-adapted Fv/Fm (i.e. $\Phi_{PSII, \max}$), the significant interaction of canopy position and species indicates that values are significantly greater in the upper canopy for *Z. mays*, but conversely lesser in the upper canopy for *M. x giganteus* (Tables 1 and 2, Fig. 2c). In *M. x giganteus*, $\Phi_{PSII, \max}$ was 0.77 ± 0.0033 and 0.79 ± 0.0035 in the upper and lower canopy, respectively. In *Z. mays*, it was 0.8 ± 0.0041 and 0.79 ± 0.0035 in the upper and lower canopy, respectively (Table 2). Although these differences are significant, the values are very similar and within 2% of each other suggesting this variation can have very little significance to photosynthetic performance. Overall, the proportion of maximal PSII capability used during light exposure (qP) appears to be greater in the upper canopy of both species (Fig. 3g and 3h).

The upper canopy of both *Z. mays* and *M. x giganteus* has a higher photosynthetic capacity at higher light levels ($Q = 500-2000$), as shown in the responses of A to Q (Fig. 3a). This difference is confirmed by the significant main effect of canopy position on A_{sat} (Table 1), with values larger in the upper canopy (Table 2). In *M. x giganteus*, A_{sat} was 32.8 ± 1.6 and 21.2 ± 1.7 in the upper and lower canopy, respectively. In *Z. mays*, it was 44.6 ± 1.5 and 29.4 ± 1.5 in the

upper and lower canopy, respectively. In both cases the decline was 34% and significant. It follows that the operating quantum yield of CO₂ assimilation (Φ_{CO_2}) at these light levels will be greater for upper canopy leaves (Fig. 3c).

Calculation of k (number of electron equivalents produced by LEF required to reduce one molecule of CO₂) indicates lower efficiency in partitioning captured light energy towards CO₂ assimilation in the lower canopy of both species (Fig. 2d). Fig. 6 shows the response of A^* to J . The inverse of the slope of this curve, calculated over the points corresponding to low light measurements, yields k . The significant main effect of canopy position indicates that k is greater in the lower canopy of both species (Tables 5 and 6, Fig. 2d). In *M. x giganteus*, k was significantly different between canopy positions ($p < .1$), at 3.94 ± 0.1 and 4.2 ± 0.1 in the upper and lower canopy, respectively. In *Z. mays*, there was no significant difference between canopy positions, at 4.1 ± 0.1 and 4.2 ± 0.1 in the upper and lower canopy, respectively (Table 1). These represented a significant increase of 7% in *M. x giganteus* and a non-significant increase of 2% in *Z. mays*.

Combining the light interception data shown in Fig. 1 with the previously shown light response curves (Fig. 3a) reveals how much carbon assimilation could occur at the leaf level of both canopy positions if capacity and efficiency were not lost in lower canopy leaves. Results are calculated from average light levels in July, 2011 ($Q = 1706 \pm 48$). At the leaf level, lower canopy photosynthesis is 11% and 20% of upper canopy photosynthesis in *M. x giganteus* and *Z. mays*, respectively. Giving the lower canopy the characteristics of upper canopy leaves would increase lower leaf carbon assimilation by 9% and 12% in *M. x giganteus* and *Z. mays*,

respectively. This would bring it up to 12% and 23% of upper canopy photosynthesis in *M. x giganteus* and *Z. mays*, respectively (Fig. 7).

The evolution of $\Phi CO_{2, \max}$ in *M. x giganteus* leaves appears unaffected by age. Over 60 days, *M. x giganteus* leaves artificially maintained in the high light environment of the upper canopy such that their only changing parameter over the course of the experiment is their increasing age, show no significant effect of time on $\Phi CO_{2, \max}$ (F-value=0.00; $p>.99$) (Fig. 8).

IV. DISCUSSION

The present study was motivated by the work of Kromdijk et al. (2007) that suggested from isotopic carbon discrimination that bundle sheath leakage of CO₂ (ϕ) in *M. x giganteus* increased in the lower canopy or shaded leaves. It follows that if ϕ increases then the maximum quantum yield of CO₂ assimilation ($\Phi_{CO_2, max}$) should decline. This prediction was tested here and it was found that $\Phi_{CO_2, max}$, measured on an absorbed light basis, declined significantly in lower canopy leaves not only in a field crop of *M. x giganteus* but also of *Z. mays*. However, the present study found no evidence that low light was the direct cause of the decrease in $\Phi_{CO_2, max}$. There was no deviation from linearity in the response of leaf CO₂ uptake (A) to photon flux (Q) at low light. This result suggested that the loss of $\Phi_{CO_2, max}$ in these C₄ crops was developmental, either due to age or acclimation to the low light environment of the lower canopy. This was further tested by artificially preventing shading as leaves aged, and here even over 60 days there was no decline in $\Phi_{CO_2, max}$ confirming that the observed loss of $\Phi_{CO_2, max}$ was a response to the shaded environment of the lower canopy.

Because of the lower light levels, it is not surprising that leaves in the lower canopy have a lower capacity for photosynthesis at light saturation. However, the demonstrated loss of photosynthetic light-use efficiency (PLUE) at low light in the lower canopy defies the pattern established for C₃ species where A_{sat} declines in shade leaves but $\Phi_{CO_2, max}$ on an absorbed light basis is maintained and increased on an apparent (i.e. on incident light) basis due to increased chlorophyll and light absorption (α) (Table 1; Fig. 2b, 3a, and 4) (Boardman, 1977; Seeman, 1989; Burkey and Wells, 1991). While the leaves of *M. x giganteus* show a 2.4% increase in fractional light absorptance in the lower canopy this is more than offset by the 27% decrease in

$\Phi CO_{2, max}$. *Z. mays* also shows a significant although smaller decline in $\Phi CO_{2, max}$ of 18% (Table 2, Fig. 2a and 2b). This smaller decline might reflect the fact that light in the lower canopy of *Z. mays* was about double that of the lower canopy of *M. x giganteus* (Fig. 1).

That both C_4 species show this unexpected decline, suggests that this may be a feature of C_4 plants, or at least within the tribe *Andropogonae*. This tribe is considered monophyletic and includes the major C_4 crops – maize, sorghum and sugarcane. Since maize and *Miscanthus* are relatively distant taxonomically within the tribe, and with sugarcane and sorghum close to *Miscanthus*, it suggests that a loss of $\Phi CO_{2, max}$ as leaves become shaded may be a common response of the crops in this tribe (Matthews et al. 2002).

While a reduction in leaf Rubisco content is likely the cause for decreased A_{sat} (light-saturated rate of CO_2 uptake) in lower canopy leaves, this factor should not depress photosynthesis in light-limited conditions (Fig. 3a and 3b). Reduced $\Phi CO_{2, max}$ in lower canopy leaves could thus be caused by reduced PSII capability, imbalances in C_3 and C_4 cycling, and alternative energy sinks such as ϕ (leakage of CO_2 from bundle-sheath cells), photorespiration, or nitrogen assimilation (Baker, 2008; Maxwell and Johnson, 2000, Oberhuber et al. 1993) (Fig. 2b, 2c and 2d). In particular, excess C_3 cycling relative to C_4 cycling could favor photorespiration, if bundle sheath cells are not able to maintain high enough $[CO_2]$ to cancel photorespiration in Rubisco. Conversely, excess C_4 cycling relative to C_3 cycling could favor ϕ , if Rubisco is not able to assimilate CO_2 as fast as it is pumped into the bundle sheath, causing some of the CO_2 to leak back to the mesophyll (Kromdijk et al., 2008).

