GLOBAL CHANGE BIOLOGY
BIOENERGY

GCB Bioenergy (2012), doi: 10.1111/j.1757-1707.2012.01167.x

Impact of nitrogen allocation on growth and photosynthesis of Miscanthus (*Miscanthus* × *giganteus*)

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Abstract

Nitrogen (N) addition typically increases overall plant growth, but the nature of this response depends upon patterns of plant nitrogen allocation that vary throughout the growing season and depend upon canopy position. In this study seasonal variations in leaf traits were investigated across a canopy profile in Miscanthus (*Miscanthus* × *giganteus*) under two N treatments (0 and 224 kg ha^{-1}) to determine whether the growth response of Miscanthus to N fertilization was related to the response of photosynthetic capacity and nitrogen allocation. Miscanthus yielded 24.1 Mg ha^{-1} in fertilized plots, a 40% increase compared to control plots. Photosynthetic properties, such as net photosynthesis (A), maximum rate of rubisco carboxylation (V_{cmax}), stomatal conductance (g_s) and PSII efficiency (F_v'/F_m'), all decreased significantly from the top of the canopy to the bottom, but were not affected by N fertilization. N fertilization increased specific leaf area (SLA) and leaf area index (LAI). Leaf N concentration in different canopy layers was increased by N fertilization and the distribution of N concentration within canopy followed irradiance gradients. These results show that the positive effect of N fertilization on the yield of Miscanthus was unrelated to changes in photosynthetic rates but was achieved mainly by increased canopy leaf area. Vertical measurements through the canopy demonstrated that Miscanthus adapted to the light environment by adjusting leaf morphological and biochemical properties independent of nitrogen treatments. GPP estimated using big leaf and multilayer models varied considerably, suggesting a multilayer model in which V_{cmax} changes both through time and canopy layer could be adopted into agricultural models to more accurately predict biomass production in biomass crop ecosystems.

Keywords: biofuel crop, canopy, GPP, nitrogen allocation

Received 30 October 2011 and accepted 31 December 2011

Introduction

Most of the world's terrestrial ecosystems are primarily or co-limited by N (LeBauer & Treseder, 2008). While it is well known that N addition typically increases plant growth, less is known about how N addition affects patterns of N allocation through the canopy. Part of this is due to the complexity of plant allocation patterns. N allocation varies both vertically in the plant canopy in response to changes in light availability (Rosati *et al.*, 2000) and also changes across the growing season (Reich *et al.*, 1991; Heaton *et al.*, 2009). Light interception by the canopy creates a vertical gradient in light levels that have a strong effect on leaf physiological and morphological processes (Ellsworth & Reich, 1993). In grasses there is an interaction between light environment and leaf age, as individual leaves shift from being sun leaves to shade leaves within a single growing season due to new growth within the plant. This growth pattern places a developmental constraint on the morphological response to light that contrasts with trees, which often exhibits large morphological differences between sun and shade leaves. The variability in foliage characteristics with canopy position and time presents a challenge when attempting to understand

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Abbreviations: A net photosynthesis (μmol m^{-2} s^{-1}); Chl *a+b* chlorophyll *a+b* content (μg cm^{-2}); Chl *a*/*b* the ratio of chlorophyll *a* to chlorophyll *b*; N_m mass-based nitrogen concentration (%); N_a area-based nitrogen concentration (g m^{-2}); V_{cmax} maximum rate of rubisco activity (μmol m^{-2} s^{-1}); k carboxylation efficiency; g_s stomatal conductance; F_v'/F_m' PSII efficiency; ${}_q$p photochemical quenching; q quantum yield; J_{PSII} PSII electron transport rate; SLA specific leaf area (m^2 kg^{-1}); LAI leaf area index (m^2 m^{-2}); Fru fructose content (μg cm^{-2}); Suc sucrose content (μg cm^{-2}); Glc glucose content (μg cm^{-2}); GPP gross primary production (μmol m^{-2} s^{-1}).

leaf development, leaf energy balance, water use, and carbon uptake, and when attempting to model physiological processes and growth of whole canopies and stands (Baldocchi & Harley, 1995).

Miscanthus [*Miscanthus* × *giganteus*] maintains high photosynthetic rates over a longer-than-average growing season and yields more than two times the biomass of other candidate biofuel grass crops (Heaton *et al.*, 2004; Price *et al.*, 2004). Miscanthus has higher nutrient-use efficiency than other C_4 species such as switchgrass (Heaton *et al.*, 2004, 2008) and corn (Dohleman & Long, 2009). There is evidence that N in the aboveground biomass is re-translocated to rhizome and recycled to the soil if delaying the harvest over the winter (Heaton *et al.*, 2009). It has also been hypothesized that Miscanthus relies partly on N-fixation to meet its annual N budget (Davis *et al.*, 2010). These characteristics make Miscanthus one of the most viable options for sustainable biofuel crops because GHG (greenhouse gases) emissions associated with fertilizers would be minimal.

It has been suggested that plants are able to optimize the allocation of N in order to preserve a balance between Calvin cycle (i.e., Rubisco) and light-harvesting (i.e., chlorophyll) capabilities (Givnish, 1988; Warren & Adams, 2001). Acclimation to light has been shown to affect N allocation within leaves (Anten *et al.*, 1998; Rosati *et al.*, 2000). Relative foliar chlorophyll concentration tends to increase with decreasing growth irradiance, while the fraction of N in Rubisco usually decreases with decreasing irradiance (Evans, 1989; Le Roux *et al.*, 1999; Turnbull *et al.*, 2007). These results however, have mostly been tested by comparing leaves from different species or sites across a wide range of growth forms (e.g., herbs, shrubs, conifers, broadleaf trees) (Anten *et al.*, 1996; Meir *et al.*, 2002; Oguchi *et al.*, 2005) or on plants grown in a controlled environment (Aerts & Decaluwe, 1994; Hikosaka *et al.*, 1994; Pons & Anten, 2004; Oguchi *et al.*, 2005), rather than on leaves from the same plant in a field growing condition.

