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## Research paper

# How vertical patterns in leaf traits shift seasonally and the implications for modeling canopy photosynthesis in a temperate deciduous forest

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Leaf functional traits are used in modeling forest canopy photosynthesis ( $A_c$ ) due to strong correlations between photosynthetic capacity, leaf mass per area (LMA) and leaf nitrogen per area ( $N_{area}$ ). Vertical distributions of these traits may change over time in temperate deciduous forests as a result of acclimation to light, which may result in seasonal changes in  $A_c$ . To assess both spatial and temporal variations in key traits, we measured vertical profiles of  $N_{area}$  and LMA from leaf expansion through leaf senescence in a sugar maple (*Acer saccharum* Marshall) forest. To investigate mechanisms behind coordinated changes in leaf morphology and function, we also measured vertical variation in leaf carbon isotope composition ( $\delta^{13}C$ ), predawn turgor pressure, leaf water potential and osmotic potential. Finally, we assessed potential biases in  $A_c$  estimations by parameterizing models with and without vertical and seasonal  $N_{area}$  variations following leaf expansion. Our data are consistent with the hypothesis that hydrostatic constraints on leaf morphology drive the vertical increase in LMA with height early in the growing season; however, LMA in the upper canopy continued to increase over time during light acclimation, indicating that light is primarily driving gradients in LMA later in the growing season. Models with no seasonal variation in  $N_{area}$  overestimated  $A_c$  by up to 11% early in the growing season, while models with no vertical variation in  $N_{area}$  overestimated  $A_c$  by up to 60% throughout the season. According to the multilayer model, the upper 25% of leaf area contributed to over 50% of  $A_c$ , but when gradients of intercellular  $CO_2$ , as estimated from  $\delta^{13}C$ , were accounted for, the upper 25% of leaf area contributed to 26% of total  $A_c$ . Our results suggest that ignoring vertical variation of key traits can lead to considerable overestimation of  $A_c$ .

**Keywords:** *Acer saccharum*, leaf mass per area, leaf nitrogen, osmotic potential, predawn water potential, turgor pressure.

## Introduction

Two leaf functional traits, leaf nitrogen ( $N_{area}$ ) and leaf mass per area (LMA), are strong predictors of leaf photosynthetic capacity across biomes and within species and are used in modeling canopy photosynthesis ( $A_c$ ) and gross primary productivity (GPP) from leaf to global scales (Kull and Jarvis 1995, de Pury and Farquhar 1997, Raulier et al. 1999, Reich et al. 1999, Wright et al. 2004, Ryu et al. 2011). Canopy photosynthesis models that incorporate leaf functional traits can vary in spatial and temporal resolutions (Thornley and Johnson 1990, Kull and

Jarvis 1995, Raulier et al. 1999, Hanson et al. 2004, Medlyn 2004). The ‘big-leaf’ (BigL) model, for example, does not take into account spatial variability in leaf traits, but is quite versatile due to the lower number of necessary parameters, whereas the multilayer (ML) model requires information about vertical variation in leaf functional traits. Modeling canopy photosynthesis can also assume a constant value of leaf photosynthetic parameters and leaf traits, which may expedite modeling efforts at larger scales, but potentially lead to modeling bias during certain times of the growing season.

As leaves acclimate to light availability following leaf expansion, leaf nitrogen and LMA often scale with light, which leads to greater canopy photosynthesis when compared with a uniform distribution (Chen et al. 1993, Ellsworth and Reich 1993, Niinemets et al. 1998, 1999, Bond et al. 1999, Jones and Thomas 2007, Coble and Cavaleri 2014, 2015). Other factors can constrain leaf acclimation to light and associated leaf features that tend to optimize nitrogen distribution across light gradients (Kull 2002, Niinemets and Valladares 2004, Niinemets 2012, Buckley et al. 2013, Coble et al. 2014). For example, leaf water potential ( $\Psi_L$ ) gradients in tall western conifers and tropical trees may constrain leaf development and stomatal conductance at higher canopy positions, as indicated by increasing LMA and leaf carbon isotope composition ( $\delta^{13}\text{C}$ ) with height (Koch et al. 2004, Woodruff et al. 2004, Ishii et al. 2008, Cavaleri et al. 2010, Kenzo et al. 2015). As stomatal aperture decreases and  $\text{CO}_2$  becomes depleted in substomatal chambers, leaves become more enriched in  $^{13}\text{C}$  (Farquhar et al. 1989). The lack of osmotic adjustments and subsequent declines in leaf turgor pressure in tall *Pseudotsuga menziesii* (Mirb.) Franco trees may be one mechanism behind constraints on leaf development and greater LMA higher in the canopy (Woodruff et al. 2004, Meinzer et al. 2008). Reduced turgor can result in smaller, denser leaves because turgor pressure facilitates the expansion and division of cells (Hsiao 1973). In shorter temperate deciduous trees such as *Quercus rubra* L., hydraulic limitation may limit leaf expansion, ultimately reducing final leaf size for leaves in the upper canopy (Zwieniecki et al. 2004). Reduced leaf expansion may also be associated with cell wall extensibility, which decreases in response to lower  $\Psi_L$  in *Robinia pseudoacacia* L. (Zhang et al. 2011, 2012). Overall, limitations to leaf development may constrain leaf photosynthesis due to the negative effects of reduced intercellular air space on mesophyll conductance of  $\text{CO}_2$  (Flexas et al. 2008, Mullin et al. 2009, Oldham et al. 2010).

In addition to vertical spatial variation observed for leaf functional traits in forest canopies, studies show that LMA (Jurik 1986, Poorter et al. 2009, Coble and Cavaleri 2015), leaf nitrogen (Ellsworth and Reich 1992a, Kull et al. 1998, Migita et al. 2007), photosynthetic capacity (Miyazawa et al. 1998, Wilson et al. 2000, Miyazawa and Terashima 2001, Kitajima et al. 2002, Grassi et al. 2005, Yasamura et al. 2006), chlorophyll content (Koike et al. 2001, Zhang et al. 2007) and  $\delta^{13}\text{C}$  (Damesin et al. 1997, Damesin and Lelarge 2003, Helle and Schleser 2004) can change considerably throughout the growing season. This temporal variability may, in turn, influence the relationships between leaf functional traits (Anten et al. 1998, Migita et al. 2007, Coble and Cavaleri 2015) and light availability and introduce modeling biases.

A key assumption behind the BigL modeling approach is that photosynthetic capacity and  $N_{\text{area}}$  are directly proportional to absorbed irradiance along the canopy light gradient (Sellers et al. 1992, Amthor 1994, de Pury and Farquhar 1997), which has been suggested to maximize canopy photosynthesis (Hirose and

Werger 1987). Otherwise, estimation of the decline in  $N_{\text{area}}$  or photosynthetic capacity with canopy depth (i.e., coefficient of leaf nitrogen allocation; Hirose and Werger 1987) would require sampling along the canopy profile. Many studies have revealed that area-based photosynthetic capacity, LMA and  $N_{\text{area}}$  are not directly proportional to light availability throughout the canopy (Bond et al. 1999, Kull 2002, Ishii et al. 2008, Cavaleri et al. 2010, Niinemets 2012, Buckley et al. 2013, Coble et al. 2014), invalidating the assumption that the light extinction coefficient is an appropriate parameter for scaling leaf nitrogen and photosynthesis from the leaf to the canopy, and potentially leading to biased estimates of canopy photosynthesis. There is evidence that the pattern of increasing  $N_{\text{area}}$  with light availability enhances canopy photosynthesis compared with a uniform distribution, but does not necessarily maximize canopy photosynthesis due to environmental pressures such as wind, herbivory or height-related constraints (Hollinger 1996, Peltoniemi et al. 2012, Buckley et al. 2013). The BigL model also does not account for the fraction of shaded leaves lower in the canopy, potentially leading to biased estimates of canopy carbon fluxes when compared with eddy-flux estimates (Sprintsin et al. 2012). Collectively, the number of errors associated with BigL models has led to the development of 'two-leaf' (2L) models that account for sunlit and shaded fractions of leaves (de Pury and Farquhar 1997, Wang and Leuning 1998) and ML models that account for spatial details of the light environment and leaf function (Leuning et al. 1995, de Pury and Farquhar 1997, Medlyn 2004, Alton et al. 2007). The 2L modeling approach has been found to improve BigL models while producing similar canopy photosynthesis estimates when compared with the more detailed ML model (de Pury and Farquhar 1997, Wang and Leuning 1998). Thus, a 2L modeling approach has been implemented in many of the current large-scale models (Dai et al. 2004, Thornton and Zimmerman 2007, Houborg et al. 2009, Ryu et al. 2011). While the spatial component of modeling is often the focus in model comparison and modifications, many modeling efforts assume a fixed photosynthetic capacity throughout the growing season, potentially leading to model biases over the course of the growing season (Muraoka et al. 2010, Bauerle et al. 2012).

