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The influence of temperature on within-canopy acclimation and variation in leaf photosynthesis: spatial acclimation to microclimate gradients among climatically divergent *Acer rubrum* L. genotypes

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Abstract

Leaf gas exchange and temperature response were measured to assess temperature acclimation within a tree canopy in climatically contrasting genotypes of *Acer rubrum* L. Over the course of two 50 d continuous periods, growth temperature was controlled within tree crowns and the steady-state rate of leaf gas exchange was measured. Data were then modelled to calculate the influence of genotype variation and vertical distribution of physiological activity on carbon uptake. The maximal rate of Rubisco carboxylation (V_{cmax}), the maximum rate of electron transport (J_{max}), leaf dark respiration rate (R_d), maximum photosynthesis (A_{max}), and the CO_2 compensation point (Γ) increased with temperature during both (i) a constant long-term (50 d) daytime temperature or (ii) ambient daytime temperature with short-term temperature control (25–38 °C). In addition, within-crown variation in the temperature response of photosynthesis and R_d was influenced by acclimation to local microclimate temperature gradients. Results indicated that carbon uptake estimates could be overestimated by 22–25% if the vertical distribution of temperature gradients is disregarded. Temperature is a major factor driving photosynthetic acclimation and within-crown gas exchange variation. Thus, this study established the importance of including spatial acclimation to temperature- and provenance-, ecotype-, and/or genotype-specific parameter sets into carbon uptake models.

Key words: Global change, photosynthetic capacity, temperature acclimation, temperature response.

Introduction

Forest trees modify their canopy temperature and humidity microclimate along a vertical gradient. Zweifel *et al*. (2002) observed about a 1 °C temperature change and 5% humidity change approximately every 4 m from the upper to lower canopy over 22 m in a *Picea abies* L. forest. Moreover, Harley *et al*. (1996) observed that over a 12 m height gradient, large differences in temperature (~12 °C) were present in a 22 m mixed hardwood–conifer woodland representative of the eastern deciduous biome. In fact, it is not uncommon for trees in the maritime Pacific climate to exceed 65 m, which would expose their crowns to substantial vertical microclimate gradients that induce variation in canopy physiological responses (Ryan and Yoder, 1997; Bauerle *et al*., 1999; Cermak *et al*., 2007).

In order to characterize the physiological adjustments to microclimate gradients correctly, there is a need to decipher the interactive effects of different crown environments while accounting for potential crown section physiological acclimation. Although it is well known that increases in ambient temperature cause enzymatic reaction rates to proceed more quickly, the size of tree crowns makes the manipulation of canopy temperature very difficult. Hence, only a few studies have looked at the variation in leaf physiology in response to temperature

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gradients within a canopy (Niinemets *et al*., 1999; Griffin *et al*., 2002). In general, researchers have hypothesized that foliar photosynthetic characteristics are modified by gradients in canopy light availability (Brooks *et al*., 1996; Bond *et al*., 1999; Niinemets *et al*., 1999, 2004). Unfortunately, this research is grounded mostly in correlative evidence. In fact, previous studies may not fully explain the mechanistic basis for responses in gas exchange variation because they did not control the atmospheric temperature.

Niinemets *et al*. (1999) were the first to study within-canopy variation in temperature. However, because an increase in height in the forest canopy is often accompanied by an increase in air temperature, irradiance, vapour pressure deficit (VPD), and wind speed (Kira and Yoda, 1989), it is hard to disentangle the concurrent changes in microclimate that contribute to the spatial variation in photosynthesis and respiration throughout a canopy. Due in part to the inability to separate microclimate variables along a canopy height gradient, there is little consensus about the mechanisms responsible for leaf photosynthetic and respiratory acclimation within canopies (Niinemets *et al*., 1999; Baldocchi *et al*., 2002; Frak *et al*., 2002; Kosugi and Matsuo, 2006). In addition, there is a paucity of studies that investigate intraspecific photosynthetic temperature response in climatically divergent forest trees (Medlyn *et al*., 2002*a*). Consequently, there is still a need to investigate temperature on a spatially explicit basis and to describe the vertical temperature acclimation response.