Successfully up-scaling photosynthesis from the leaf to the canopy level requires understanding the rate-determining factors in leaf photosynthesis (Laisk *et al.*, 2005). Photosynthetic carbon gain of leaves is mainly affected by light availability and N concentration (Field & Mooney, 1986). This observation is supported by positive relationships between N concentration and net photosynthesis observed on many different species (Field & Mooney, 1986; Meir *et al.*, 2002; Turnbull *et al.*, 2007). For many species, this relationship between N content and light holds true when N is expressed per unit leaf area, N_a (Field, 1983; Reich & Walters, 1994). By contrast, when expressed per unit mass, N concentration (N_m) increased (Turnbull *et al.*, 2007), remained unchanged (Reich & Walters, 1994), or decreased (Ellsworth & Reich, 1993) with increasing light availability. The different relationships between N_a and N_m with light reflect the variable effect of light on SLA. For fast-growing Miscanthus, light availability varies significantly within the canopy. Few data, however, are available on the ontological changes in the correlations between leaf N concentration (expressed per unit leaf area or mass) and light availability at different canopy levels and their relationship to photosynthetic processes for Miscanthus. Systematic measurements of photosynthesis across the growing season are needed for validation of growth models and to elucidate the physiological basis for observed differences in productivity (Dohleman *et al.*, 2009).

Photosynthesis models have been implemented in big leaf and multilayer-canopy models in order to predict canopy photosynthetic production by scaling up from individual leaves (Leuning *et al.*, 1995). In big leaf models single-leaf photosynthesis calculations are applied to the whole canopy assuming that photosynthetic capacity and absorbed photosynthetically active radiation (PAR) have a homogeneous distribution through the canopy (Sinclair *et al.*, 1976; Farquhar, 1989); conversely, multilayer canopy models integrate leaf-level photosynthesis calculations over discrete canopy layers that vary in PAR and photosynthetic properties (Norman, 1993). Because of their greater complexity and data demands multilayer models may not be as applicable as big leaf models for global-scale projections across numerous vegetation types, they may be more desirable in agricultural models to predict crop biomass productivity. In both multilayer and big leaf models, parameters such as V_{cmax} may change across the growing season and, in multilayer models, with depth in the canopy. However, time- or canopy- changing parameters are often lacking in these models (Amthor, 1994; Miguez *et al.*, 2009). In this study, a simple GPP model was implemented based on field observations to investigate the effects of varying V_{cmax} with, time, canopy position or the combination of time and canopy position.

In addition to or in combination with changes in biochemistry, leaves within a canopy change foliage structure in order to acclimate to within-canopy light gradients (Niinemets, 1999; Pons & Anten, 2004). Leaves that develop in high light levels have lower SLA as a result of increased leaf thickness and increased mesophyll cell density (Witkowski & Lamont, 1991). High SLA at low light levels is beneficial for obtaining a more extensive foliar display that captures more light for constant biomass investment (Niinemets, 1999). However, unlike trees, grass leaves develop first in high light and later shift to become shade leaves as they are overtopped by new growth. The effect of irradiance on the relationships between photosynthesis, SLA, N_a, N_m has not been extensively studied in grasses and little is known about whether morphology or biochemistry plays the leading role in their photosynthetic performance at different canopy layers and nitrogen treatments for Miscanthus. Therefore, in this study we investigated the acclimation of leaves of Miscanthus to within-canopy light levels, and the effect of N fertilization on these relationships. Specifically, we hypothesize: (1) growth and photosynthetic capacities will be increased by N fertilization; (2) The increase of photosynthetic parameters will be driven by increased N content; (3) N allocation within the canopy will be affected by N fertilization, with more N allocated at the top layer in N-applied plots than control plots. We also aim to test how big leaf and multilayer models with different time- and canopy-dependent V_{cmax} will vary in predicting GPP.

Methods

Study site

Four-year-old Miscanthus [*Miscanthus × giganteus*] stands were grown in an agricultural study site in Savoy, IL (40°10′20″ N, 88°11′40″ W, 228 m above sea level). Details of planting stock were described in detail in Heaton *et al.* (2008). The soil at the site is Flanagan series silt loam (Fine, smectitic, mesic Aquic Argiudolls). Before the experiment, Miscanthus stands have never been fertilized and the soil nitrate level is about 4 ppm for 0–6 inch depth and 1 ppm for 6–12 inch depth. The experiment design was split plot arrangements in randomized complete block with four replications to test for the effect of harvesting times on the yield of Miscanthus. Subplots (4.6 × 2.1 m) were blocked by N fertility levels (0 and 224 kg N ha^{-1}) and N in the form of urea was applied on May 12, 2009. Plots were harvested with a plot harvester (Model Cibus S; Wintersteiger, Ried, Austria) on March 17, 2010, by cutting a 1.22 m swath through the middle of the plots. A subsample was collected from each plot to determine moisture content on which the calculation of dry biomass was based. Throughout the growing season, one plant was randomly selected for physiological measurements within each plot on day 168, 205, 240, 261, and 279. Fully expanded leaves were sampled from two layers (top: 0–0.5 m; bottom: 0.5 m lower from the top) on day 168 and from three layers distributed through the nonsenescent portion of canopy (top: 0–0.5 m, middle: 0.5–1 m, and bottom: 1.5 m lower from the top, approximately) on other four sampling days, depending on the light levels leaves were exposed to.