Considering the observed changes in leaf function over time and along canopy gradients, there is a need to assess the potential consequences of both temporal and spatial variation in leaf functional traits on canopy photosynthesis. The objective of this study was to identify seasonal changes in leaf functional traits in a deciduous broad-leaved tree species (*Acer saccharum* Marshall) and potential consequences of not accounting for this temporal variability in leaf function when modeling canopy photosynthesis. In addition, we compare BigL and 2L models with an ML model in order to identify potential modeling biases associated with assumptions regarding the distribution in leaf nitrogen within a forest canopy. We tested the following hypotheses: (i) leaf morphology is constrained due to greater hydrostatic tension in the upper canopy;

(ii) seasonal variation in area-based leaf nitrogen ( $N_{\text{area}}$ ) distribution is primarily dictated by seasonal changes in LMA, where upper canopy leaves require time to acclimate to high light availability; and (iii) modeled early-season canopy photosynthesis is overestimated when light acclimation and subsequent changes in leaf functional traits are not accounted for, and this bias is equally as important as not accounting for vertical gradients in leaf functional traits.

## Materials and methods

### Study site

This study was conducted at the Michigan Technological University Ford Center and Forest in Alberta, MI (46.65°N, 88.48°W). Mean annual temperature and precipitation at the Ford Forestry Center are 4.8 °C and 810 mm, respectively (NOAA, WS ID 15608). Sugar maple (*A. saccharum*) contributed 97% of tree density of this stand, which also included *Ulmus americana* L., *Tilia americana* L., *Betula alleghaniensis* Britton and *Ostrya virginiana* (Mill.) K. Koch. The mean height of the stand was 23.0 m and the mean height to live crown was 13.8 m. Additional details of this stand are described in Coble and Cavaleri (2014).

### Study design and timing of environmental and leaf measurements

We constructed a 19-m mobile aluminum walk-up tower (Upright, Inc., Selma, CA, USA) that provided crown access to a total of five trees. Sampling occurred in 2013 and along 10 tower platforms (including ground level) that were spaced ~1.8 m apart. Light availability measurements (details below) were made in two sampling periods following leaf expansion (22 July and 20 August), which were averaged to represent light conditions following leaf expansion in subsequent analysis. During leaf expansion from 29 May to 24 June, leaf area was repeatedly measured on the same leaves from two to three branches per height interval (10 height intervals). Toward the end of leaf expansion (17 June), two leaves at every other height interval (12 leaves total) were collected for measurements of predawn leaf water potential ( $\Psi_{\text{Pre}}$ ) and derived estimates of osmotic potential ( $\Psi_{\pi}$ ) and turgor pressure (detailed methods given below).

From leaf expansion to senescence, we used a combination of whole-leaf collection and hole-punches for leaf trait measurements to minimize the impact of repeated whole-leaf collection from the same tower locations and trees. For LMA measurements, we collected two to three hole-punches from two to three leaves (26 and 29 May; 1, 4, 7, 10, 17 and 24 June; 1, 8 and 22 July; 20 August) and two to three whole leaves (26 May; 1, 7 and 17 June; 1 and 22 July; 20 August; 14 and 30 September; 4 October) at each height interval per tree. Using a subset of the whole leaves, we measured leaf  $N_{\text{area}}$ , mass-based nitrogen ( $N_{\text{mass}}$ ) and  $\delta^{13}\text{C}$  at 7–11 height intervals on eight to nine dates throughout the growing season. Leaf gas exchange measurements were made at three height intervals (0, 11.5 and 20.3 m) on four sep-

arate days (6 and 16 June; 7 and 8 July) for a total of two to three leaves at each height interval (detailed methods given below).

### Environmental measurements

Leaf area index was measured at each height in the canopy using two plant canopy analyzers (LAI-2000 and LAI-2200, LI-COR, Inc., Lincoln, NE, USA) either in the hour following sunrise or in the hour preceding sunset. The LAI-2000 was mounted on a tripod in an open field (~400 m from the tower) to collect 'above canopy' measurements at 30-s intervals, and simultaneous below canopy measurements along the vertical tower transect were measured using an LAI-2200 with a 180° view cap to prevent the tower from obstructing the view. 'Above canopy' measurements were not taken from the top of the tower because it was shorter than adjacent trees. From the LAI measurements, we estimated the relative cumulative leaf area index (Relative LAI<sub>ABOVE</sub>) at each canopy height. Relative LAI<sub>ABOVE</sub> (0: top of canopy, 1: bottom of canopy) was estimated as the cumulative LAI at each height divided by the total canopy LAI. Air temperature was measured along the vertical tower transect at nine heights using temperature sensors covered with radiation shields at 10-min intervals (HOBO U23 ProV2, Onset Computer Corporation, Bourne, MA, USA).

Photosynthetic photon flux density (PPFD,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in the open field (described above) was measured at 10-min intervals using a photosynthetically active radiation light sensor (Onset Computer Corporation) mounted on a post. In 2013, PPFD data were only available from 16 July to 22 September, but a more complete data set was available in 2012 (11 May–19 October). We found that mean daily PPFD at Alberta, MI, was within 2.5% of the mean over a 15-year period (1991–2005) at a site in Hancock, MI, ~40 miles from the study (site ID 727440; National Solar Radiation Database, Department of Energy National Renewable Energy Laboratory). Thus, we merged the 2012 data with the existing 2013 data to develop a more complete data set of PPFD for canopy photosynthesis modeling. Corresponding air temperature data from 2012 that were measured in a nearby canopy (~1 km from the tower) were also merged with the 2013 data for canopy photosynthesis modeling.

### Leaf morphology

For leaf area measurements, three expanding leaves per branch were marked with one to three dots with an acid-free permanent paint marker (Sharpie, Newell Rubbermaid, Oak Brook, IL, USA) in order to identify leaves for repeated measurements. Leaves were placed next to a ruler between two sheets of plexiglass, and black felt was placed under each leaf to enhance contrast along leaf margins. Photographs were taken when the plexiglass was perpendicular to the line of sight of the camera and analyzed for leaf area using ImageJ v1.44j (Schneider et al. 2012). A few leaves were excluded from the analysis due to branch dieback.

Samples used for LMA measurements were placed in sealed plastic bags immediately following hole-punch and whole-leaf



collections. Areas of leaf discs were measured with digital calipers. Whole-leaf area was measured by digitally scanning leaves using an Epson Expression 10000XL flatbed scanner (Seiko Epson Corporation, Nagano, Japan), and analyzing images using ImageJ v1.44j (Schneider et al. 2012). Leaves were dried at 65 °C for 48 h and weighed to the nearest 0.1 mg for calculating LMA [leaf dry mass (g)/leaf area (m<sup>2</sup>)]. For a subset of leaf samples, LMA of hole-punches was strongly correlated ( $R^2 = 0.99$ ) with LMA of the same whole leaves, but LMA of the small discs was consistently lower by  $2.41 \pm 0.63 \text{ g m}^{-2}$ , as derived from the intercept of the regression between hole-punch discs and whole-leaf LMA. Thus, we adjusted LMA of hole-punches by adding  $2.41 \text{ g m}^{-2}$ .

### Leaf water relations

Leaves were collected between 04:30 and 05:30 h for in situ  $\Psi_{\text{Pre}}$  measurements using a pressure chamber (PMS Instrument, Co., Corvallis, OR, USA). Leaves were cut near the base of the petiole, placed in sealed plastic bags until  $\Psi_{\text{Pre}}$  measurements were taken near the base of the tower (within 1 h), and stored in plastic bags until initial fresh weight (g) measurements were taken in the laboratory within 10–20 min of the  $\Psi_{\text{Pre}}$  measurements.

To estimate leaf  $\Psi_{\pi}$  and turgor pressure ( $\Psi_p$ ), we developed pressure–volume curves by plotting relative water content (RWC, %) against the inverse of  $\Psi_L$  ( $-1/\Psi_L$ ) as measured from dehydrating leaves on the laboratory bench (Tyree and Hammel 1972, Turner 1981). Following in situ  $\Psi_{\text{Pre}}$  measurements,  $\Psi_L$  and fresh weight measurements were collected approximately every 2 h for each leaf until  $\Psi_L$  values exceeded  $-3.0 \text{ MPa}$ . Leaves were not brought to full turgor prior to generating pressure–volume curves to avoid biased  $\Psi_{\pi}$  associated with artificial rehydration and excessive apoplastic water (Kubiske and Abrams 1990, 1991, Woodruff et al. 2004). Saturated leaf weight (necessary for estimates of RWC) was estimated by plotting leaf fresh weight by  $\Psi_L$  and extrapolating to zero  $\Psi_L$  (Kubiske and Abrams 1990). Leaves were then placed in a drying oven at 65 °C for 48 h and weighed to the nearest 0.1 mg for leaf dry weight. Turgor loss point was identified for each pressure–volume curve as the transition between curved and linear portion curve (Kubiske and Abrams 1990, 1991). For each leaf, a regression equation [ $-1/\Psi_L = \beta_1(100 - \text{RWC}) + \beta_0$ , where  $\beta_0$  = inverse of  $\Psi_{\pi}$  at full turgor] was developed for the linear portion below the turgor loss point. To estimate  $\Psi_{\pi}$ , we used the regression equation above and the RWC value that corresponded with the initial fresh weight measurement (in situ weight). The difference between in situ  $\Psi_{\text{Pre}}$  and  $\Psi_{\pi}$  was calculated to estimate  $\Psi_p$  (Woodruff et al. 2004, Zhang et al. 2012).