Recent studies show that growth temperature affects the temperature dependencies of Rubisco kinetics and activation state (Yamorie *et al*., 2005, 2006; Hikosaka *et al*., 2006; Weston *et al*., 2007). Therefore, the maintenance of a higher rate of Rubisco carboxylation (V_{cmax}) and/or the maximum rate of electron transport (J_{max}) may play a key role in photosynthetic heat acclimation (Hikosaka *et al*., 2006). Moreover, the temperature response of each factor differs among species (Berry and Björkman, 1980; Ferrar *et al*., 1989; Hikosaka *et al*., 1999). For that reason, the aim was to characterize and understand the shift in the temperature dependence of photosynthesis that is a result of acclimation to growth temperature.

Here a crown section warming experiment that separates temperature from crown light interception, VPD, and wind within a crown is reported. Although there have been reports of adaptation in ecotypic and species-level photosynthesis and respiration (Berry and Björkman, 1980; Larigauderie and Körner, 1995), the primary objective was to quantify the spatial acclimation to temperature gradients. In addition to isolating the conditions that pertain to canopy position, the temperature dependence of photosynthetic capacity after leaf expansion was examined in two climatically contrasting genotypes of a common eastern biome continuously flushing species (*Acer rubrum* L.). In so doing, an attempt was made to address scaling assumptions regarding the variation between a big leaf model as opposed to a spatially explicit layered canopy model. This inquiry is needed to ascertain which model is more suitable to obtain reliable estimates of canopy flux in response to temperature and light (Sharkey *et al*., 1996).

The purpose of this study, therefore, was to mimic the relative natural crown daytime temperature gradient of tall trees (~13 °C) to (i) separate positional influence versus specific temperature effects; (ii) investigate the effect of short- versus long-term temperature manipulation; (iii) decipher the consequences of differences in temperature coefficients for various photosynthetic parameters on the calculated canopy carbon gain; and (iv) examine the carbon uptake estimate difference between a genotype-specific spatially explicit layered canopy model versus a big leaf model. Because experimental systems for regulating temperature are difficult to use on trees, the influence of temperature on photosynthetic capacity within the foliage of individual trees has never been decoupled from light under outdoor conditions. Thus, the present study asked whether growth temperature alone influences the photosynthetic temperature optimum within a canopy and whether within-species variation from climatically contrasting habitats can influence acclimation to elevated temperatures. The results are expanded on in the context of the net effect of the within-canopy distributed temperature response on total carbon exchange.

Materials and methods

Plant material and study site layout

Measurements were carried out during the 2003 growing season in a 0.58 ha outdoor gravel pad of open terrain at the Clemson University Calhoun Field Laboratory in Clemson, SC, USA (latitude 34°40′8″; longitude 82°50′40″). A full description of the site is given in Bauerle *et al*. (2002). Two genotypes from thermally contrasting parentage were used for intensive sampling in this study: *A. rubrum* L. (red maple) cv 'October Glory' (OG), native to Massachusetts (latitude 40°27′18″; longitude 74°29′3″), USA, and red maple cv 'Summer Red' (SR), native to Georgia (latitude 31°27′27″; longitude 83°33′41″), USA. A row–column design was used, which resulted in two genotypes, two treatments (temperature-controlled and the mimic of ambient outdoor conditions), and four replicate trees per treatment and genotype with randomly assigned temperature profiles. Each genotype was processed in canopy bags over two separate 50 d periods. To ensure that the trees never experienced substrate water-limiting conditions, each tree was watered three times daily to container capacity with pressure-compensating micro emitters (ML Irrigation Inc., Laurens, SC, USA) and spaced 1.5 × 1.5 m. Substrate volumetric water content was monitored on a daily basis (Theta Probe type ML2, Delta-T Devices, Cambridge, UK) and root zone volumetric water content was maintained at 0.4–0.5 $m^3\ m^{-3}$ in each 114 litre tree container.

Red maple, a continuous flushing species, developed at least one new fully expanded leaf per branch every 7 d during the experiment. Therefore, leaf measurements took place on leaves that developed under treatment conditions. To assess the extent to which long-term (50 continuous days of temperature control with leaf gas exchange measured on continuously developed fully expanded leaves) versus short-term (the same type of gas exchange

measurement as long-term but temperature was controlled only for short durations by the leaf level gas exchange cuvette) temperature exposure influences photosynthetic and respiration responses, this study controlled the daytime growth temperature of whole crown sections. The crown sections without growth temperature control (run at ambient conditions) witnessed a mean growth temperature of 25 °C during the 2003 growing season.