LAI measurement

Leaf area index (LAI) and the proportion of photosynthetic active radiation (PAR, 400–700 nm) intercepted by the canopy were measured on day 205, 240, 261, and 279. The measurements were taken by measuring the PAR outside the crop canopy using an external sensor (Model LI-190; LI-COR Biosciences, Lincoln, NE, USA) connected to a linear ceptometer (Model PAR-80; Decagon Devices, Inc., Pullman, WA, USA) which was used to measure the amount of PAR not intercepted by the crop canopy. These measurements were taken at four depths within the crop canopy, between 10:00 and 14:00 hours on mostly sunny days when the minimum PAR was at least 1400 μmol m^{-2} s^{-1}. One to three subsamples were taken in each plot for each measurement date and each subsample was the average of ~20 independent readings. Light interception was determined by calculating the proportion of PAR intercepted by the crop canopy. Leaf area index was estimated for each subsample using the observations of radiation interception beneath and outside the canopy, and zenith angle and leaf angle distribution (Deblonde *et al.*, 1994).

Gas exchange measurements

Shoots from different canopy layers were sampled before dawn and returned to the lab partially submerged in water and put in the dark before measurement. Gas exchange and chlorophyll fluorescence were measured on leaves with a portable infrared gas analyzer (LI-COR 6400LCF; Li-COR, Lincoln, NE, USA). During measurements, leaves were exposed to a CO_2 concentration of 370 μmol mol^{-1}, temperature at 25 °C, vapor pressure deficit (VPD) at the leaf surface 1.5 kPa and airflow through the chamber 250 μmol s^{-1}. Leaves were acclimated to a photosynthetic photon flux (PPFD; 1500 μmol m^{-2} s^{-1}) until photosynthetic rates stabilized. The rate of photosynthesis at a PPFD of 1500 μmol m^{-2} s^{-1} was defined as the net photosynthetic rate (A). For the CO_2 response (A–C_i) curves, leaves were acclimated for 30–60 min before adjusting the CO_2 concentrations. Thereafter, CO_2 concentration was decreased in five steps (400, 300, 200, 100, and 50 ppm CO_2) and then increased in three steps (400, 600, and 800 μmol mol^{-1} CO_2). For the light response (A–Q) curves, photosynthetic photon flux was decreased from 1500 to 50 μmol m^{-2} s^{-1} in eight steps (1500, 1000, 800, 500, 300, 200, 100, 50) and measurements were logged after the photosynthetic rates were stabilized. Post-PSII electron transport (J_{PSII}), PSII efficiency (F_v'/F_m') and photochemical quenching (q_p) in light-adapted leaves were also measured using a Licor 6400-40 Leaf Chamber Fluorometer (LI-COR Biosciences). A-C_i and A-Q curves were fitted to a coupled photosynthesis-stomatal conductance model by Collatz *et al.* (1992). The initial slope and rate saturated region of the A-C_i curves were used to estimate carboxylation efficiency (k) and maximum Rubisco activity (V_{cmax}) (Miguez *et al.*, 2009). The initial slope of the A-Q curves was used to estimate quantum efficiency (q) (Miguez *et al.*, 2009).

Leaf harvest, specific leaf area (SLA), chlorophyll, and C, N measurements

Immediately following gas-exchange measurements, ten 0.5 cm^2 leaf punches from each canopy layer were taken and oven-dried at 65 °C for 2 weeks for measurement of SLA and two 0.5 cm^2 leaf punches were taken for chlorophyll measurements. N and C concentration were measured with a Perkin Elmer CHN Analyzer (Model 2400; PelkinElmer Inc, Waltham, MA, USA). Chlorophyll was extracted with 80% ethanol (Richardson *et al.*, 2002) and measured at absorbance of 645 and 663 nm (Varian Cary 300 spectrophotometer, Varian, Walnut Creek, CA, USA). Chlorophyll *a* and *b* concentrations were calculated with the equations of Wellburn (1994) and expressed as μg cm^{-2} leaf area.

GPP estimation

We used the Collatz *et al.* (1992) C_4 photosynthesis model to predict leaf CO_2 uptake rate. GPP in the control plots was estimated by treating the canopy as either big leaf or multilayer. Big leaf 1 and 2 models differed by taking either constant V_{cmax} calculated by the average of V_{cmax} collected from the top canopy in June, July, and August or varied V_{cmax} collected from top canopies through growing season. For multilayer model 1, 2, and 3, we compared the results by taking a constant V_{cmax}, a V_{cmax} measured from top canopy through time or V_{cmax} varying through both time and among canopy position (Table 2). Air temperature, PAR, and relative humidity measured at 30 min intervals were obtained from a meteorological station installed two miles away on another Miscanthus field plot. Other parameters in the C_4 photosynthesis model were set according to the methods described in Miguez *et al.* (2009). The radiation profile within the canopy was approximated by Beer's Law (Jones, 1992):

$$I = I_0 e^{-KL}$$

where K is the extinction coefficient, L the LAI (m^2 m^{-2}), I the irradiance at a given depth in the canopy (mmol PAR m^{-2} s^{-1}), and I_0 the irradiance above the canopy (mmol PAR m^{-2} s^{-1}). GPP was calculated by multiplying leaf CO_2 uptake rate by leaf area. For multilayer models GPP was calculated by summing up the GPP of different layers.