### Leaf chemistry and photosynthesis

Leaf nitrogen, carbon and stable carbon isotope composition for sampled leaves were measured at the Michigan Technological University Forest Ecology Stable Isotope Laboratory. Dried whole

leaves collected from each tower section from each day from each tree were combined (~3–4 leaves) and ground to a fine powder (8000 M Mixer/Mill, Spex SamplePrep LLC, Metuchen, NJ, USA). Mass-based leaf nitrogen and carbon and stable carbon isotope composition ( $\delta^{13}\text{C}$  ‰) were determined using a Costech Elemental Combustion System 4010 connected to a Thermo Finnigan ConFloIII Interface and Delta + Continuous Flow-Stable Isotope Ratio Mass Spectrometer (Thermo Scientific, Waltham, MA, USA). Leaf  $N_{\text{area}}$  (g N m<sup>-2</sup>) was determined as the product of  $N_{\text{mass}}$  and LMA divided by 100. Leaf  $\delta^{13}\text{C}$  was calculated as  $\delta^{13}\text{C} = 1000(R_{\text{sample}}/R_{\text{standard}} - 1)$  (‰), where  $R_{\text{sample}}$  was the  $^{13}\text{C}/^{12}\text{C}$  of the sample, and  $R_{\text{standard}}$  was the ratio for a standard. The analytical precision for the  $\delta^{13}\text{C}$  measurements was  $\pm 0.25$ ‰.

Photosynthesis measurements were taken with a LI-6400XT (LI-COR Biosciences) infrared gas analyzer on attached branches from the tower. Relative humidity was kept between 50 and 60%, and  $\text{CO}_2$  concentrations were at 400 p.p.m. Prior to measurements, leaves were allowed to equilibrate to maximum light conditions of  $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  until photosynthesis and stomatal conductance was stable. We measured net photosynthesis at the following PPFD values: 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 100 and  $0 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . The curvature of light response of electron transport ( $\theta$ ) and the quantum yield of electron transport ( $\alpha$ ; assuming four electrons needed per  $\text{CO}_2$  molecule fixed; Valentini et al. 1995) were both estimated from the light response curves.

### Canopy photosynthesis models

We assessed the potential effects of seasonal changes in leaf functional traits on canopy photosynthesis using three models: BigL, 2L and ML models, and each of the three models were run under two conditions. The first set of models did not account for seasonal changes in leaf functional traits (BigL, 2L and ML), and the second set of models did account for seasonal changes (BigL-Seas, 2L-Seas, ML-Seas; ‘Seas’ for seasonal changes). The BigL and 2L models were based on the models of de Pury and Farquhar (1997), and the ML models were based on the model of Leuning et al. (1995). The 2L model of de Pury and Farquhar (1997) forms the basis of many of the recent large-scale modeling approaches in predicting carbon flux (Dai et al. 2004, Thornton and Zimmerman 2007, Houborg et al. 2009, Sprintsin et al. 2012). The mathematical equations and approaches to modeling leaf and canopy photosynthesis ( $A_c$ ) are described in detail in Canopy photosynthesis model section available as Supplementary Data at *Tree Physiology* Online. Briefly, all models incorporated the Farquhar–von Caemmerer–Berry biochemical model (Farquhar et al. 1980) that estimates photosynthesis based on limitations by Rubisco activity or regeneration of ribulose-1,5-bisphosphate. Three key parameters involved in the photosynthesis model include the maximum rate of Rubisco activity ( $V_{\text{cmax}}$ ), the potential rate of electron transport

( $J_{\max}$ ), dark respiration ( $R_d$ ) and the intercellular  $\text{CO}_2$  concentration ( $C_i$ ) (Farquhar et al. 1982, 1989). Maximum rate of Rubisco activity was estimated using  $N_{\text{area}}$  as measured in this study,  $J_{\max}$  was estimated from the ratio of  $J_{\max}$  to  $V_{\text{cmax}}$ ,  $R_d$  was estimated from the ratio of  $R_d$  to  $V_{\text{cmax}}$  and  $C_i$  was estimated from leaf  $\delta^{13}\text{C}$  and an assumed air  $\delta^{13}\text{C}$ . The BigL and 2L models scale  $V_{\text{cmax}}$ ,  $J_{\max}$  and  $R_d$  to the canopy using  $N_{\text{area}}$  of the topmost leaves of the canopy ( $N_o$ ) and the extinction coefficient of  $N_{\text{area}}$  ( $k_n$ ) with  $\text{LAI}_{\text{ABOVE}}$  (see Environmental measurements) as measured in this study. The extinction coefficient of  $N_{\text{area}}$  ( $k_n$ ) describes the rate of change in  $N_{\text{area}}$  with relative  $\text{LAI}_{\text{ABOVE}}$  (Hirose and Werger 1987). These parameters are then used to predict canopy photosynthesis ( $A_c$ ) using the series of equations associated with the Farquhar–von Caemmerer–Berry model (see Tables S1 and S2 available as Supplementary Data at *Tree Physiology* Online). The primary difference between the BigL and 2L models is that the 2L model predicts canopy photosynthesis for both sunlit and shaded leaves.

The ML model predicts leaf  $V_{\text{cmax}}$ ,  $J_{\max}$  and  $R_d$  for sunlit and shaded leaves at multiple canopy layers using height-specific  $N_{\text{area}}$  values, models leaf photosynthesis using these parameters and then scales leaf photosynthesis to the canopy using the fraction of sunlit and shaded leaves and LAI values of each canopy layer to estimate  $A_c$ . Two driving environmental parameters, absorbed irradiance at the leaf surface ( $I_a$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and air temperature, were incorporated into all models. In all models, we accounted for the diffuse and direct radiation absorbed by leaves, and the absorbed irradiance for sunlit and shaded leaves were estimated in the 2L and ML models. Models that accounted for seasonal changes in leaf traits allowed  $k_n$  and  $N_o$  to vary based on seasonal and measured changes in leaf traits, whereas models that did not account for seasonal changes used one  $k_n$  and  $N_o$  value that were estimated based on leaf trait values at one point in time in the growing season (22 July). The date 22 July was selected because it corresponds with a leaf collection made during the middle of the growing season that occurred after leaf maturation, and the  $k_n$  value (0.97) at this point in time was similar to the summer average  $k_n$  (1.0) (see Table S3 available as Supplementary Data at *Tree Physiology* Online).

### Data analysis

Leaf area expansion rates along the vertical gradient (see Figure S1 available as Supplementary Data at *Tree Physiology* Online) were assessed by fitting a three-parameter asymptotic sigmoid function for leaf area as function of days following bud break (Day):

$$\text{Leaf area} = \frac{\text{Final leaf area}}{1 + e^{-b(\text{Day}-c)}}, \quad (1)$$

where Final leaf area ( $\text{cm}^2$ ) represents the leaf area value at the asymptote as the denominator ( $1 + e^{-b(\text{Day}-c)}$ ) approaches 1. Parameters  $b$  and  $c$  are coefficients that describe the steepness

of the curve and the number of days to reach 50% of final leaf area ( $\text{Days}_{50\% \text{Area}}$ ), respectively. Using this equation, we estimated the number of days to reach 95% of final leaf area ( $\text{Days}_{95\% \text{Area}}$ ) by setting leaf area divided by final leaf area equal to 0.95 and solving for Day. We plotted mean leaf area as a function of days after bud break within canopy positions using Eq. (1) in order to display general patterns in leaf expansion among canopy positions. We used regression analysis to develop relationships between height and final leaf area,  $\text{Days}_{50\% \text{Area}}$  and  $\text{Days}_{95\% \text{Area}}$ , which were averaged across leaves within each height.

In presenting seasonal patterns in LMA,  $N_{\text{area}}$ ,  $N_{\text{mass}}$  and  $\delta^{13}\text{C}$  at three canopy positions (lower, mid- and upper canopy), we averaged values from 1.3 to 5.2 m (lower), 7.1 to 12.6 m (mid-) and 14.5 to 20.3 m (upper) at each point in time. We separated seasonal changes in leaf functional traits into four groups during distinct seasonal phases of the growing season: leaf expansion (26 May–9 June), early growing season (10 June–7 July), late growing season (8 July–29 September) and senescence (30 September–4 October). We used linear and nonlinear regression analysis to develop relationships between the independent variables, height and light, and the response variables, LMA,  $N_{\text{area}}$ ,  $N_{\text{mass}}$  and  $\delta^{13}\text{C}$ . We used  $t$ -tests to determine significance among slopes and intercepts and to identify seasonal changes. In order to assess seasonal patterns in the vertical distribution in leaf area, leaf area density ( $\text{m}^2 \text{m}^{-3}$ ) was calculated by dividing cumulative LAI ( $\text{m}^2 \text{m}^{-2}$ ) of each layer by the height of each tower platform (1.83 m). The percent difference of  $A_c$  between models was calculated as:

$$\% \text{Difference} = \left[ \frac{A_1 - A_2}{(A_1 + A_2)/2} \right] \times 100, \quad (2)$$

where  $A_1$  and  $A_2$  are the corresponding daily canopy photosynthesis values as estimated from two separate models. R statistical software was used for all statistical analysis and model simulations (R Development Core Team 2013).