Both Φ_{CO_2} (operating quantum yield of CO_2 assimilation) and Φ_{PSII} (operating quantum yield of PSII photochemistry) are lower at nearly all light levels in the lower canopy of both species (Fig. 3c, 3d, 3e, and 3f). While this could suggest that PSII inefficiency is responsible for reduced overall photosynthetic efficiency, $\Phi_{PSII, \max}$ (maximum dark-adapted quantum yield of PSII photochemistry) is greater in the *M. x giganteus* lower canopy than in the upper canopy, and only slightly inferior in the *Z. mays* lower canopy than in the upper canopy (Fig. 2c; Table 1). Under light limiting conditions, a decline in $\Phi_{CO_2, \max}$ would result if $\Phi_{PSII, \max}$ was decreased, since it would indicate a lower efficiency of PSII, and therefore rate of whole chain electron transport. However, the differences in $\Phi_{PSII, \max}$ are very small (<2% changes between canopy levels), indicating that PSII electron transport capacity is not responsible for the decline in $\Phi_{CO_2, \max}$ (Table 2). This is particularly evident in *M. x giganteus*, where there is an increase in $\Phi_{PSII, \max}$ in the lower canopy leaves. As a result, qP (proportion of maximal PSII capability used) is greater in the upper canopy of both species (Fig. 3g and 3h).

Results of k (number of electrons required through LEF per CO_2 assimilated) are based on several assumptions (Oberhuber et al. 1993): mitochondrial respiration must be constant across light fluxes (at least under low light) and equal to R_d (dark respiration), and f (ratio of quanta absorbed by PSII per quanta absorbed by PSI) must be constant across light fluxes (at least under low light) and identical in the upper and lower canopy. At a given canopy level, k should decrease with f (equations 5 and 10): if f is different between canopy levels, then the difference in k between canopy levels will be affected. The canopy level with a lower f will consequently see its k reduced.

This is a critical assumption, which may not be valid: ϕ (Leakage of CO₂ from bundle-sheath cells) increases ATP requirements but not NADPH requirements. If ϕ does increase in the lower canopy, as is hypothesized, the ATP/NADPH ratio of the energetic cost of CO₂ assimilation in these leaves should also increase. LEF is a form of electron transport employing PSII and PSI to produce NADPH and ATP; CEF is a form of electron transport employing PSI that produces no NADPH, but produces more ATP per quanta of absorbed light than LEF (Von Caemmerer and Furbank, 1999). Increasing CEF relative to LEF by diverting more light towards PSI could balance ATP and NADPH production to match demand, and would decrease f (because more light is partitioned towards PSI than PSII) (Edwards and Baker, 1993; Furbank et al., 1990). Assuming that f is equivalent between canopy levels could therefore overestimate k in the lower canopy relative to the upper canopy.

In this scenario, LEF (NADPH and ATP production) would be optimized, and the excess cost caused by ϕ would be covered only by CEF (ATP production) (Furbank et al., 1990). This would cause the efficiency of CEF (but not of LEF) to decrease. Importantly, this would make k an inadequate measure of ϕ , since it only estimates the efficiency of LEF. ϕ would increase the number of electrons required through CEF per CO₂ assimilated, but not affect the efficiency of LEF. Plants could in theory control energy partitioning between LEF and CEF through dynamic control of f , in particular through state transitions, which displace light receiving antenna complexes between PSI and PSII (Taiz and Zeiger, 2002; Von Caemmerer and Furbank, 1999). f is assumed equal between canopy levels despite these reservations, because actual levels of this partitioning factor cannot be estimated.

Variation in mitochondrial respiration and f with light flux could cause the response of J (electron transport rate) to A^* (Gross rate of CO_2 uptake) to vary, affecting the calculation of k (Fig. 6; equations 5 and 10) (Oberhuber et al. 1993). However, the assumption that there is no such variation should hold equivalently for both canopy levels, such that comparing the upper and lower canopy is still possible.

k is lower in the upper canopy of both species (Fig. 2d and 6; Table 1). Values in general are quite low, and close to the theoretical minimum of 4: this may point to CEF being used to compensate for alternative energy sinks, allowing LEF to be optimized, as previously discussed (Furbank et al., 1990). Despite these further reservations, assuming that variations in k are not due to variations in mitochondrial respiration and f , this result suggests that chemical energy is less efficiently partitioned towards CO_2 assimilation in the lower canopy. This points to a prevalence of alternative energy sinks in these leaves (Oberhuber et al. 1993). While this could be representative of any energy sink other than carbon assimilation (e.g. nitrogen assimilation), Kromdijk et al. (2008) identified ϕ as an important cause for lost PLUE in light-limited conditions in *M. x giganteus*. The findings in this study therefore agree with ours, and point towards increased ϕ in the lower canopy of *M. x giganteus*, also occurring in related C_4 crops such as *Z. mays*. Increased α in the lower canopy of *M. x giganteus* should marginally help compensate for its reduced PLUE, however (Fig. 2a; Table 1).

There is an identified loss of PLUE in the lower canopy of both *M. x giganteus* and *Z. mays*, likely caused by increased ϕ in the lower, more light-limited leaves (Fig. 2b and 2d). However, this limitation of the lowest leaves does not appear to have much effect on whole

plant productivity: because of the effectiveness of canopy closure in both species (particularly *M. x giganteus*), lower canopy leaves receive very little light (Fig. 1). As a result, even if they had the PLUE and $\Phi CO_{2, max}$ of upper-canopy leaves, the added contribution to overall photosynthesis would in fact be negligible (Fig. 7). This estimation ignores differences in leaf area index (LAI) between the upper and lower canopy, however: lost productivity from lower canopy leaves should be more substantial if the lower canopy has a greater total surface of photosynthetic leaves than the upper canopy.

Light interception in the uppermost leaves is more prominent in *M. x giganteus* than in *Z. mays*, likely due to the horizontal orientation of leaves in the former compared to the more erect nature of the leaves in the latter. Because the majority of light is intercepted in the top meter of a mature *M. x giganteus* canopy, it is the evolution of the decline in $\Phi CO_{2, max}$ in this canopy layer which is likely to determine the bulk of whole-plant productivity, rather than the very lowest leaves (Fig. 1). Among these, only the uppermost are not exposed to shade; therefore the majority of these leaves could potentially be affected by the loss of PLUE observed in the lowest leaves. Dohleman and Long (2009) identify photosynthesis under non-light saturated conditions as responsible for a significant portion of CO_2 assimilation, not only in shaded leaves (i.e. middle or lower canopy), but also in all leaves at dusk and dawn and on overcast days. It is therefore important to determine to what extent the factors causing a loss of $\Phi CO_{2, max}$ in the lower canopy leaves could affect leaves at intermediate depths (.5-1 m), but also whether this effect is linked to low-light conditions in the short-term (e.g. upper canopy leaves transiently exposed to low light at dusk) and/or in the long term (e.g. upper canopy leaves permanently exposed to lower light as they are overshadowed by newer leaves).