Statistical analysis

Fixed effects of date, fertilization (N), and canopy position (CA) and their interactions on the morphological, biochemical and physiological parameters were tested by ANOVA [PROC GLM, SAS 9.1, (SAS Institute, Cary, NC, USA)]. Post-hoc Tukey HSD tests were made on specific contrasts to examine significant treatment effects among groups. The relationship between light level, N concentration, and photosynthetic activity was tested by linear regression.

Results

Miscanthus yielded 24.1 Mg ha^{-1} in fertilized plots, a 40% increase compared to control plots (17.0 Mg ha^{-1}). LAI peaked in late July at about 6.8 and 6.0 at fertilized and control plots, respectively (Fig. 1). Light levels decreased from the top to the base of the canopy irrespective of fertilizer treatment, shown by increased LAI from the top to the base of the canopy. The increase in LAI with N is higher during early developmental stages (June and July) than late developmental stages (Aug and Sep). Specific leaf area was increased by fertilization at the late growing season (Sep and Oct) and was higher at the base than at the top of the canopy (Fig. 1; Table 1).

The values of J_{PSII} and q_P decreased over the course of the growing season. Fertilization had no significant effect on J_{PSII}, F_v'/F_m', q, and q_p (Fig. 2; Table 1). Leaf photosynthetic traits varied appreciably along the vertical gradient from the top to the bottom of the canopy. The value of q_p, q, and J_{PSII} were significantly higher at the top canopies than at the middle and base canopies. There were no significant interactive N and canopy effect on J_{PSII}, F_v'/F_m', q, and q_P.

Photosynthetic parameters (A, g_s, V_{cmax}, and k) all decreased throughout the growing season (Fig. 3; Table 1). Net photosynthesis (A) at the top layer averaged about 30 μmol m^{-2} s^{-1} in the early growing season and decreased to about 20 μmol m^{-2} s^{-1} in the late growing season. Canopy position had a significant effect

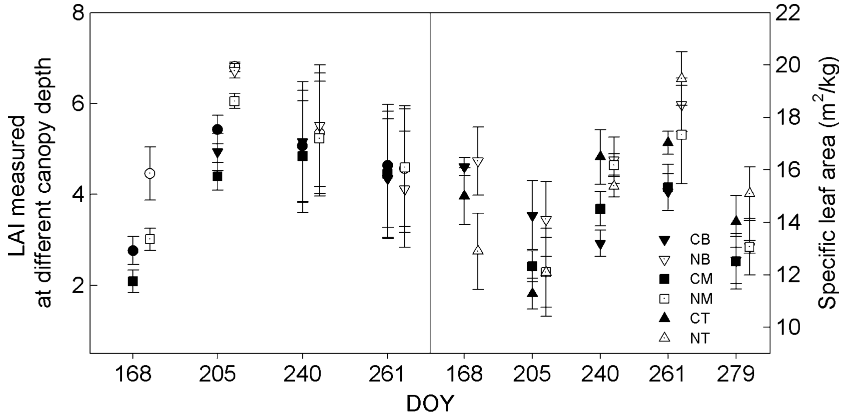

Fig. 1 Effects of nitrogen (▿□Δ – with nitrogen (N); ▼■▲ – without nitrogen (C)) and canopy position (▿▼ – bottom canopy (B); □■ – middle canopy (M); Δ▲ – top canopy (T)) on LAI and SLA throughout the growing season. Values are means ± 1 SE; *n* = 4.

Table 1 Degrees of freedom (numerator, denominator) and F-statistics from ANOVA on the fixed effect of date, fertilization (N), canopy position (CA), and their interactions on the morphological, biochemical, and physiological parameters

Factors	LAI	SLA	F_v'/F_m'	q_P	J_{PSII}	q	g_s	A	k	V_{cmax}	N_m	N_a	Chl *a*+*b*	Chl *a*/*b*	Suc	Fru	Glc
Date	3.125	4.94	4.73	4.73	4.77	4.75	4.77	4.75	4.73	4.69	4.70	4.70	4.88	4.88	3.138	3.148	3.146
	30.6*	23.9*	2.0	4.2*	3.8*	1.6	4.6*	4.0*	5.43*	6.3*	4.3*	20.6*	19.2*	53.1*	9.1*	34.2*	29.9*
N	1.125	1.94	1.73	1.73	1.77	1.75	1.77	1.75	1.73	1.69	1.70	1.70	1.88	1.88	1.138	1.148	1.146
	12.4*	5.9*	0.0	0.7	2.4	2.3	0.9	1.2	0.2	0.5	42.1*	27.3*	93.3*	0.6	4.5*	0.1*	4.8*
CA	1.125	2.94	2.73	2.73	2.77	2.75	2.77	2.75	2.73	2.69	2.70	2.70	2.88	2.88	2.138	2.14	2.146
	9.7*	4.1*	0.2	41.0*	56.2*	4.7*	19.9*	43.3*	16.1*	43.2*	38.6*	43.2*	1.6	15.6*	3.3*	7.0*	1.0
Date × N	3.125	4.94	4.73	4.73	4.77	4.75	4.77	4.75	4.73	4.69	4.70	4.70	4.88	4.88	3.138	3.148	3.146
	7.6*	3.3*	0.5	1.6	1.3	2.0	1.1	1.4	0.6	0.9	0.1	0.4	2.0	5.6*	1.9	1.9	1.1
Date × CA	7.125	8.94	7.73	7.73	7.77	7.75	7.77	7.75	7.73	7.69	7.70	7.70	8.88	8.88	5.138	5.148	5.146
	5.0*	3.7*	1.9	0.7	2.0	2.0	1.0*	2.7*	1.7	2.2*	2.7*	6.3*	1.9	1.0	1.3	3.1	0.1
N × CA	3.125	2.94	2.73	2.73	2.77	2.75	2.77	2.75	2.73	2.69	2.70	2.70	2.88	2.88	2.138	2.146	2.146
	0.6	1.3	0.4	1.4	0.4	1.8	0.1	1.2	1.3	0.2	0.2	0.6	0.4	0.8	0.6	0.6	0.6
Date × N × CA	7.125	7.94	7.73	7.73	7.73	7.75	7.77	7.75	7.73	7.69	7.70	7.70	7.88	7.88	5.138	5.148	5.146
	0.8	1.0	0.5	1.2	1.3	1.1	1.0	2.3*	0.9	1.33	0.5	0.6	0.4	0.6	1.7	0.8	1.4