## Results

### Leaf area expansion and leaf water relations

The increase in leaf area displayed a sigmoid function at each canopy position (see Figure S1 available as Supplementary Data at *Tree Physiology* Online). The number of days to reach 50 and 95% of final leaf area increased with height, showing that leaf expansion rates decreased with height. Near the end of leaf expansion, predawn water potential ( $\Psi_L$ ) linearly decreased with height, and the decline of  $\Psi_L$  with height ( $-0.011 \text{ MPa per meter}$ ) was similar to the gravitational component ( $-0.01 \text{ MPa per meter}$ ). Leaf  $\Psi_\pi$  linearly decreased with height, while  $\Psi_p$  linearly increased with height (Figure 1).

### Seasonal changes in leaf morphology and chemistry with light and height

Leaf mass per area decreased during the leaf expansion phase followed by an increase, with the upper canopy displaying the largest increase in LMA and the greatest maximum LMA, while the mid- and lower canopy showed gradual increases in LMA following leaf expansion (Figure 2a). Leaf mass per area decreased during leaf senescence, which began prior to the last two leaf collection periods (30 September and 4 October). Leaf mass per area exponentially decreased with relative  $LAI_{ABOVE}$  (Figure 2b), and the slope of this relationship decreased from leaf expansion through the late season, but did not change during senescence (Table 1). The intercept of this relationship increased from the early to late growing season (Table 1). Leaf mass per area linearly increased with height during leaf expansion and exponentially increased with height for the remainder of the growing season (Figure 2c). The slope of this relationship also increased from leaf expansion through the late season, but the intercept did not change over time (Table 1).

Leaf nitrogen per area followed slightly different patterns with time, relative  $LAI_{ABOVE}$  and height than observed for LMA (Figure 3a–c). Leaf  $N_{area}$  decreased during the leaf expansion phase and senescence at all canopy positions (Figure 3a). Following leaf expansion and prior to senescence, leaf  $N_{area}$  steadily increased for the upper canopy, did not change over time for the mid-canopy and decreased in the lower canopy (Figure 3a). Leaf nitrogen per area exponentially decreased with relative  $LAI_{ABOVE}$  during the early and late seasons, but  $N_{area}$  did not change with relative  $LAI_{ABOVE}$  during leaf expansion and senescence (Figure 3b). The slope of this relationship decreased from leaf expansion to the late growing season, while the intercept decreased from the late growing season through leaf senescence (Table 1). Leaf nitrogen per area exponentially increased

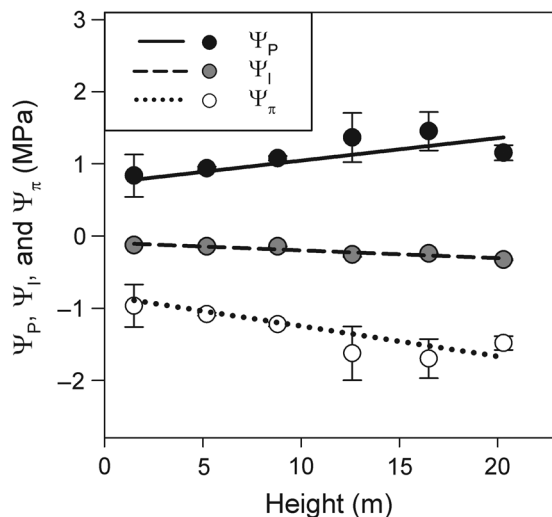


Figure 1. Relationships between height and  $\Psi_p$ ,  $\Psi_l$  and  $\Psi_\pi$  (MPa) on 17 June near the end of leaf expansion.  $R^2$  and  $P$ -values:  $\Psi_p$ ,  $R^2 = 0.58$ ,  $P < 0.05$ ;  $\Psi_l$ ,  $R^2 = 0.87$ ,  $P < 0.01$ ;  $\Psi_\pi$ ,  $R^2 = 0.74$ ,  $P < 0.01$ .

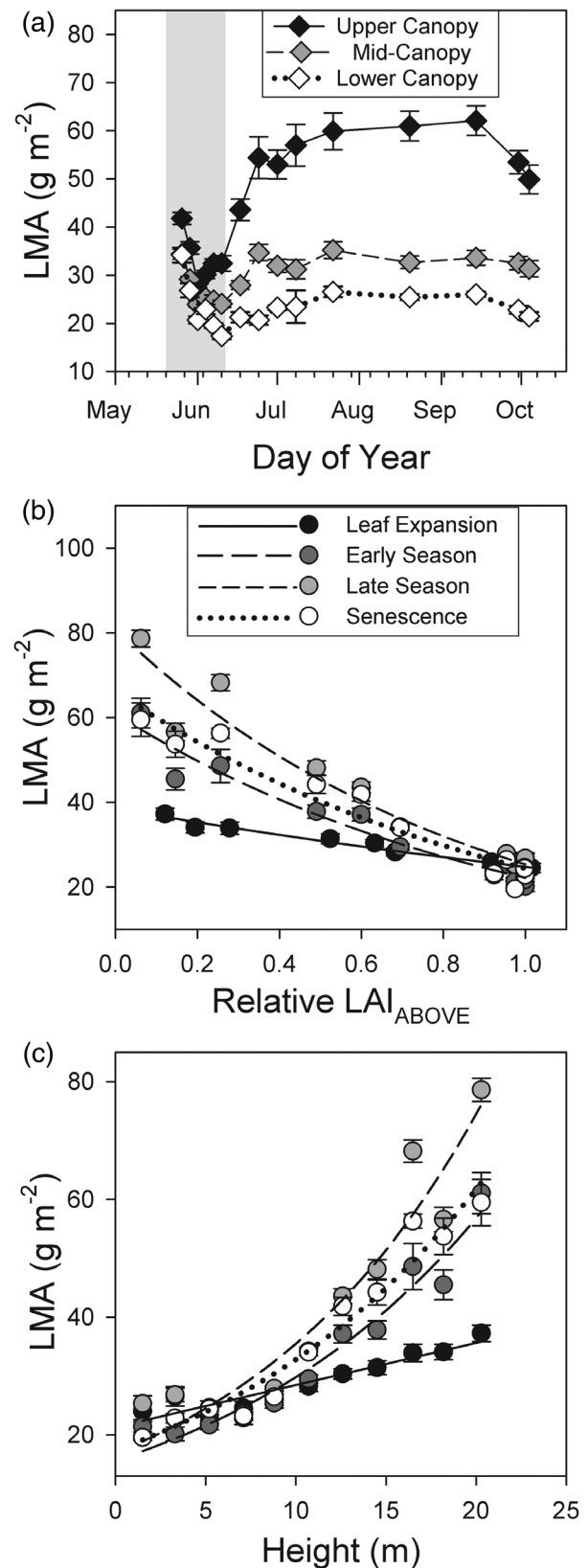


Figure 2. Changes in LMA ( $\text{g m}^{-2}$ ) at three canopy positions beginning on 26 May and ending on day 4 October (a). The gray bar represents the leaf expansion phase. Relationships between relative  $LAI_{ABOVE}$  and LMA (b) and height and LMA (c) during four phases of the growing season.

Table 1. Summary of slopes and intercepts for the relationships among height, relative  $LAI_{ABOVE}$ ,  $LMA$ ,  $N_{area}$ ,  $N_{mass}$  and  $\delta^{13}C$  for four parts of the growing season: leaf expansion, early, late and senescence (see Materials and methods for full description). Different letters indicate significant differences in slopes or intercepts within each row among the four seasons. Asterisks indicate that the slope or intercept is significantly different from zero.  $**P < 0.01$ ,  $***P < 0.001$ .