Chamber construction and temperature control

Whole crown chambers were placed on four replicate trees per treatment. Crown chambers were subdivided by dividing each crown into three volumetrically equal area layers (Fig. 1). Each individual tree crown chamber, dimensions $1\times1\times2$ m^3, was constructed from 5 cm diameter polyvinyl chloride (PVC) tubing and covered with clear 0.025 mm Mylar® (DuPont, Wilmington, DE, USA). The Mylar® photon flux density (PFD) characteristics were checked with a spectroradiometer (model 1800, Li-Cor Inc., Lincoln, NE, USA). Results similar to Corelli and Magnanini (1993), were found where PFD was >90% of outside incident PFD, with midday levels exceeding 1800 μmol m^{-2} s^{-1}, and the spectral composition was unchanged over the 400–900 nm range. To create the self-contained crown sections, horizontal sheets of Mylar® divided each crown layer and were secured to the trunk with foam rubber gaskets. Hence, each layer subchamber was plumbed independently to maintain growth temperature on an individual crown layer basis (Fig. 1). Three Twintemp® 16300/10700 BTU/h cooling and heating air conditioners (model ES16, Friedrich Inc., San Antonio, TX, USA) were plumbed to 12 Mylar® enclosed crown sections (three sections per crown) and together they continuously controlled temperature in each crown section (Fig. 1). Chamber temperatures were sampled at 10 s intervals with fine wire thermocouples (CR21X, Campbell Sci. Inc., Logan, UT, USA) and a control switch triggered the air conditioners to either heat, cool, or run at ambient temperature (fan only) to maintain the temperature within 1 °C of the set point. Crown section temperatures were assigned randomly per subchamber within each crown, where each crown had three different temperatures controlled at 25, 33, or 38 °C (Fig. 1).

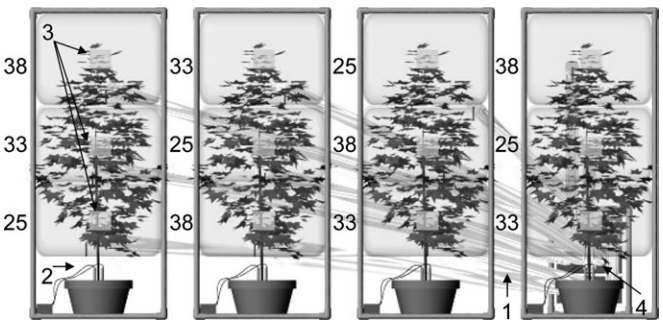

Fig. 1. A side view diagram of the Mylar® crown section temperature treatment chambers. Simultaneously, each of three prescribed temperatures was controlled on four separate replicate tree crowns per genotype. An additional four trees with paired crown sections run at ambient conditions (short-term duration of temperature control by the gas exchange cuvette during leaf level gas exchange measurements) were used as a control in the adjacent row (not shown). The controlled temperature of each crown section is denoted to the immediate left of the section in °C (25, 33, and 38). Arrows with reference numbers denote the following: (1) separately plumbed air ducts per crown section; (2) micro irrigation emitters; (3) ventilation and crown access ports; and (4) location of air conditioners. Please note, the transparency of chambers is darkened compared with actual experimental conditions for visual clarity of crown sections.

The airflow produced >3 volumes min^{-1} per layer and created a slight positive pressure on the Mylar®, which kept it in a wrinkle-free state for maximum light penetration. A preliminary experiment found the amount of air exchange more than adequate to ensure that CO_2 levels did not deviate from outside ambient conditions. At night, temperature was returned to ambient in order to prevent variation in temperature acclimation of dark respiration (Turnbull *et al*., 2002).

Calculation of leaf-intercepted radiation and optical characteristics

Photosynthetically active radiation (PAR) was estimated for each crown layer with a three-dimensional light absorption model validated on red maple (Bauerle and Bowden, 2004; Bauerle *et al*., 2004*a*). Crown spacing and leaf PAR absorption characteristics of the crown were set up to minimize lower crown shading, where PAR variation between the upper and lower crown position was <10%. Absorbed PAR was calculated by summing the crown subvolumes. Red maple leaf reflectance, transmittance, and absorption values were determined using the non-linear correlation equations of Bauerle *et al*. (2004*b*) by averaging five SPAD readings per leaf (Minolta SPAD 502 chlorophyll meter, Minolta Camera Co., Ramsey, NJ, USA). Under the outdoor experimental conditions, leaf PAR was light saturating for red maple at all canopy positions when incident radiation was >550 μmol m^{-2} s^{-1} (Bauerle *et al*., 2003).