*Denotes significance at $P < 0.05$. See text for abbreviations.

on g_s, A, k, and V_{cmax} (Fig. 3; Table 1). The value of A, g_s, k, and V_{cmax} were higher at the top than at the middle and base canopy. Nitrogen fertilization had no significant effect on A, g_s, k, and V_{cmax}. There were no significant interactive N and canopy effect on A, g_s, k, and V_{cmax}.

Both N_a and N_m decreased throughout the growing season (Fig. 4). Fertilization increased N_m and N_a. Both N_a and N_m decreased continuously throughout the canopy from upper to lower canopy levels (Fig. 4; Table 1). There were no significant interactive N and canopy effect on N_m and N_a.

Fertilization increased Chl *a*+*b* content ($P < 0.005$); Canopy position did not affect Chl *a*+*b* content (Fig. 5; Table 1). The value of Chl *a*/*b* was not affected by fertilization but was higher at the top than at the middle and bottom canopy (Fig. 5; Table 1).

Daily GPP estimated from different models varied considerably (Fig. 6). The accumulated GPP for the

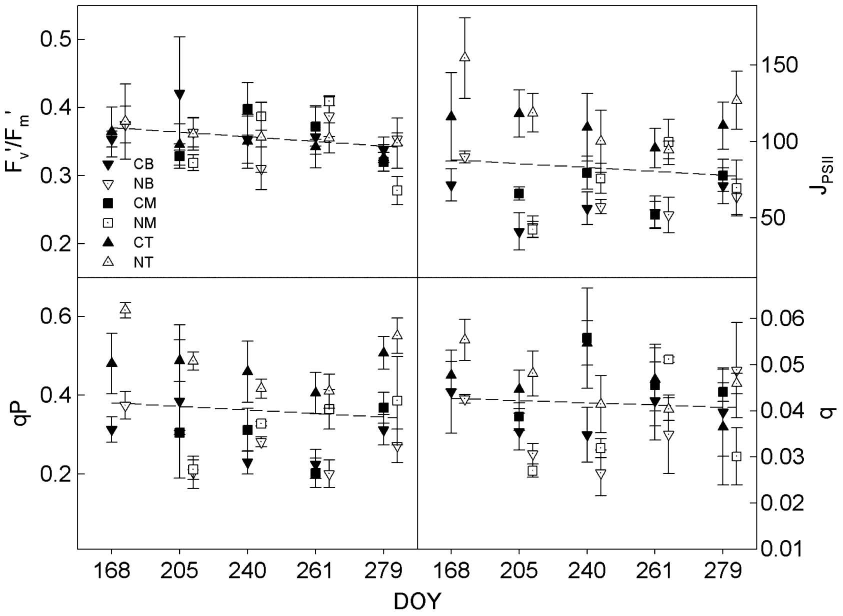

Fig. 2 Effects of nitrogen (▿□Δ – with nitrogen (N); ▼■▲ – without nitrogen (C)) and canopy position (▿▼ – bottom canopy (B); □■ – middle canopy (M); Δ▲ – top canopy (T)) on F_v'/F_m', $_q$p, J_{PSII} and q throughout the growing season. Values are means ± 1 SE; $n = 4$.