Response variable	Independent variable	Slopes of relationship ( $\pm SE$ ) Intercepts of relationships ( $\pm SE$ )			
		Expansion	Early	Late	Senescence
(ln)LMA ( $g\ m^{-2}$ )	$LAI_{ABOVE}$	*** $-0.45 \pm 0.04^a$ *** $3.65 \pm 0.03^a$	*** $-0.98 \pm 0.04^b$ *** $4.10 \pm 0.03^b$	*** $-1.16 \pm 0.04^c$ *** $4.40 \pm 0.03^c$	*** $-1.01 \pm 0.05^{bc}$ *** $4.21 \pm 0.04^b$
	Height (m)	*** $0.03 \pm 0.00^a$ *** $3.05 \pm 0.03^c$	*** $0.06 \pm 0.00^b$ *** $2.79 \pm 0.06^a$	*** $0.07 \pm 0.00^c$ *** $2.86 \pm 0.07^a$	*** $0.06 \pm 0.00^{bc}$ *** $2.82 \pm 0.06^a$
(ln) $N_{area}$ ( $g\ m^{-2}$ )	$LAI_{ABOVE}$	$-0.35 \pm 0.24^a$ $0.43 \pm 0.18^b$	*** $-0.59 \pm 0.06^{ab}$ *** $0.24 \pm 0.05^{ab}$	*** $-0.81 \pm 0.05^b$ *** $0.38 \pm 0.04^b$	$-0.59 \pm 0.29^{ab}$ $-0.11 \pm 0.22^a$
	Height (m)	$0.02 \pm 0.01^a$ $-0.01 \pm 0.17^c$	*** $0.03 \pm 0.00^{ab}$ *** $-0.52 \pm 0.05^b$	*** $0.05 \pm 0.00^b$ *** $-0.67 \pm 0.04^b$	** $0.05 \pm 0.02^b$ *** $-1.05 \pm 0.19^a$
$N_{mass}$ ( $mg\ g^{-1}$ )	$LAI_{ABOVE}$	$4.16 \pm 4.89^a$ *** $40.47 \pm 3.55^c$	*** $11.19 \pm 1.99^a$ *** $20.53 \pm 1.46^b$	*** $8.53 \pm 1.15^a$ *** $17.32 \pm 0.84^{ab}$	$8.43 \pm 4.21^a$ ** $12.78 \pm 3.18^a$
	Height (m)	$-0.19 \pm 0.28^a$ *** $45.36 \pm 3.52^c$	*** $-0.67 \pm 0.12^a$ *** $35.18 \pm 1.46^b$	*** $-0.47 \pm 0.08^a$ *** $28.06 \pm 0.94^a$	$-0.31 \pm 0.28^a$ *** $21.89 \pm 3.41^a$
$\delta^{13}C$ (‰)	$LAI_{ABOVE}$	*** $-5.78 \pm 0.89^a$ *** $-23.51 \pm 0.65^a$	*** $-5.08 \pm 0.39^a$ *** $-25.48 \pm 0.28^b$	*** $-5.61 \pm 0.21^a$ *** $-25.86 \pm 0.21^b$	*** $-4.94 \pm 0.45^a$ *** $-26.67 \pm 0.33^b$
	Height (m)	*** $0.30 \pm 0.04^a$ *** $-30.51 \pm 0.56^b$	*** $0.28 \pm 0.02^a$ *** $-31.71 \pm 0.30^a$	*** $0.30 \pm 0.02^a$ *** $-32.66 \pm 0.30^a$	*** $0.26 \pm 0.04^a$ *** $-32.61 \pm 0.54^a$

with height during the early and late growing seasons, but did not change with height during leaf expansion and senescence (Figure 3c). The slope and intercept of this relationship increased and decreased, respectively, over time (Table 1). Leaf  $N_{mass}$  decreased at all canopy positions during the first half of the growing season (Figure 3d). Leaf  $N_{mass}$  linearly increased with relative  $LAI_{ABOVE}$  and linearly decreased height throughout most of the growing season during the early and late growing seasons and did not change with light or height during leaf expansion and senescence (Figure 3e and f). The slope of the relationships between  $N_{mass}$ , relative  $LAI_{ABOVE}$  and height did not change throughout the growing season, but the intercepts decreased from leaf expansion through leaf senescence (Table 1). Leaf  $\delta^{13}C$  decreased through time at all canopy positions (Figure 4a) and was consistently greater and more variable in the upper canopy position. Leaf  $\delta^{13}C$  linearly decreased with relative  $LAI_{ABOVE}$  (Figure 4b) and linearly increased with height throughout the growing season (Figure 4c). The slope of the relationship between height and  $\delta^{13}C$  did not change through time, and the intercept decreased following leaf expansion (Table 1).

### Modeling leaf functional traits and canopy photosynthesis

We compared BigL, 2L and ML models that did and did not account for seasonal changes in  $N_{area}$  to assess any bias for not accounting for seasonal variation in  $N_{area}$ . Seasonal variations in  $A_c$  were similar among the BigL and BigL-Seas, 2L and 2L-Seas, and ML and ML-Seas (Figure 5a–c). The largest percent differences in daily  $A_c$  between models that did and did not account for seasonal variation ranged from 7 to 11% (Figure 5d). There were no differences in modeled  $A_c$  at the corresponding time of

the reference  $N_{area}$  on 22 July for all models (Figure 5d). The percent differences in monthly  $A_c$  earlier in the growing season (12 June–12 July) between models ranged from 2 to 5%, while the percent differences in monthly  $A_c$  later in the growing season (12 July–12 August) were <1% between BigL and BigL-Seas, 2L and 2L-Seas, and ML and ML-Seas (Figure 5d). Corresponding changes in  $k_n$  are provided in Table S3 available as Supplementary Data at [Tree Physiology Online](http://www.treephysiology.org).

We next compared BigL, 2L and ML models to assess any bias for not accounting for details of the canopy profile. The general patterns in  $A_c$  among the BigL, 2L and ML models were similar (Figure 6a). However,  $A_c$  as estimated with the ML models was consistently lower than  $A_c$  as with the 2L and BigL models, particularly during days with high  $A_c$  (Figure 6a). The mean percent difference in  $A_c$  between BigL and ML models was 51%, and the mean percent difference between the 2L and ML models was 31%. Overall, biases associated with spatial variation in  $N_{area}$  (Figure 6b) were consistently greater than biases associated with seasonal variation in  $N_{area}$  (Figure 5d).

We assessed the leaf area distribution and contribution of each canopy layer to total  $A_c$  over time using the ML-Seas model. Leaf area density was lower during leaf expansion, did not markedly change following leaf expansion in July and August and was generally greatest in the middle of the canopy (Figure 7a). In contrast, the contribution to total  $A_c$  was greater at higher canopy positions when a constant  $C_i$  was assumed along the vertical gradient (Figure 7b). In fact, the upper 25% of leaf area contributed 57% to total  $A_c$  in August, and the contribution to  $A_c$  increased over time for upper canopy leaves beginning in June, but decreased for mid-canopy leaves (Figure 7b). However,

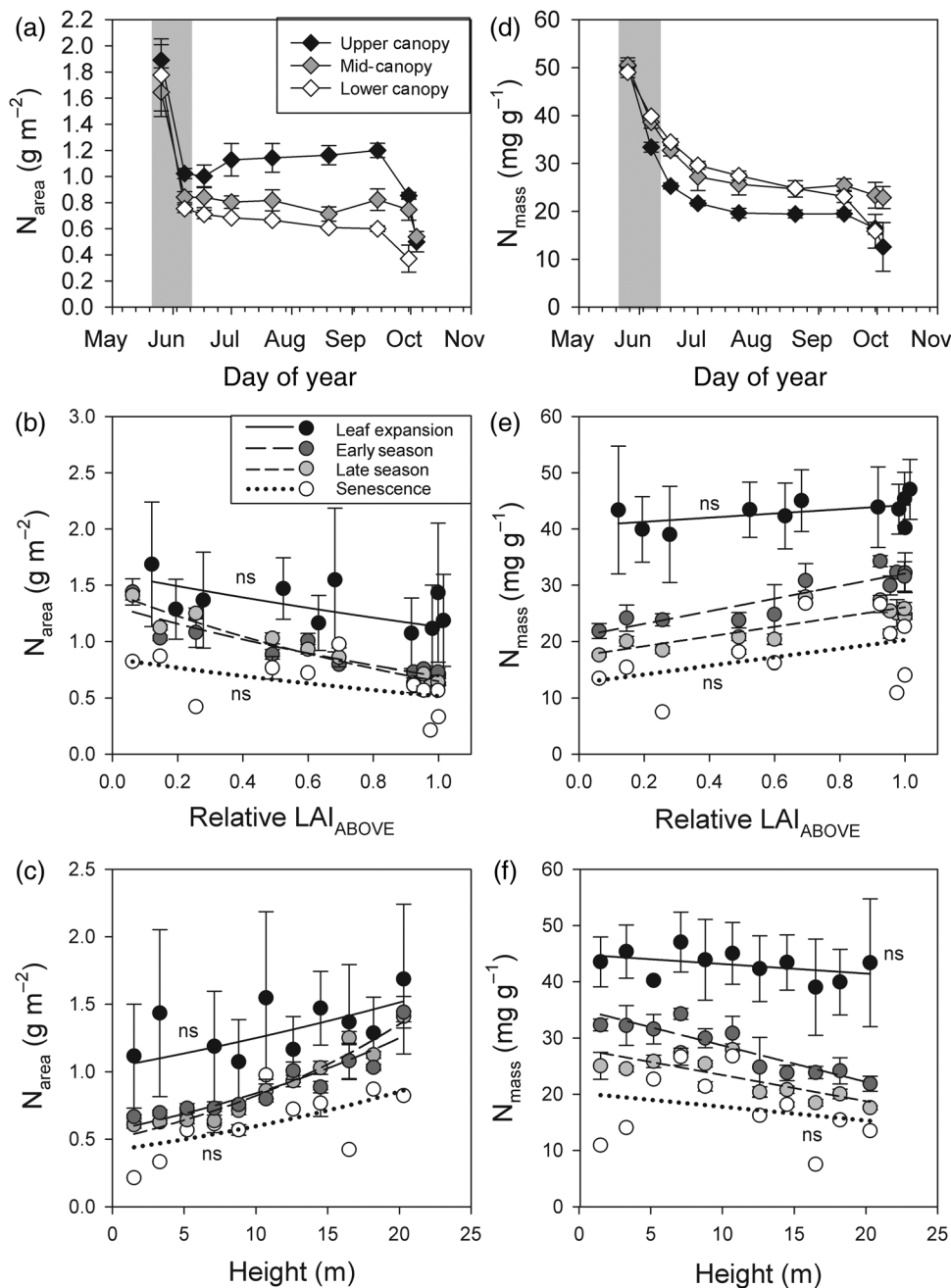


Figure 3. Changes in  $N_{\text{area}}$  ( $\text{g m}^{-2}$ ) and  $N_{\text{mass}}$  ( $\text{mg g}^{-1}$ ) at three canopy positions beginning on 26 May and ending on 4 October (a and d). The gray bar represents the leaf expansion phase. Relationships between relative  $\text{LAI}_{\text{ABOVE}}$ ,  $N_{\text{area}}$  and  $N_{\text{mass}}$  (b and e) and height,  $N_{\text{area}}$  and  $N_{\text{mass}}$  (c and f) during four phases of the growing season.

when we accounted for decreasing  $C_i$  with increasing height, we found that the upper 25% of leaf area contributed 26% to total  $A_c$  in August (Figure 7c).