Loss of $\Phi CO_{2, max}$ in leaves transitioning from the upper to intermediate and lower canopy depths could be caused by leaf age or by their environment (light exposition, light quality, atmospheric $[CO_2]$ and $[O_2]$, etc.). If age is the cause for lost efficiency, the relatively young leaves in the top meter of the canopy should be unaffected. However, a leaf's age does not appear to affect its photosynthetic efficiency (Fig. 8), indicating that the leaf environment is the cause for the loss of PLUE in the lower canopy of field-grown plants. Either the lower photon flux or altered spectral composition of light in the lower canopy is therefore the likely driver of this change (Fig. 1). These findings agree with results of Long et al. (1993) Bjorkman and Demmig (1987) in C_3 and C_4 plants, who confirm that leaf age does not affect $\Phi CO_{2, max}$. Ehleringer and Pearcy (1983), however, found no effect of light conditions during growth on $\Phi CO_{2, max}$ of C_3 or C_4 plants: it is thus likely that the reduced $\Phi CO_{2, max}$ in the lower canopy of *Z. mays* and *M. x giganteus* is not due to the absolute photon flux received by these leaves.

Light quality is another factor that changes within the canopy, with ratios of far-red to red light increasing with canopy depth (Boardman, 1977). PSI typically absorbs far-red light more easily than PSII (Baker, 2008; Maxwell and Johnson, 2000), such that in-field light quality of the lower canopy could favor CEF relative to LEF. As previously discussed, this could play a role in balancing ATP/NADPH requirements and improving overall PLUE if φ is more prevalent in the lower canopy. Measurements were taken under identical light conditions, such that the inherent effect of light quality between canopy levels should not affect our results. However, light quality changes do trigger developmental changes within leaves, which could play an important part in the decrease of PLUE with canopy depth (Smith, 1982).

Kromdijk et al. (2008) suggest that φ increases during short term light flux decline (e.g. several minutes or hours). If so, photosynthesis should be increasingly depressed as light intensity decreases, causing the response of A to Q to always be curved, even at low light. However, this response is linear from 40-140 Q (Fig. 5). This indicates that photosynthesis is linearly correlated to light flux from 40-140 Q , such that there is no progressive increase of φ as light flux decreases in the short term. We therefore conclude that short-term light flux changes, such as dawn and dusk, or overcast days, do not cause a loss of productivity through increased φ .

While there is no apparent response of φ to short term light flux changes (e.g. several minutes or hours), it is possible that φ could increase in a manner that equivalently affects photosynthesis from 40-140 Q as leaves are progressively and permanently exposed to lower overall light levels (Fig. 5). Leaves develop at the top of the canopy, where they are exposed and adapted to high light levels (Suarez, 2010). They are then progressively overshadowed as younger leaves are produced above them, but may not be able to acclimate to optimize low light use. Since there is no loss of PLUE due to age, it appears that the leaves adapt to these reduced light levels in a way that decreases their PLUE at all light fluxes (Fig. 3a and 3b). φ therefore appears as a factor that increases due to a leaf's long term progressively diminished light environment, but remains constant over the various short term light flux changes the leaf is then exposed to.

The relation between leaf nutrient content and photosynthetic rate makes vertical nutrient distribution throughout the canopy a key factor determining whole-plant productivity.

Plants that develop canopies typically export nutrients from older leaves to younger leaves so they can better utilize higher light intensities; this can be seen with the higher A_{sat} of the upper canopy (Table 2, Fig. 3a) (Suarez, 2010). While lower canopy leaves should not benefit from high saturating photosynthetic potential due to their inherently low light environment, they could maintain significant productivity if able to photosynthesize well at low light. In a more optimal scenario, upper canopy leaves would have greater high-light photosynthesis and inferior low-light photosynthesis, whereas lower canopy leaves would have inferior high-light photosynthesis and greater high-light fluxes. Assuming that leaves can independently modify high light and low light photosynthesis, this would better balance nutrient distribution throughout the canopy and maximize production; however it appears that lower canopy leaves are less effective at nearly all measured light fluxes (Fig. 3a and 3b).

It is therefore possible that leaves cannot balance nutrients in a way that affects high light and low light photosynthesis independently of one another, and ϕ is increased by imbalances caused by remobilization of proteins to supply N to the developing upper canopy leaves. However, the *Z. mays* crop was well fertilized and of a modern cultivar bred to respond to high rates of N fertilization. We therefore propose that low-light PLUE is lost when leaves that develop and acclimate to any given light level are progressively and permanently exposed to a lower light level as they are overshadowed by younger leaves. This may be a maladaptation of the NADP-ME C_4 photosynthesis of this group of plants. However if this effect is intrinsic to these plants, the small loss of productivity that results would be unlikely to result in selection against this trait given the large advantage of a higher A_{sat} conferred by C_4 photosynthesis in the upper canopy leaves (Table 2; Fig. 3a). The tradeoff between A_{sat} in the

upper canopy and $\Phi CO_{2, max}$ in the lower canopy should therefore have a net positive effect on whole plant productivity.

While the loss of PLUE of the lowest leaves due to decreased light flux has little impact on whole-plant productivity (Fig. 7), it could be a more important factor in intermediate leaves (e.g. .5-1 m canopy depth), where relatively young leaves are exposed to intermediate light levels. These leaves receive enough sunlight to significantly photosynthesize, such that any loss of their PLUE could affect whole-plant productivity. Because of the effectiveness of canopy closure, they are rapidly exposed to intermediate light levels (e.g. 50-100% of above-canopy levels, as seen in Fig. 1). This decreased light environment has been shown to decrease their PLUE: therefore, we conclude that the long term decreasing light environment of leaves as they are progressively overshadowed by newer leaves reduces their PLUE in a way that likely significantly decreases whole-plant productivity. This effect, seemingly caused by increased φ as leaves adapt to their changing environment, should only be of importance in intermediary canopy depths, where leaves receive enough light to be significantly productive, but little enough light that they have lost PLUE due to φ .

Zhu et al. (2010) and Ort et al., (2011) suggest that vertical light distribution is suboptimal in closed-canopy crops because too much light is absorbed by upper-canopy leaves. They propose that decreasing the chlorophyll content (and consequently α) of upper canopy leaves could allow more light to filter to the lower leaves: this would marginally decrease upper canopy photosynthesis (shifting it from saturating to nearly saturating levels, which are virtually equivalent), but significantly boost middle and lower canopy photosynthesis (as these leaves

are more light-limited, and thus benefit more from additional light), such that whole-plant photosynthesis would increase. This technique could affect C_4 crops such as *Z. mays* or *M. x giganteus* differently than C_3 : because ϕ is not an issue for C_3 plants; their lower canopy leaves should not have decreased low light PLUE, allowing them to benefit more from added light than C_4 lower canopy leaves. However, increasing light levels for shaded leaves would not only bring their photosynthesis closer to saturating rates, it would also decrease ϕ , thus improving their low-light PLUE, providing an additional boost to photosynthesis. While it seems likely that decreasing upper canopy chlorophyll content in closed canopy C_4 crops would increase their productivity, it is unclear whether they will benefit more or less from this technique than their C_3 counterparts. Interestingly, this mechanism seems to be inherent in *M. x giganteus*, whose lower canopy leaves have marginally greater α (leaf fractional light absorptance) than upper canopy leaves (Table 2; Fig. 2a).