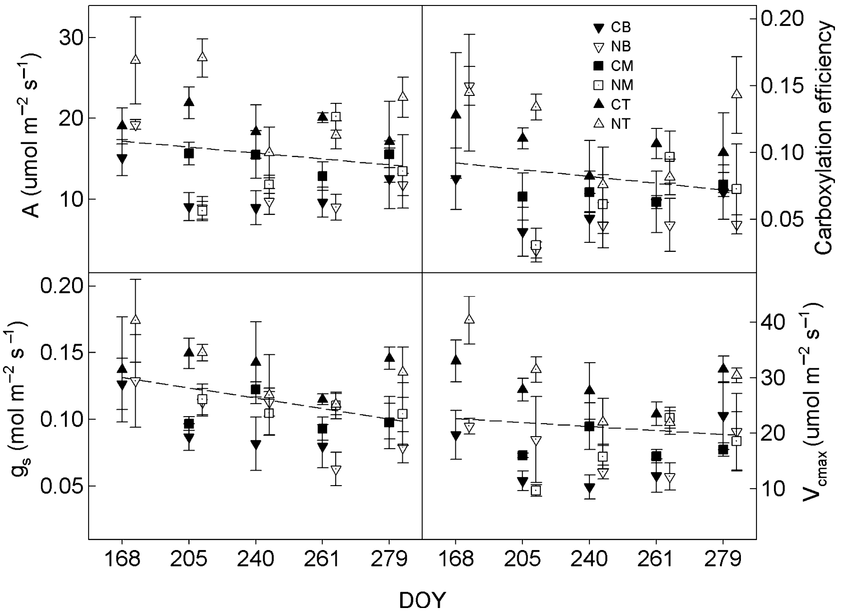

Fig. 3 Effects of nitrogen (▿□Δ – with nitrogen (N); ▼■▲ – without nitrogen (C)) and canopy position (▿▼ – bottom canopy (B); □■ – middle canopy (M); Δ▲ – top canopy (T)) on g_s, A, k, and V_{cmax} throughout the growing season. Values are means ± 1 SE; $n = 4$.

control plots from day 168 to day 279 was 5.6 and 5.3 kg C m^{-2} estimated by big leaf model 1 (time-independent parameters) and 2 (time-dependent parameters), respectively. By comparison, accumulated GPP estimated from multilayer model 1 (time-independent and canopy-depth dependent), 2 (time-dependent, canopy-depth independent), and 3 (time and canopy-depth dependent) was 5.1, 4.8 and 4.6 kg C m^{-2}, respectively (Table 2).

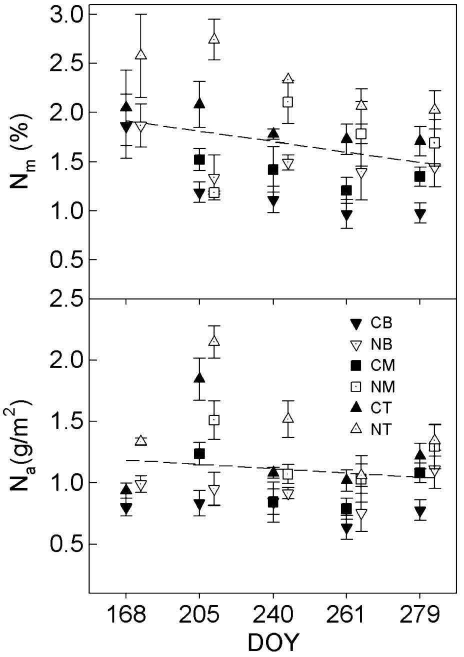

Fig. 4 Effects of nitrogen (▿□Δ – with nitrogen (N); ▼■▲ – without nitrogen (C)) and canopy position (▿▼ – bottom canopy (B); □■ – middle canopy (M); Δ▲ – top canopy (T)) on N_a and N_m throughout the growing season. Values are means ± 1 SE; $n = 4$.

Discussion

The aim of this work is to investigate the effect of N on the growth of Miscanthus and to show whether the yield response was correlated with the effect of N fertilization on the photosynthetic performance and nitrogen allocation within the canopy profiles. In biomass feedstock production, the use of N fertilizer must be optimized to balance the economics, energy, and environmental costs of fertilizer use with the resulting gains in yield (Wang *et al.*, 2010). Consistent with the hypothesis that the yield of Miscanthus will be stimulated by N fertilization, we found in this study that N increased the yield of Miscanthus by 40% compared to control plots. Positive effect of N was also shown in a parallel study with additional N levels (50, 100, and 150 kg ha^{-1}) applied and in the experiment conducted in the subsequent years (A. Parrish and D. K. Lee, unpublished data). The positive effect of N fertilization on the yield of Miscanthus was unrelated to A and V_{cmax}, instead, productivity gains were achieved mainly by increased canopy leaf area, brought out mostly by the effect of N fertilization on the expansion of individual leaves, as shown in other species (Gastal and Lemaire 2002; Taylor *et al.*, 1993). The effect of N on plant

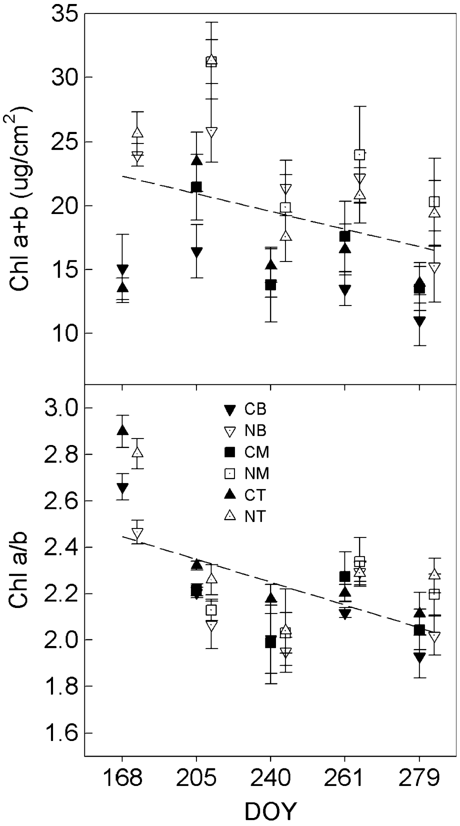

Fig. 5 Effects of nitrogen (▽□△ – with nitrogen (N); ▼■▲ – without nitrogen (C)) and canopy position (▽▼ – bottom canopy (B); □■ – middle canopy (M); △▲ – top canopy (T)) on Chl *a+b* and Chl *a/b* throughout the growing season. Values are means ± 1 SE; *n* = 4.