## Discussion

### Vertical and seasonal variation in leaf functional traits

Our results provide support for our first hypothesis that limitations on leaf morphology higher in the canopy early in the growing season were due to greater hydrostatic tension. Leaf

expansion and morphology appeared to be constrained in the upper canopy as indicated by lower expansion rates (see Figure S1 available as Supplementary Data at *Tree Physiology* Online) and a linear increase in LMA with height, which was inconsistent with the exponential trend of increasing light availability with height. This may be due to declining  $\Psi_L$  with height during leaf expansion or biophysical properties of cells, which influence the force required to irreversibly expand cells (Lockhart 1965, Cosgrove 2000). Coble and Cavaleri (2015) observed a linear decrease in midday  $\Psi_L$  with height that corresponded with a



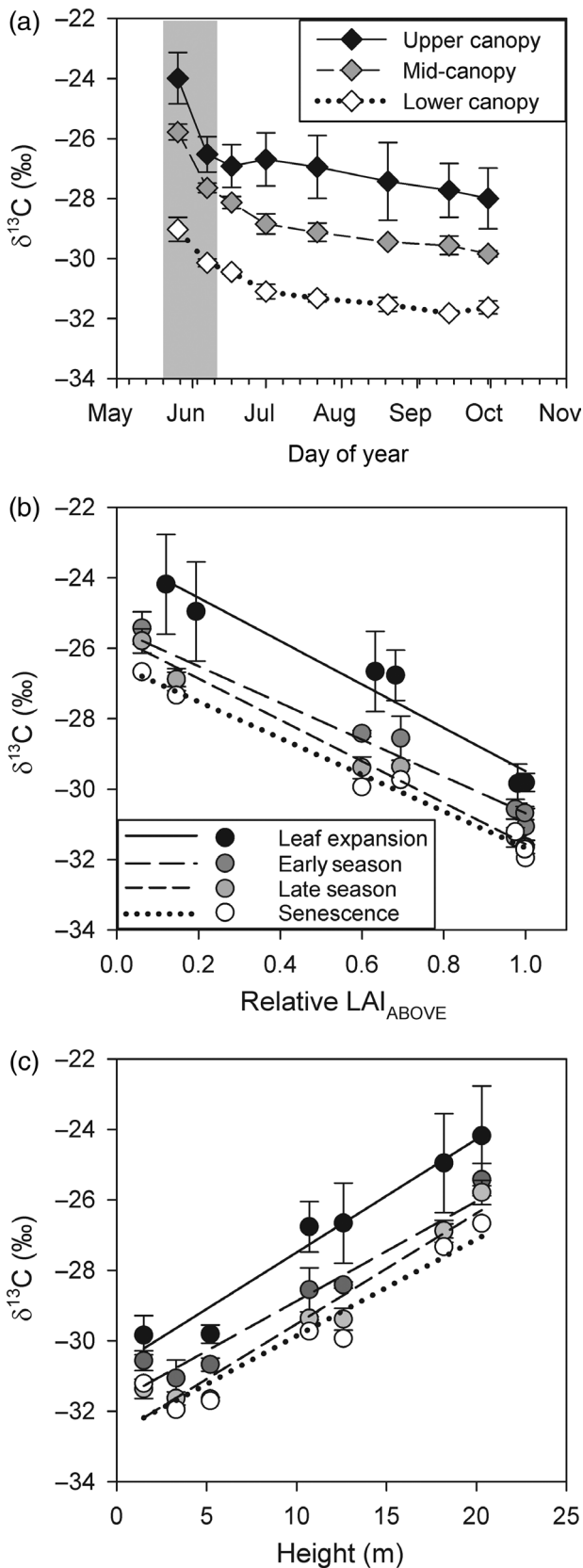


Figure 4. Changes in  $\delta^{13}\text{C}$  (‰) at three canopy positions beginning on 26 May and ending on 4 October (a). The gray bar represents the leaf expansion phase. Relationships between relative LAI<sub>ABOVE</sub> and  $\delta^{13}\text{C}$  (b) and height and  $\delta^{13}\text{C}$  (c) during four phases of the growing season.

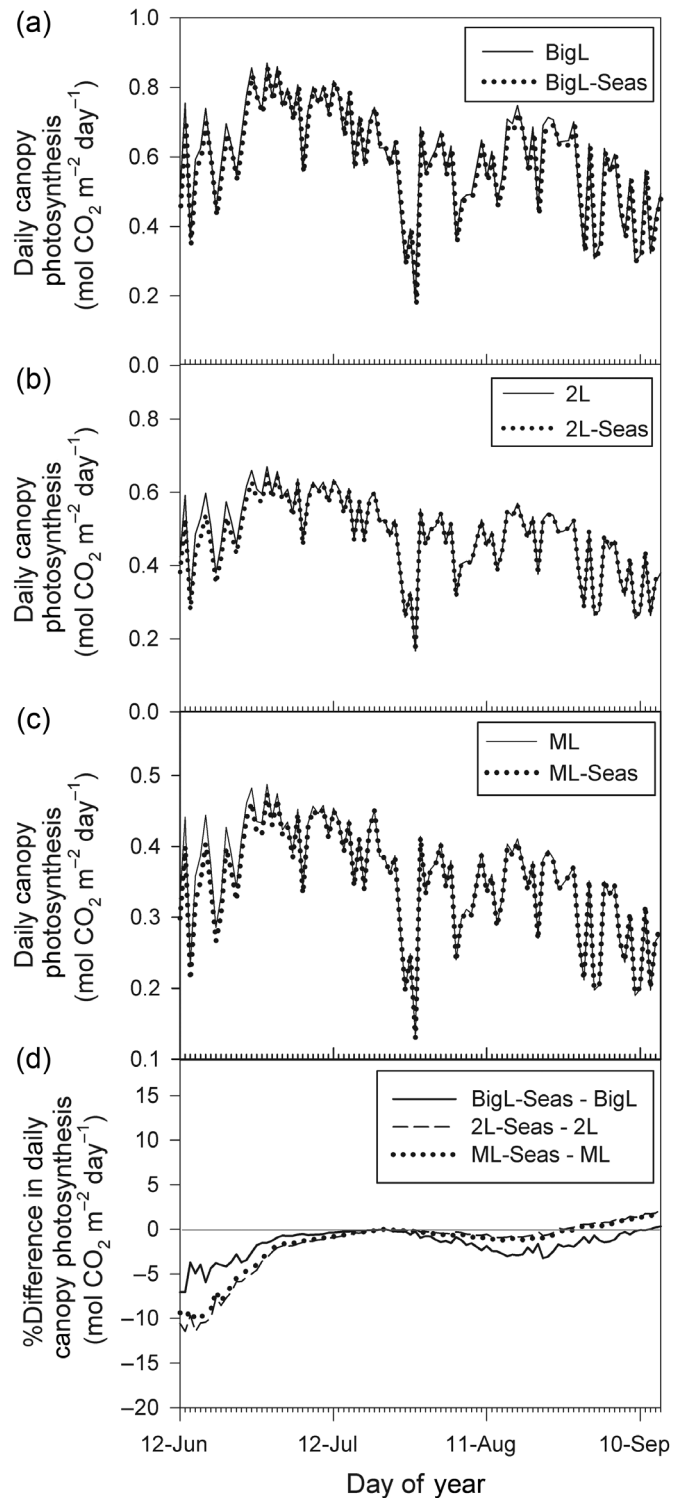


Figure 5. Simulated daily canopy photosynthesis (mol CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>) using a BigL, 2L and ML models that did (BigL-Seas, 2L-Seas and ML-Seas) and did not (BigL, 2L and ML) account for seasonal changes in  $N_{\text{area}}$  distribution (a–c). Percent differences in daily canopy photosynthesis between models that did and did not account for seasonal changes in  $N_{\text{area}}$  distribution (d). The time period investigated does not include the period of leaf expansion and senescence. Negative and positive differences between models (d) indicate that models that do not account for seasonal changes (BigL, 2L and ML) overestimate and underestimate canopy photosynthesis, respectively.