V. CONCLUSION

Lower canopy leaves of both species suffer from a loss of $\Phi CO_{2, \max}$, i.e. efficiency of light use under limiting light. This is not due to a loss of maximum photochemical efficiency, but is consistent with increased bundle sheath leakage of CO_2 (φ). This loss of $\Phi CO_{2, \max}$ appears to result as a developmental response to the altered light quantity and quality of the lower canopy. This could be caused by nutrient transfer from the older to the newer leaves. Loss of PLUE should therefore not occur during transient low-light conditions, such as dawn, dusk, or overcast days; nor is it caused by increasing leaf age. Indeed the apparently perfectly linear decline in A with absorbed Q ($<140 \mu\text{mol m}^{-2} \text{s}^{-1}$), shown here, suggests there is no impact of an instantaneous decrease in Q on φ , since any increase in φ would divert more of the limiting absorbed energy away from CO_2 assimilation into the C_3 cycle, toward re-assimilation of CO_2 into the C_4 cycle.

The lower canopy leaves used during these experiments receive so little light that their loss of PLUE has a negligible impact on whole-plant productivity. Giving the lower canopy the characteristics of upper canopy leaves would only bring it up from 11% to 12% and 20% to 23% of upper canopy photosynthesis in *M. x giganteus* and *Z. mays*, respectively.

This research shows a potential limit to the productivity of C_4 crops that form closed canopies, reducing their competitive advantage relative to C_3 crops. These results would be strengthened by obtaining measurements from intermediary canopy depths, and by using modeling techniques to estimate the extent of productivity lost due to reduced PLUE throughout the canopy. In particular, measurements of photosynthetic efficiency should be

coupled with measurements of LAI throughout the canopy. Similarly, measurements should be taken prior to canopy closure to assess variations in PLUE at younger plant stages.

These results add new complexity to the question of optimizing vertical light distribution through closed canopy C_4 crops (Ort et al., 2011). Reducing upper canopy leaf chlorophyll content could be considered as a means to both reduce this effect and better optimize production from light-limited leaves. Further research should seek to quantify the potential to boost productivity by decreasing upper canopy fractional light absorptance.

If the lost PLUE during canopy closure can be mitigated, it may favor greater planting densities, which would heighten the advantages linked to canopy closure (in particular, weed management in young stands). Solving these inefficiencies could thus allow significant improvement in overall productivity. Because similar results were found in both *M. x giganteus* and *Zea mays*, there is a high likelihood that other C_4 crops are also affected by reduced PLUE throughout the canopy. We therefore identify this factor as an important determinant of overall productivity in many crops, which should be considered as a target for improvement in future breeding efforts.

TABLES AND FIGURES

Table 1: F-values from the main effect of canopy position and the interaction of canopy position and species of A_{sat} , $\Phi CO_{2, max}$, $\Phi PSII, max$, k , and α of upper and lower canopy leaves in *M. x giganteus* and *Z. mays*. Statistically significant difference at $P < .1$ is indicated by #; at $P < .05$ by *, at $P < .001$ by **, and at $P < .0001$ by ***.

Effect	A_{sat}	$\Phi CO_{2, max}$	$\Phi PSII, max$	k	α
canopy position main effect	97.29***	36.65***	0.16	3.06#	3.86#
canopy position*species interaction	1.84	2.03	27.55***	0.57	5.23*

Table 2: Least significant means of 51 plants for A_{sat} , $\Phi CO_{2, max}$, $\Phi PSII, max$, k , and α of upper and lower canopy leaves in *M. x giganteus* and *Z. mays*. Statistically significant difference between canopy positions at $P < .1$ is indicated by #; at $P < .05$ by *, at $P < .001$ by **, and at $P < .0001$ by ***: in the case of a significant difference the greater of the two (upper or lower canopy) is written in bold.

<i>M. x giganteus</i>	A_{sat}		$\Phi CO_{2, max}$		$\Phi PSII, max$		k		α	
	mean	standard error	mean	standard error	mean	standard error	mean	standard error	mean	standard error
upper canopy	32.7795***	1.5929	0.05598***	0.002839	0.7743	0.003272	3.94	0.1042	0.8502	0.004128
lower canopy	21.2375	1.6509	0.04107	0.002999	0.7926**	0.003521	4.196#	0.1004	0.87***	0.004368

<i>Z. mays</i>	A_{sat}		$\Phi CO_{2, max}$		$\Phi PSII, max$		k		α	
	mean	standard error	mean	standard error	mean	standard error	mean	standard error	mean	standard error
upper canopy	44.5899***	1.4964	0.05454*	0.002246	0.8042**	0.004102	4.1112	0.1004	0.9062	0.004884
lower canopy	29.3634	1.5159	0.0453	0.00232	0.7829	0.004102	4.2132	0.1042	0.9047	0.005148

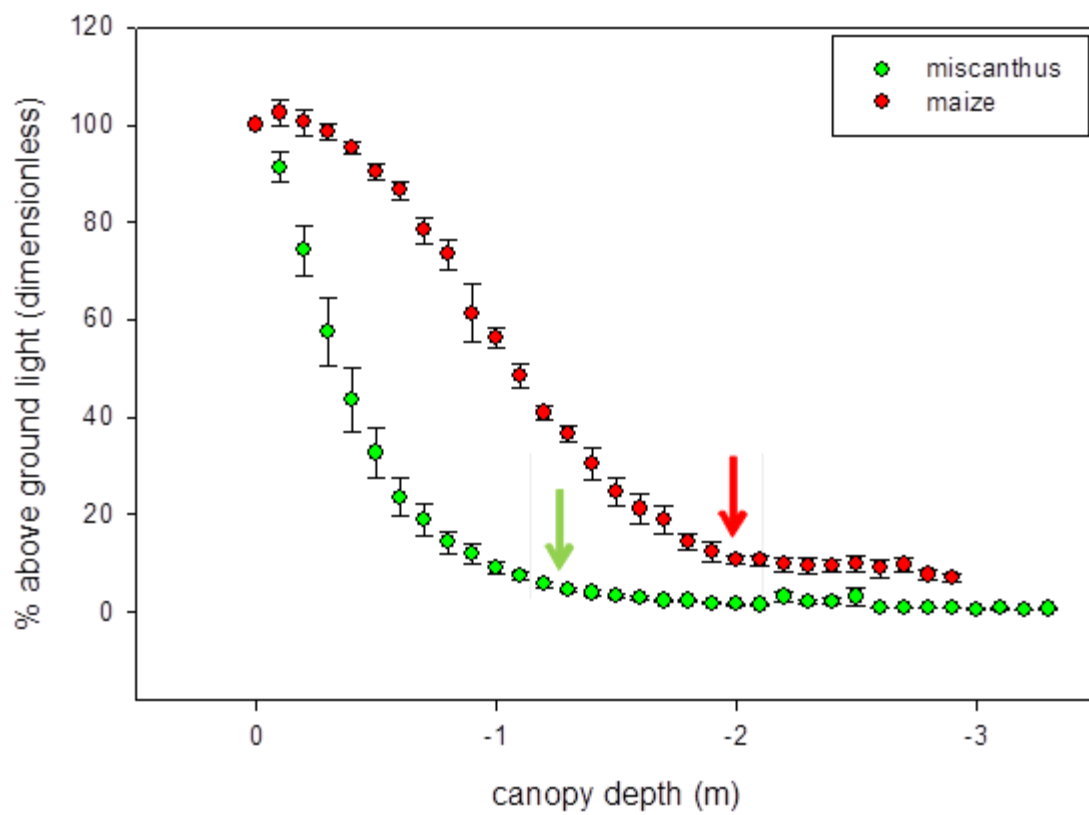


Figure 1: Light interception by *M. x giganteus* and *Z. mays* canopies in July. Arrows indicate approximate canopy depths where lower canopy leaves were selected from both species: 1.3 m for *M. x giganteus* and 2 m for *Z. mays*.