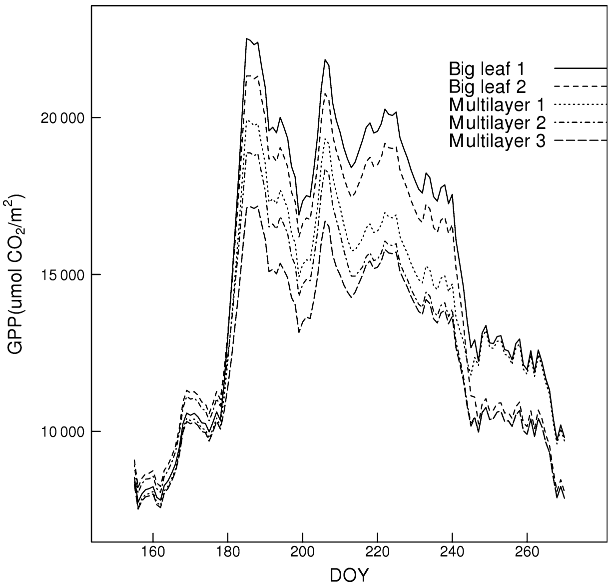

Fig. 6 Daily GPP estimated from day 168 to day 279 for control plots by different models (model description in Table 2). Each line was smoothed by averaging data for 7 days.

Table 2 Accumulated GPP from day 168 to day 279, estimated with different models which had V_{cmax} or light varying through time or among canopy

	Big leaf 1	Big leaf 2	Multilayer 1	Multilayer 2	Multilayer 3
V_{cmax}-Time	No	Yes	No	Yes	Yes
V_{cmax}-Canopy	No	No	Yes	No	Yes
Light-Canopy	No	No	Yes	Yes	Yes
GPP (kgC m^{-2})	5.6	5.3	5.1	4.8	4.6

Yes or no indicates whether V_{cmax} or light changes or does not change over time or through canopy.

growth is generally due to both an effect on photosynthesis and leaf growth (Gastal & Lemaire, 2002), which was mostly confirmed on C_3 species (MacDonald *et al.* 1986; Dreccer *et al.*, 2000). As pointed out previously (Sinclair & Horie, 1989), there is a trade-off between allocation of N to photosynthesis of existing leaves and allocation of N to develop additional leaf area. For Miscanthus, leaf extension rate has been reported to play a more critical role than single leaf photosynthesis in selecting more productive genotypes (Clifton-Brown & Jones, 1997). However, this theory has been poorly investigated; as a consequence, it remains unclear what the relative impact of N on growth is due to leaf area increase or to leaf and canopy photosynthesis for other species or functional types.

Alterations in leaf structure are an important mode of acclimation to shade in many species (BjÖrkman, 1981). Specific leaf area (SLA) of Miscanthus in this study increased from the top to the base of the canopy, as found in other species (Ellsworth & Reich, 1993; Evans & Poorter, 2001). It has been suggested that in fertilized plots, plants tend to have thinner or less dense leaves (Knops & Reinhart, 2000). Consistent with this we found that SLA was higher in fertilized plots than in control plots, which suggested that partitioning of leaf mass was related to both canopy light gradients and N fertilizations. From a canopy perspective, higher SLA allows a more extensive foliar display for a similar biomass investment in leaves, resulting in improved light absorption (Niinemets, 1998).

In previous studies Miscanthus photosynthesis at saturating light level ranged between 20 and 27 μmol m^{-2} s^{-1} on clear days between May and July and attained a peak mean value of 34 μmol m^{-2} s^{-1} in late

June in southern England (Beale *et al.*, 1996). Dohleman & Long (2009) and Dohleman *et al.* (2009) examined the diurnal variation of photosynthesis of Miscanthus on multiple dates across 2 years on a nearby field plot. Similar to this present study, they showed average values of A at about 30 μmol m^{-2} s^{-1} for Miscanthus. As found in other studies (Henskens *et al.*, 2001; Close *et al.*, 2004), net photosynthesis (A) in this study declined from the top to the lower canopy, which was attributed by many factors, including g_s, F_v'/F_m', $_qp$, J_{PSII}, k and V_{cmax}. We found that stomatal conductance (g_s), efficiency of PSII (F_v'/F_m'), the fraction of open PSII ($_qp$) and the electron transport rate (J_{PSII}) in the light reactions all decreased throughout the canopy. The photosynthetic acclimation within the canopy was also biochemical; i.e., V_{cmax} and k both increased within canopy height.

Contrary to our hypothesis, the photosynthetic processes were not altered by N fertilization. The effect of N on A and V_{cmax} of upper-canopy leaves is particularly important, since they contribute most to canopy photosynthesis. The results showed that there was no N effect on A and V_{cmax} on either the upper- or lower -canopy leaves (indicated by no N × CA effect in Table 1), though fertilization increased N_a and N_m for Miscanthus. This variability in the response of photosynthesis and N content to N fertilization may have several causes. First, not all N in the leaf is part of the photosynthetic machinery, and the inorganic N content in leaves may have been built up (Evans & Poorter, 2001; Lawlor, 2002). It has been shown that the fraction of non-photosynthetic N increased significantly with decreasing irradiation for *Chenopodium album* (Hikosaka & Terashima, 1996) and *Spinacia oleracea* (Evans, 1989) and decreased for *Betula pendula* (Eichelmann *et al.*, 2005). Secondly, the proportion of N allocation to Rubisco may not increase, as shown by the carboxylation efficiency, which was not altered by N fertilization. Thirdly, rubisco may not be fully active, and the enzyme protein may be used partially for N storage (Eichelmann *et al.*, 2005). Soluble sugar accumulation has been related to the down-regulation of A in several species, including tobacco (Paul & Driscoll, 1997), maize (Jeannette *et al.*, 2000), and *Poa alpine* L. (Baxter *et al.*, 1995). The more accumulated soluble sugar contents (mostly Suc, methods and results in supporting material S1 and S2) may have played a negative feedback on the photosynthetic capacity in the fertilized Miscanthus.