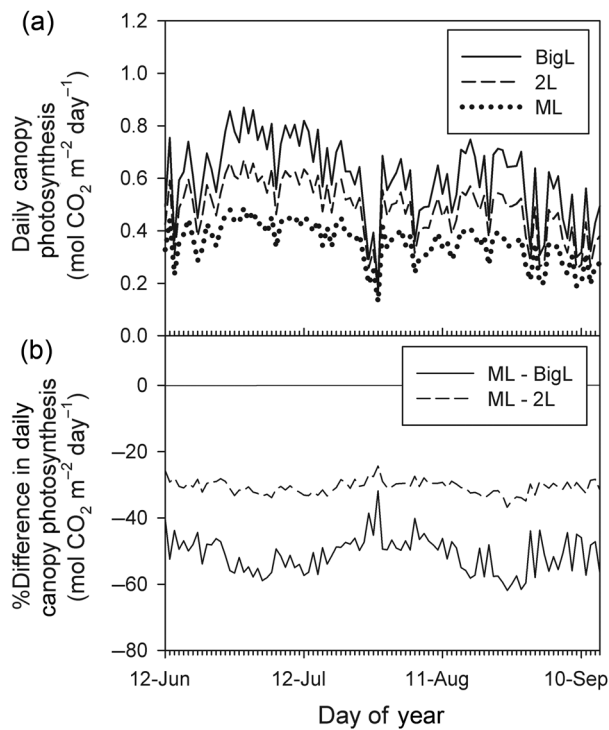


Figure 6. Simulated daily canopy photosynthesis ( $\text{mol CO}_2 \text{ m}^{-2} \text{ day}^{-1}$ ) for the BigL, 2L and ML models (a). Differences in daily canopy photosynthesis using models that did (ML) and did not (BigL and 2L) account for the detailed distribution of  $N_{\text{area}}$  along the canopy profile (b). The time period investigated does not include the period of leaf expansion and senescence. Negative percent differences between models (b) indicate that the BigL and 2L models overestimate canopy photosynthesis.

linear increase in LMA with height early in the growing season within the same stand, but did not detect a significant decline in midday water potential with height later in the growing season. The trend of increasing turgor pressure with height in this study opposed the trend of decreasing turgor with height in much taller trees (Woodruff et al. 2004). Limitations to leaf development and morphology in this study could not be attributed to declining turgor pressure. In other studies, changes in biophysical properties such as reduced cell wall extensibility (irreversible cell expansion) and higher yield turgor threshold (force required to expand cells) in response to soil drying and reduced  $\Psi_L$  were primarily responsible for limitations in leaf expansion and morphology (Nonami and Boyer 1990a, 1990b, Zhang et al. 2012). Thus, reduced  $\Psi_L$  in the upper canopy as observed in this study likely regulated (directly or indirectly) leaf expansion and morphology. Overall, we speculate that the driver of increasing LMA with height shifted from the hydrostatic gradient during the leaf expansion phase to the light gradient following leaf expansion.

Our results provide partial support for our second hypothesis that changes in the vertical distribution in  $N_{\text{area}}$  were primarily due to changes in LMA, where upper canopy leaves required time to acclimate to high light availability. Changes in LMA were likely contributing to seasonal changes in  $N_{\text{area}}$  due to the strong link

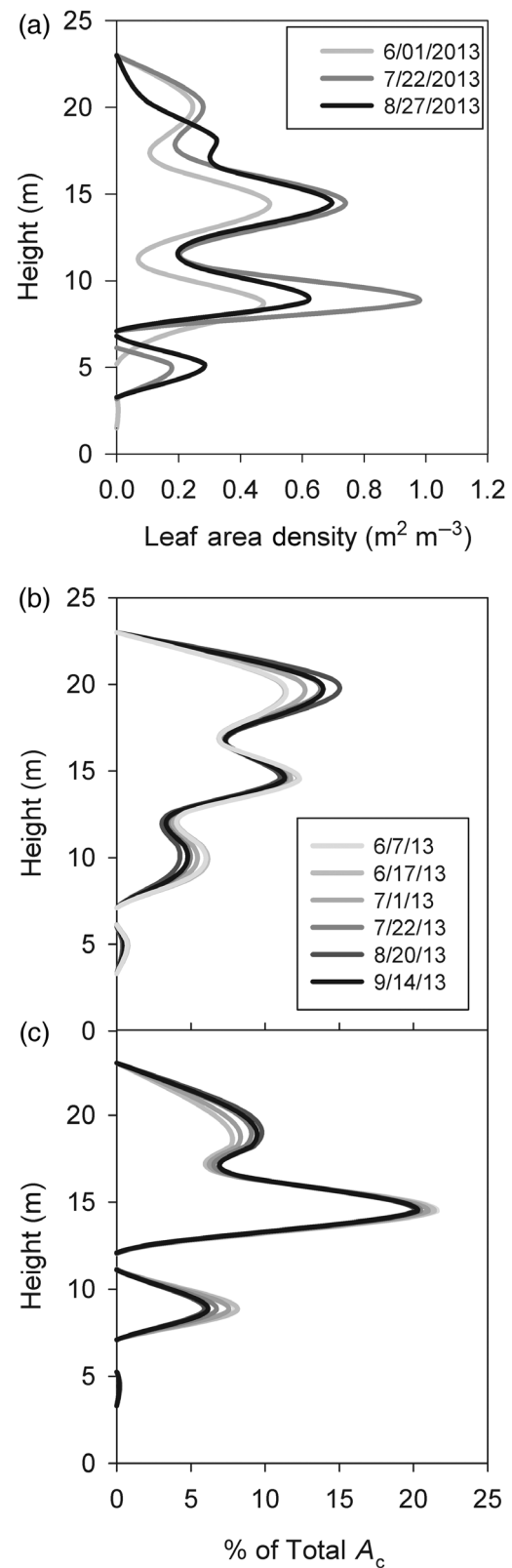


Figure 7. Distribution of leaf area index ( $\text{m}^2 \text{ m}^{-3}$ ) along the canopy profile at ~50% leaf expansion (1 June 2013) and after leaf expansion (22 July 2013 and 27 August 2013) (a). Distribution of the percentage of total canopy photosynthesis ( $A_c$ ) along the canopy profile as estimated from the ML-Seas model when  $C_i$  was held constant (b) and when  $C_i$  was allowed to vary along the canopy profile (c).

between LMA and  $N_{\text{area}}$  ( $N_{\text{area}} = \text{LMA} \times N_{\text{mass}}$ ). We observed a steeper LMA and  $N_{\text{area}}$  slope with height and relative  $\text{LAI}_{\text{ABOVE}}$  later in the growing season when compared with early-season distributions. Seasonal changes in LMA have been shown to correspond with an accumulation of structural proteins (Yasamura et al. 2006) and calcium (Kitajima et al. 2002), which are particularly important in the deposition of carbon-rich compounds such as lignin and noncellulosic polysaccharides in cell walls (Eklund and Eliasson 1990). Thus, seasonal patterns in LMA may be due to changes in cell wall structure and composition in addition to thickness. We speculate that these changes may be contributing to improved tolerance of lower water potentials of *A. saccharum* typically observed from mid- to late-summer (Ellsworth and Reich 1992b, Coble and Cavaleri 2015). Seasonal changes in  $N_{\text{area}}$  distribution were not as apparent as observed with LMA, likely due to the trend of decreasing  $N_{\text{mass}}$  with height and over time. Leaf  $N_{\text{mass}}$  is typically assumed to be uniform throughout forest canopies; however, our results suggest that this is not always the case.

Investigations into the optimality of leaf nitrogen distributions have been gaining attention due to the potential application to large-scale canopy process modeling (Dewar et al. 2012, Niinemets 2012, Peltoniemi et al. 2012). Within-crown or canopy distributions of leaf photosynthesis are required for modeling canopy photosynthesis, and  $N_{\text{area}}$  distributions are often used because  $N_{\text{area}}$  strongly correlates with photosynthetic capacity, yet it is easier to measure (Cornelissen et al. 2003), especially in mature tree canopies. When integrating photosynthesis over the entire crown or canopy using BigL models, a critical assumption is that  $N_{\text{area}}$  is 'optimally' distributed with available light in order to maximize canopy photosynthesis (Field 1983, Kull and Jarvis 1995, Kull et al. 1998). There are shortcomings in defining an optimal distribution in leaf nitrogen, however, because the absorbed irradiance along the canopy profile can change considerably over the course of a day and season (Chen et al. 1993, Leuning et al. 1995). Nevertheless, investigation into optimality may provide a basis for understanding constraints on canopy photosynthesis (Niinemets 2012). While  $N_{\text{area}}$  distributions that maximize canopy photosynthesis were not explicitly investigated in this stand, our results suggest that canopy photosynthesis increased as a result of seasonal patterns in leaf nitrogen distribution following leaf expansion (Figure 5d), which consisted of a transition from a uniform  $N_{\text{area}}$  distribution to a  $N_{\text{area}}$  distribution that followed the general pattern of exponentially decreasing light availability with relative  $\text{LAI}_{\text{ABOVE}}$ . Seasonal changes in  $N_{\text{area}}$  distribution have been reported in other studies where senescence corresponded with uniform distributions of  $N_{\text{area}}$  (Migita et al. 2007). Uniform distributions during senescence are likely due to greater nitrogen resorption in the upper canopy, which has been attributed to greater investments in leaf nitrogen in leaves growing at higher light availability (Migita et al. 2007).