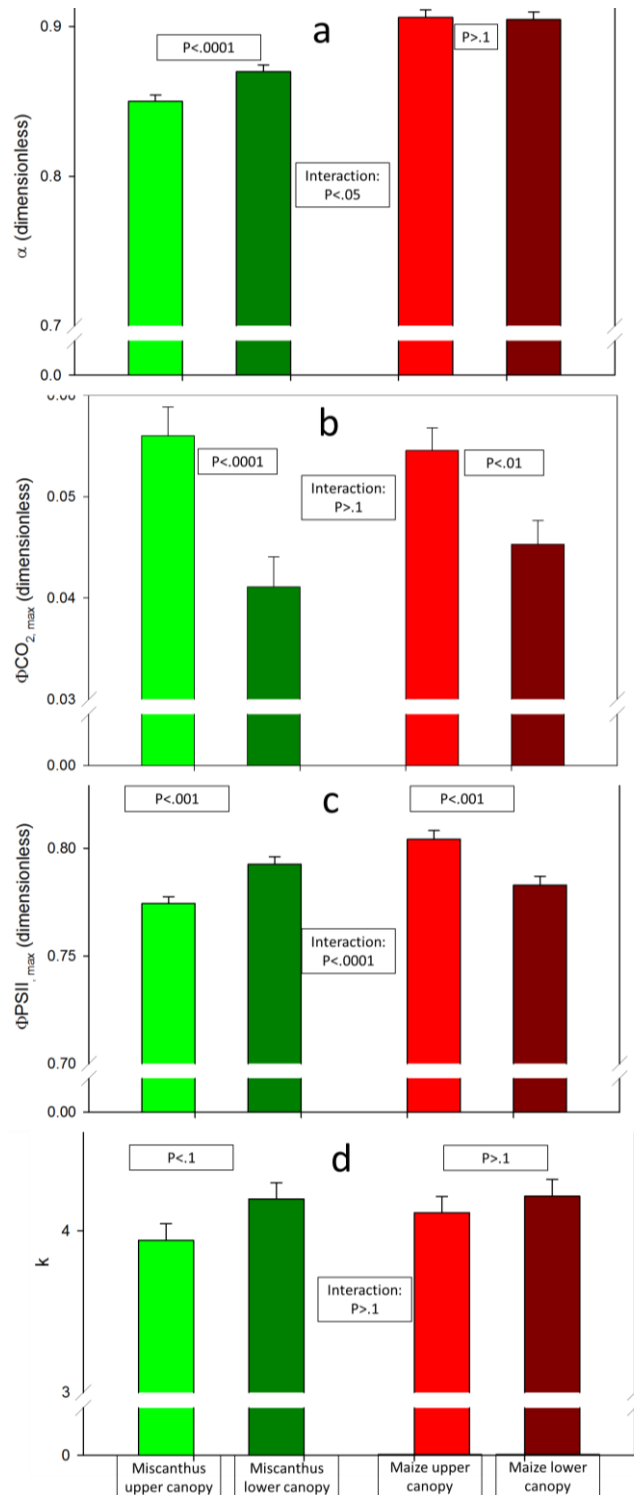


Figure 2: a), leaf fractional light absorbance (α) measured in an integrating sphere using the exact wavelengths of light used in the measurements of photosynthesis. b), maximum quantum yield of CO_2 assimilation ($\Phi_{CO_2, max}$). Results are corrected for α as explained in materials and methods. c), maximum quantum yield of PSII photochemistry, i.e. dark adapted F_v/F_m ($\Phi_{PSII, max}$). d), number of electrons required through whole chain electron transport per CO_2 assimilated (k). Results are obtained from the inverse of the slope of A^* to J at low light (40-140 Q). All results are shown in the upper and lower canopy of *Z. mays* and *M. x giganteus*. Each bar is the mean of approximately 25 replicate leaves $\pm$ 1 s.e.