The distribution of N_a was positively affected by light levels at different canopy layers (confirmed by the significant relationship between N_a and light in S3), lending support to the light-nitrogen hypothesis (Ellsworth & Reich, 1993; Rosati *et al.*, 2000; Frak *et al.*, 2001). Variation in N_a was not solely a result of increasing SLA with decreasing height in the canopy (Hollinger, 1989; Rosati *et al.*, 2000), because N_m also increased significantly from the base to the top of the canopy (Table 1). These results suggest that the maximization of carbon gain should be studied by analyzing patterns of investment in both leaf dry weight per area and N_m. We proposed that more N will be allocated to the top canopy at the N-applied plot, considering that competition for light increases at high N when the competition for soil N decreases. However, in this study, N fertilization did not result in preferential allocation of N (indicated by no effect of N × CA in Table 1), but rather caused a general increase in N_m and N_a at all canopy levels which is consistent with other studies (Palmroth *et al.*, 2002; Calfapietra *et al.*, 2005), indicating that N allocation among leaves in a canopy is fixed regardless of N availability and does not contribute to adaptation to irradiance.

There is generally a trade-off between the capacities for light and carbon capture (Niinemets, 2006). In this study, leaf Chl *a+b* concentration did not change significantly from the base to the top canopy, but the relative content of Chl *a*/*b* decreased from the top to the base of the canopy. In weak light, optimization of leaf function calls for greater investment of leaf resources in light harvesting rather than energy processing. As a result the relative abundance of Chl *b* will increase and the Chl *a*/*b* ratio will be lower compared with that in strong light. The fraction of N allocation to chlorophyll a (indicated by Chl a/N_a, data not shown) was also higher at the lower canopy. The increase in N investment in the light-harvesting in the low light took place at the expense of Rubisco indicated by decreased rate of k and V_{cmax}, as shown in other studies (Eichelmann *et al.*, 2005).

Compared with single leaf model, multilayer models require knowledge of the spatial distribution of temperature, humidity, and boundary layer conductance (Leuning *et al.*, 1995), which is beyond the scope of this paper. In this present study, we aimed in providing information about the uncertainties introduced by using single leaf vs. multilayer model. While the model structure and many of the functions used in the model may be critical, choice of one parameter value for those functions alone can significantly affect model prediction. Many previous modeling studies of canopy photosynthesis assumes V_{cmax} invariant with time, usually using either a single mean value of V_{cmax} over the season or a single measurement of V_{cmax} for a brief period of time (Amthor *et al.* 1994; Williams *et al.*, 1994). With regard to multilayer models, most studies focused on PAR characterization, including the amount of direct and diffuse radiation and foliage angle, and contained simplified photosynthetic production functions (Kull & Kruijt, 1998; Larocque, 2002), which implies that all the leaves within the canopy are characterized by the same physiological responses. This assumption may result in an unrealistic representation of canopy photosynthesis, as biochemical, anatomical, and foliage characteristic vary substantially within the canopy. As indicated in this study the multilayer model without a canopy-depth dependent V_{cmax} (multilayer model 1) overestimated 6% GPP compared with the multilayer model 2 with a canopy-depth dependent V_{cmax}. When taking both time- and canopy-depth dependent V_{cmax}, multilayer model 3 predicts 20% less GPP than the big leaf model which has an averaged V_{cmax} taken at the top layer of the canopy. The results indicate that a better understanding of the photosynthetic process temporally and spatially could significantly alter the model in representing the canopy function and predicting the ecosystem productivity.

In conclusion, fertilization increased the yield of Miscanthus. Contrary to the hypothesis, greater productivity following fertilization resulted largely from increased canopy leaf area, but not from increased photosynthetic capacities. Photosynthetic parameters, such as A and V_{cmax}, were not affected by N fertilization, regardless whether photosynthetic measurements were taken from upper-, middle- or lower- canopy leaves. N fertilization did not favor N allocation in the upper canopy, rather caused a consistent increase of N concentration throughout the canopy. Photosynthetically, the acclimation to irradiance was both biochemical (increasing V_{cmax} and k with increasing light) and physiological (increasing g_s with increasing light). Morphological acclimation to light was achieved by decreasing SLA and increasing the ratio of Chl a/b with increasing light. Due to the difficulty of GPP measurements, the model results stress the need for systematic canopy-scale measurements of Miscanthus in the field over the entire growing season to parameterize and calibrate mechanistic models of biomass production under either control or fertilized conditions. The variation in biomass yield presumably created by increased soil N levels in response to fertilization provided an opportunity to estimate biochemical and physiological parameters and development of canopy leaf area in plots that differed by 40% for biomass yield during a single growing season in the Midwestern USA.

Acknowledgement

We thank Dr. Andrew Leakey for his constructive comments on an earlier draft of this paper. We thank Mike Masters and John Drake for their assistance with C/N measurements. This work was funded by the Energy Bioscience Institute.

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