The pattern of increasing  $\delta^{13}\text{C}$  with height has been attributed to reduced stomatal aperture in very tall trees (Koch et al. 2004,

Ishii et al. 2008) or higher photosynthetic capacity of upper canopy leaves (Berry et al. 1997, Duursma and Marshall 2006). There is evidence that high vapor pressure deficit and reduced  $\Psi_L$  in the upper canopy may lead to reduced stomatal conductance (Koch et al. 2004, Niinemets et al. 2004). In contrast to these findings, there is evidence that leaves with higher photosynthetic capacity generally maintain lower  $C_i$  values, which could lead to greater  $\delta^{13}\text{C}$  values (Berry et al. 1997, Duursma and Marshall 2006). Based on the vertical patterns of  $\Psi_L$  and  $N_{\text{area}}$  as observed in this stand, an argument could be made for either case for increasing  $\delta^{13}\text{C}$  with height. However, the relationship between  $\delta^{13}\text{C}$  and height did not change from leaf expansion to the early growing season despite changing light conditions. Furthermore, Coble and Cavaleri (2015) found that experimentally altered light conditions in the upper canopy influenced LMA,  $N_{\text{area}}$  and, likely, photosynthetic capacity, but had no effect on  $\delta^{13}\text{C}$  within the same stand during the same year as this study. This suggests that  $\delta^{13}\text{C}$  is not primarily influenced by photosynthetic capacity, and suggests that stomatal behavior likely influences  $\delta^{13}\text{C}$ . Regardless of the mechanism involved with increasing  $\delta^{13}\text{C}$  with height, implementation of  $\delta^{13}\text{C}$  into the model simulations accounted for decreasing  $C_i$  with height and feedbacks on photosynthesis. Seasonal declines in  $\delta^{13}\text{C}$  through time may be due to a transition to carbon autonomy (nonreliance on reserves) of leaves, accumulation of structural compounds (lipids and lignin) or accumulation of epidermal cuticular waxes low in  $\delta^{13}\text{C}$  (Damesin and Lelarge 2003, Bai et al. 2008). High leaf  $\delta^{13}\text{C}$  in the spring may be a result of carbon fractionation during sugar export from the leaves to stems during the previous growing season, which is then imported back to the leaves in the spring (Damesin and Lelarge 2003).

### Effects of temporal and spatial variations of leaf functional traits on canopy photosynthesis

Our modeling results partially supported our third hypothesis that model estimates of  $A_c$  are slightly biased, especially early in the growing season, when not accounting for seasonal changes in leaf functional traits. The increase in  $N_{\text{area}}$  with decreasing relative  $\text{LAI}_{\text{ABOVE}}$  (as described by  $k_n$ ) through time had a detectable effect on daily modeled  $A_c$ . An overestimation likely occurred early in the growing season because  $N_{\text{area}}$  was increasing in the upper canopy, partially reflected by increasing LMA in upper canopy positions. Due to the dynamic nature of  $N_{\text{area}}$  distributions over the course of the growing season, we show that the use of a constant  $N_{\text{area}}$  (as derived from mid-summer) would likely overestimate canopy photosynthesis early in the growing season. Our results are consistent with Leuning et al. (1995) who observed a 10–16% increase in daily  $A_c$  as a result of increasing  $k_n$ . Similarly, Muraoka et al. (2010) found that the use of a constant value of photosynthetic capacity overestimated GPP early in the growing season at the stand-level. Bauerle et al. (2012) also showed that global net primary productivity was

overestimated with the use of constant photosynthetic capacity in Earth systems models. Across multiple AmeriFlux sites in deciduous forests, there is general agreement between modeled canopy photosynthesis (2L model) and eddy-flux measurements during the middle of the growing season, but GPP tends to be overestimated earlier and later in the growing season (Houborg et al. 2009). The discrepancy between modeled and observed values was attributed to seasonal changes in leaf function with leaf age and development (Houborg et al. 2009). Our results suggest that accounting for seasonal changes in  $N_{\text{area}}$  may refine canopy process models, but overestimation of  $A_c$  due to these changes was likely not sufficient to explain large early-season discrepancies between observed and modeled GPP.

Biases associated with not accounting for vertical variability in leaf functional traits were greater than biases associated with models that did not account for seasonal changes in traits. Consistent with our findings, de Pury and Farquhar (1997) found that the BigL model overestimated  $A_c$  by as much as 45% at high LAI (6.0) when compared with the ML model. In contrast, other studies have found that BigL models underestimate  $A_c$  (Friend 2001, Sprintsin et al. 2012). In these BigL models, modeled photosynthesis was scaled to the canopy using the LAI of sunlit leaves (Sellers et al. 1992). Also consistent with our findings, Wang and Leuning (1998) found that the 2L model, as presented by de Pury and Farquhar (1997), overestimated  $A_c$  relative to the ML model and suggested that this was primarily due to overestimation of absorbed irradiance of shaded leaves. Overall, our results indicate that the assumption that photosynthetic capacity is directly proportional to relative irradiance is invalid for BigL models.

By incorporating vertical and seasonal components to  $A_c$ , we were able to assess the contribution of leaves to total canopy photosynthesis along the canopy profile. We showed that the upper 25% canopy leaf area contributed over 50% of total net canopy photosynthesis if a constant  $C_i$  was assumed. Earlier in the growing season, leaves higher in the canopy contributed less to total  $A_c$  prior to morphological acclimation to light for upper canopy leaves, when height was the primary limitation to leaf traits. However, as upper canopy leaves acclimated to light environments, their contribution to total  $A_c$  increased. These results are consistent with other studies that have detected large contributions to modeled canopy photosynthesis from leaves in the uppermost canopy (Acock et al. 1979, Ellsworth and Reich 1993, Bond et al. 1999). When we incorporated the vertical changes in  $\delta^{13}\text{C}$ , the upper 25% canopy leaf area only contributed to 26% of total canopy photosynthesis. Thus, understanding limitations to photosynthesis for upper canopy leaves is crucial in assessing seasonal and environmental constraints on canopy  $\text{CO}_2$  exchange.

### Additional considerations in modeling canopy photosynthesis

In our model simulations of canopy photosynthesis, the distribution of  $N_{\text{area}}$  along the canopy profile varied in accordance with

leaf measurements made throughout the growing season. We assumed a constant ratio of photosynthetic capacity to  $N_{\text{area}}$ , which was found to vary throughout the growing season for temperate deciduous species (Wilson et al. 2000). Wilson et al. (2000) observed a decrease in the ratio of photosynthetic capacity to leaf nitrogen over the course of the growing season for numerous temperate deciduous tree species, which may influence the model simulations and assessment of the effects of seasonal variation in  $N_{\text{area}}$  on canopy photosynthesis. Photosynthesis and nitrogen relationships may be altered under anomalous temperature increases in the early growing season, as evident by decreases in photosynthesis with no changes in leaf nitrogen (Filewod and Thomas 2014). Furthermore, there is evidence that reproduction can strongly influence canopy physiological processes (Thomas 2011), potentially altering the allocation of leaf nitrogen within tree crowns, which was not accounted for in this study.

## Conclusions

This study describes a series of environmental limitations to leaf functional traits that ultimately influence canopy photosynthesis. During leaf expansion, the number of days to reach 50 and 95% of final leaf area increased with height, which was likely due to decreasing  $\Psi_L$  with height and subsequent effects on cell wall extensibility. This may explain the linear increase in LMA with height early in the growing season. During the acclimation period following leaf expansion,  $N_{\text{area}}$  and LMA increased for leaves growing at high light availability throughout the growing season until the initiation of senescence. These observed trends indicate that the driver of vertical gradients in LMA shifted from hydrostatic gradients to gradients in light availability.

We showed that models that do not account for seasonal changes in  $N_{\text{area}}$  may overestimate daily  $A_c$  early in the growing season by up to 11%. However, biases associated with models that do not account for canopy profiles in leaf structure and function are much greater (up to 51%) throughout the majority of the growing season. When we assumed a constant  $C_i$  along the canopy profile, the upper 25% of the canopy contributed to over 50% of canopy photosynthesis, but this was reduced to 26% after we incorporated vertical decreases in  $C_i$  with height using leaf  $\delta^{13}\text{C}$ . In conjunction with prior research in this stand (Coble and Cavaleri 2014, 2015), our results indicate that limitations on stomatal behavior by reduced  $\Psi_L$  or relative humidity in the upper canopy reduce  $C_i$ , and this reduction in  $C_i$  limits the contribution to total canopy photosynthesis by upper canopy leaves.

## Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.



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## Conflict of interest

None declared.

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