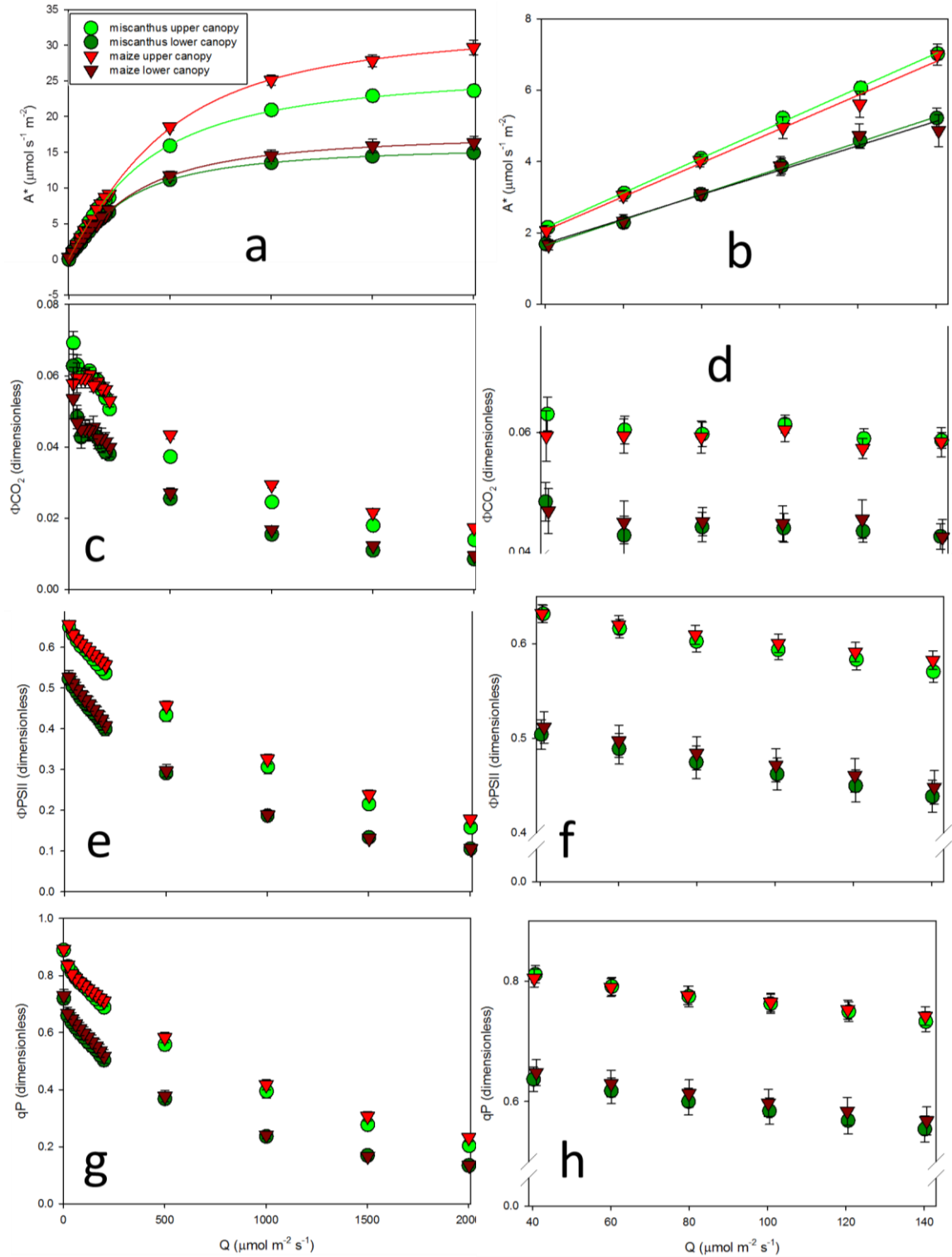


Figure 3: Response to Q of a), b) gross CO_2 assimilation (A^*), c), d) operating quantum yield of CO_2 assimilation (Φ_{CO_2}), and e), f) operating quantum yield of PSII photochemistry (Φ_{PSII}), and g), h) proportion of PSII capability used (qP) in the upper and lower canopy of *M. x giganteus* and *Z. mays*. The strictly light limiting phase of the response of photosynthesis to Q is shown for $Q = 40 - 140$ in the adjacent panels b, d, f, h respectively. Each point is the mean of approximately 25 replicate leaves ± 1 s.e.

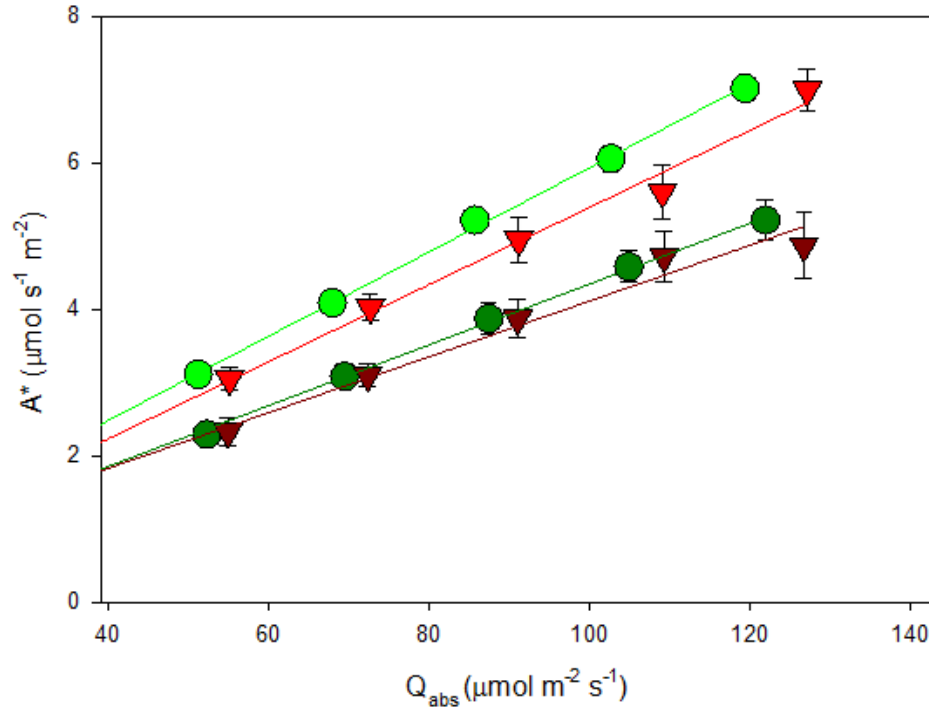


Figure 4: Response of gross CO_2 assimilation (A^*) to absorbed light (Q_{abs}) in the upper and lower canopy of *M. x giganteus* and *Z. mays*. The strictly light limiting phase of the response of photosynthesis to Q is shown for $Q = 40\text{-}140$. These slopes correspond to the maximum quantum yield of CO_2 assimilation ($\Phi_{\text{CO}_2, \text{max}}$). Each point is the mean of approximately 25 replicate leaves ± 1 s.e.

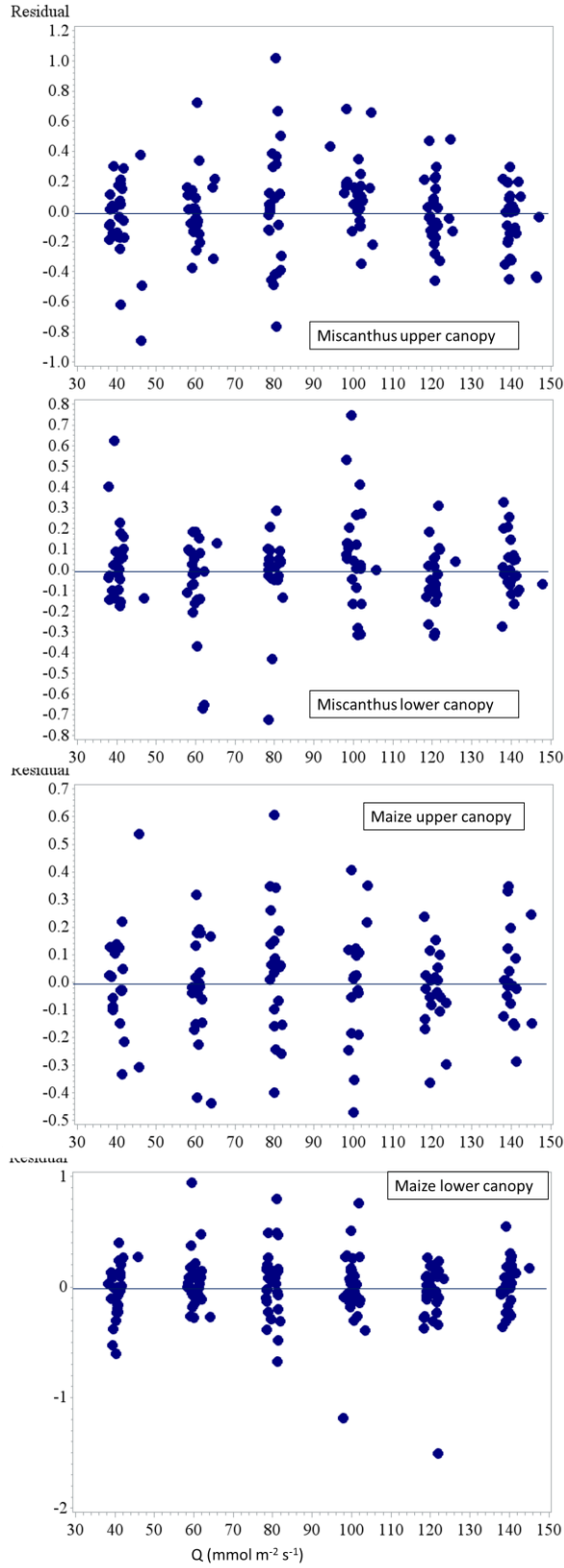


Figure 5: residuals obtained from the linear regression of the response of A to Q at low-light ($Q = 40-140$) used to determine the maximum quantum yield of leaf CO_2 uptake ($\Phi\text{CO}_{2, \text{max}}$) for field-grown *Z. mays* and *M. x giganteus* plants. Distribution of residuals shows whether there is any evidence of non-linearity over the range of Q used.

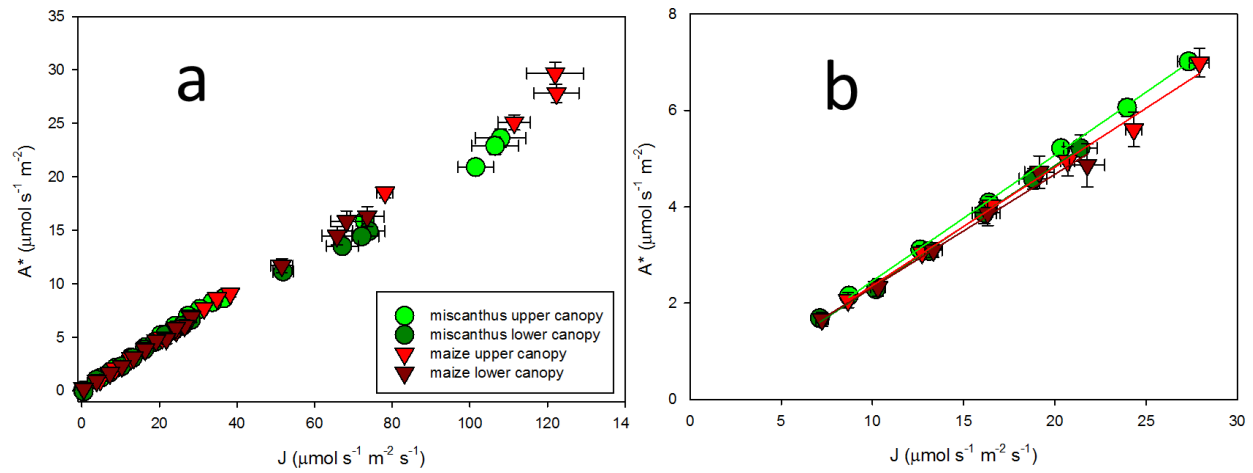


Figure 6: net CO₂ assimilation (A^*) as a function of photosynthetic electron transport rate (J) in the upper and lower canopy of *Z. mays* and *M. x giganteus*. The strictly light limiting phase of the response of photosynthesis to Q is shown for $Q=40-140$ in the adjacent panel b. Each point is the mean of approximately 25 replicate leaves ± 1 s.e.

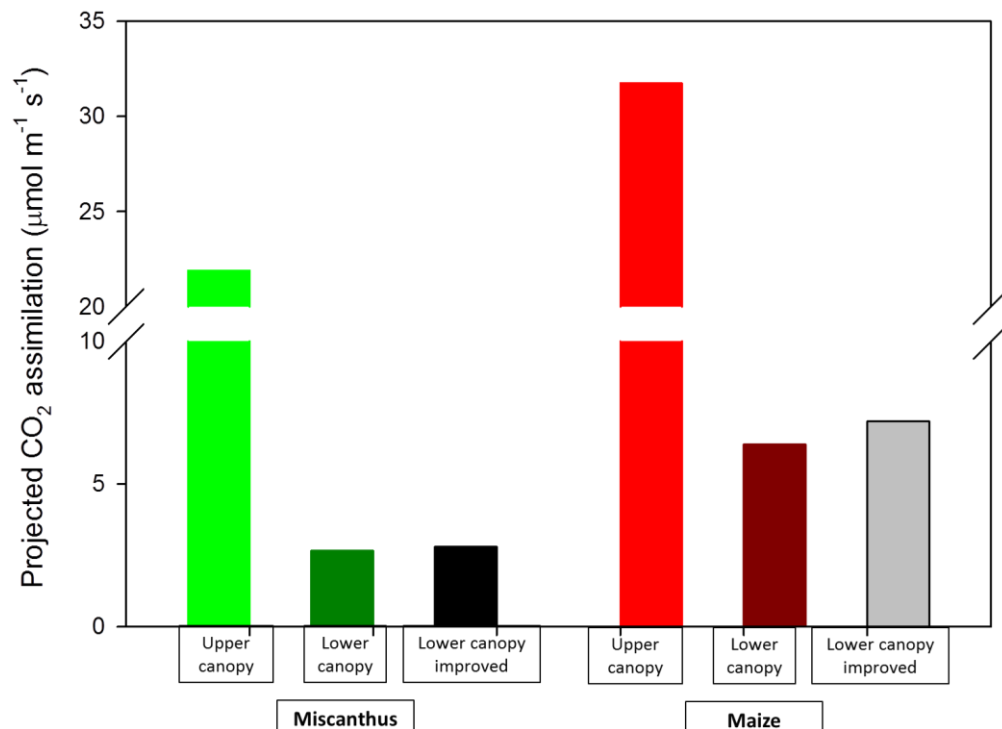


Figure 7: Projected CO₂ assimilation at different canopy levels of *M. x giganteus* and *Z. mays* canopies. “Lower canopy improved” denotes assimilation levels that would occur in the lower canopy if these leaves had the characteristics of the upper canopy in terms of photosynthetic efficiency (as shown in Fig. 3a). The upper canopy is considered to receive full above-canopy sunlight at levels measured in July, 2011 which averaged $1706 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 48$. The lower canopy is placed at 1.3 m and 2 m canopy depth for *M. x giganteus* and *Z. mays*, respectively. Each bar is the mean of approximately 25 replicate leaves ± 1 s.e.

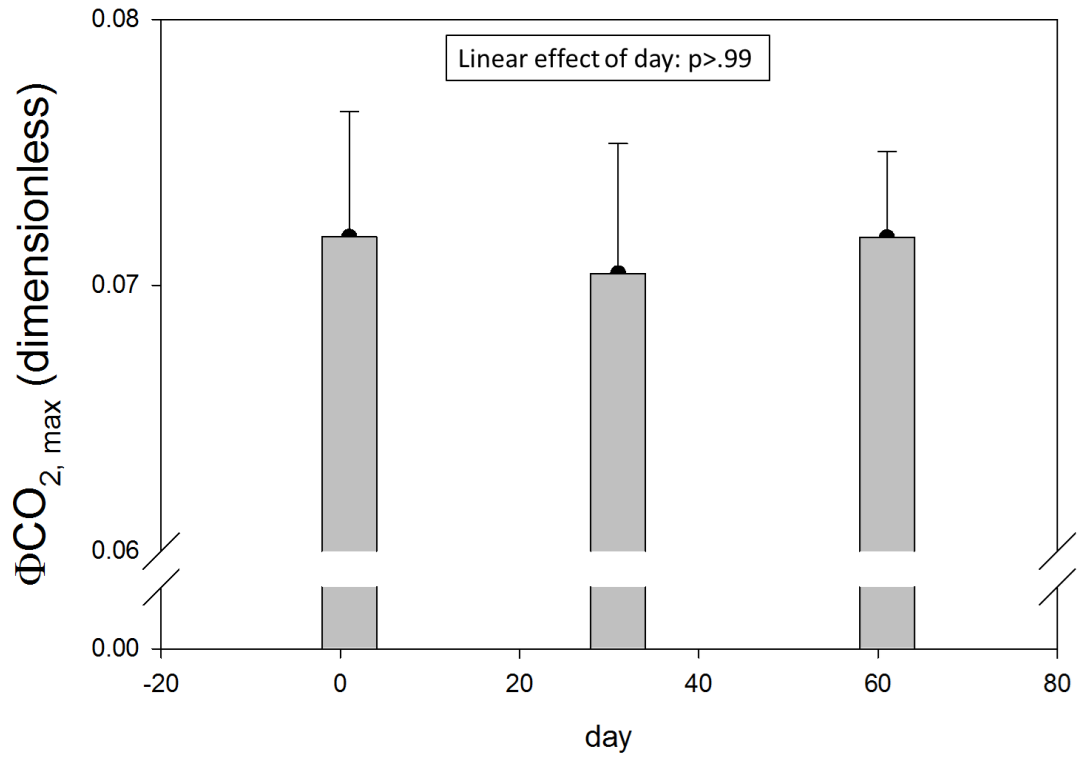


Figure 8: Maximum quantum yield of CO_2 assimilation ($\Phi CO_{2, \max}$) with days after emergence of the leaf ligule in *M. x giganteus* leaves that are artificially maintained in a high light environment, to separate increase in age from decrease in light, as would occur with sequential production of leaves above. Results are corrected for fractional light absorptance (α) as explained in materials and methods. Each bar is the mean of approximately 8 replicate leaves ± 1 s.e.

REFERENCES

- Baker, N. R. (2008). Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.*, 59, 89-113.
- Beale, C. V., Bint, D. A., & Long, S. P. (1996). Leaf photosynthesis in the C₄-grass *Miscanthus x giganteus*, growing in the cool temperate climate of southern England. *Journal of Experimental Botany*, 47(2), 267-273.
- Björkman O, Demmig-B (1987) Photon yield of O₂ evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. *Planta*. 170:489–504
- Boardman, N. (1977). Comparative photosynthesis of sun and shade plants. *Annual Review of Plant Physiology*, 28(1), 355-377.
- Burkey, K. O., & Wells, R. (1991). Response of soybean photosynthesis and chloroplast membrane function to canopy development and mutual shading. *Plant Physiology*, 97(1), 245-252.
- Von Caemmerer, Farquhar D (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153:376–387
- Von Caemmerer. & Furbank T. (1999) Modeling C₄ photosynthesis. In *C₄ Plant Biology* (eds R.F. Sage & R.K. Monson), pp.173–211. Academic Press, San Diego, CA, USA. Von Caemmerer., 2000 Biochemical models of leaf photosynthesis
- von Caemmerer, S., & Furbank, R. T. (2003). The C₄ pathway: an efficient CO₂ pump. *Photosynthesis Research*, 77(2), 191-207.

Dohleman, F. G., & Long, S. P. (2009). More productive than maize in the Midwest: how does *Miscanthus* do it?. *Plant Physiology*, 150(4), 2104-2115.

Donner SD, Kucharik CJ (2008) Corn-based ethanol production compromises goal of reducing nitrogen export by the Mississippi River. *Proc Natl Acad Sci USA* 105: 4513–4518

Edwards, G. E., & Baker, N. R. (1993). Can CO₂ assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis?. *Photosynthesis Research*, 37(2), 89-102.

Ehleringer, J., & Pearcy, R. W. (1983). Variation in quantum yield for CO₂ uptake among C₃ and C₄ plants. *Plant Physiology*, 73(3), 555-559.

Farquhar, G. D., Caemmerer, S. V., & Berry, J. A. (1980). A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta*, 149(1), 78-90.

Farquhar D (1983) On the nature of carbon isotope discrimination in C₄ species. *Aust J Plant Physiol* 10: 205–226

Farquhar, G. D., Ehleringer, J. R., & Hubick, K. T. (1989). Carbon isotope discrimination and photosynthesis. *Annual review of plant biology*, 40(1), 503-537.

Furbank, R. T., Jenkins, C. L. D., & Hatch, M. D. (1990). C₄ photosynthesis: quantum requirement, C₄ and overcycling and Q-cycle involvement. *Functional Plant Biology*, 17(1), 1-7.

Greef, J. M., & Deuter, M. (1993). Syntaxonomy of *Miscanthus x giganteus* GREEF et DEU. *Angewandte Botanik*, 67.

Hatch et al. (1995) Measurement of the Leakage of CO₂ from Bundle-Sheath Cells of Leaves during C₄ Photosynthesis. *Plant Physiol.* (1995) 108: 173-181

Heaton, E., Voigt, T., & Long, S. P. (2004). A quantitative review comparing the yields of two candidate C₄ perennial biomass crops in relation to nitrogen, temperature and water. *Biomass and Bioenergy*, 27(1), 21-30.

Heaton EA, Dohleman FG, Long SP (2008) Meeting US biofuel goals with less land: the potential of *Miscanthus*. *Glob Change Biol* 14: 2000–2014

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

Long SP, Postl WF, Bolharnordenkamp HR. (1993) Quantum yields for uptake of carbon-dioxide in C₃ vascular plants of contrasting habitats and taxonomic groupings. *Planta* 189:226–34

Long, S. P., ZHU, X. G., Naidu, S. L., & Ort, D. R. (2006). Can improvement in photosynthesis increase crop yields?. *Plant, Cell & Environment*, 29(3), 315-330.

Long, S. P., & Ort, D. R. (2010). More than taking the heat: crops and global change. *Current opinion in plant biology*, 13(3), 240-247.

Mathews, S., Spangler, R. E., Mason-Gamer, R. J., & Kellogg, E. A. (2002). Phylogeny of *Andropogoneae* inferred from phytochrome B, GBSSI, and NDHF. *International Journal of Plant Sciences*, 163(3), 441-450.

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of experimental botany*, 51(345), 659-668.

Meinzer and Zhu (1998) Nitrogen stress reduces the efficiency of the C₄ CO₂ concentrating system, and therefore quantum yield, in *Saccharum* (sugarcane) species. *Journal of Experimental Botany*, Vol. 49, No. 324, pp. 1227–1234

Oberhuber, W., Dai, Z. Y., & Edwards, G. E. (1993). Light dependence of quantum yields of photosystem II and CO₂ fixation in C₃ and C₄ plants. *Photosynthesis research*, 35(3), 265-274.

Ort, D. R., Zhu, X., & Melis, A. (2011). Optimizing antenna size to maximize photosynthetic efficiency. *Plant physiology*, 155(1), 79-85.

Seemann, J. R. (1989). Light adaptation/acclimation of photosynthesis and the regulation of ribulose-1, 5-bisphosphate carboxylase activity in sun and shade plants. *Plant physiology*, 91(1), 379-386.

Smith, H. (1982). Light quality, photoperception, and plant strategy. *Annual review of plant physiology*, 33(1), 481-518.

Suárez, N. (2010). Leaf lifetime photosynthetic rate and leaf demography in whole plants of *Ipomoea pes-caprae* growing with a low supply of calcium, a 'non-mobile' nutrient. *Journal of experimental botany*, 61(3), 843-855.

Ubierna et al. (2011) The efficiency of C₄ photosynthesis under low light conditions: assumptions and calculations with CO₂ isotope discrimination. *Journal of Experimental Botany*, Vol. 62, No. 9, pp. 3119–3134

Wang D, Naidu SL, Portis AR, Jr, Moose SP, Long SP (2008) Can the cold tolerance of C4 photosynthesis in *Miscanthus x giganteus* relative to *Zea* may be explained by differences in activities and thermal properties of Rubisco? *Journal of Experimental Botany* doi:10.1093/jxb/ern074

Zhu, X. G., Long, S. P., & Ort, D. R. (2010). Improving photosynthetic efficiency for greater yield. *Annual review of plant biology*, 61, 235-261.