Gas exchange measurements

From 27 May to 8 September 2003 (Julian days 147–251), leaf net photosynthesis (A_{net}) versus CO_2 response curves (A_{net}–C_i curves, where A_{net} is net photosynthetic rate in μmol m^{-2} s^{-1} and C_i is the internal CO_2 concentration expressed as the mol fraction of CO_2) were measured. The measurements were conducted twice weekly on a 2 d time block. Gas exchange was measured using a portable steady-state gas exchange system (CIRAS-I, PP Systems, Amesbury, MA, USA) equipped with a light-, humidity-, and temperature-controlled cuvette [model PLC5 (B); PP Systems]. Measurements were made on the youngest fully expanded leaf at all three crown layers between 09.00 and 14.30 h. The leaves were tagged and, on any given day, measurements were taken in random order to compensate for any effects caused by time of sampling. At each crown layer, A_{net}–C_i response curves were constructed at saturating PFD (1200 μmol m^{-2} s^{-1}). Measurements began at a cuvette CO_2 concentration of 370 mmol mol^{-1} and were decreased as follows: 370, 175, 150, 100, and 50 μmol mol^{-1}; after this sequence was completed, the cuvette CO_2 was returned to 370 μmol mol^{-1} and sequentially increased at the following intervals: 600, 800, 1000, and 1200 μmol mol^{-1} to generate the A_{net}–C_i curves. Leaf temperatures were controlled at 25, 33, or 38 °C by the leaf level gas exchange cuvette, and VPD was controlled at 1.3 ± 0.4 kPa at each target temperature by adjusting the water vapour inside the cuvette. It is also important to note that although it was possible to control cuvette VPD, crown section VPD co-varies with temperature. To prevent elevated crown section VPD values from influencing canopy gas exchange, VPD was kept below values that influence red maple stomatal conductance responses (<2.5 kPa) (Bauerle *et al*., 2004*c*). Also, it should be noted that red maple is not responsive to VPD changes below 2.5 kPa under well-watered conditions (WL Bauerle *et al*., unpublished results).

A total of 672 A_{net}–C_i curves were measured (336 for each genotype, 168 per long- or short-term temperature treatment, and 56 at each genotype long-term or short-term temperature treatment of 25, 33, and 38 °C). To estimate biochemical limitations to carbon assimilation from the curves, the methodology of Wullschleger

(1993) was followed. Temperature response curves were constructed by changing the Peltier blocks in a manner similar to Weston *et al*. (2007), with an initial measurement at 25 °C and then stepwise increases in temperature to 42 °C. Specifically, leaf temperature within the cuvette was increased to 27, 30, 33, 36, 38, and finally to 42 °C. Data were fit to thermodynamic models (see below). For each curve, non-linear regression explained >91% of the variation in A_{net}–C_i data.

Temperature dependence of gas exchange parameters

To derive temperature dependencies of the Michaelis–Menten coefficient for CO_2 (K_c: μmol mol^{-1}), for O_2 (K_o: μmol mol^{-1}), the CO_2 compensation point (Γ: μmol mol^{-1}), dark respiration (R_d: μmol m^{-2} s^{-1}), and the Rubisco specificity factor (τ), the parameters were fit with the exponential function:

$$\text{Parameter} = \exp(c - \Delta H_a/RT_l) \quad (1)$$

where T_l is absolute leaf temperature (K), R is the gas constant (8.314 J mol^{-1} K^{-1}), c is the scaling constant, and ΔH_a is the energy of activation. If a parameter decreased at elevated temperature, such as V_{cmax}, J_{max}, and maximum photosynthesis (A_{max}), Equation (1) was modified to include entropy (ΔS) and energy of deactivation (ΔH_d) (Harley and Tenhunen, 1991; Harley *et al*., 1992):

$$\frac{\exp(c - \Delta H_a/RT_l)}{1 + \exp[(\Delta ST_l - \Delta H_d)/RT_l]} \quad (2)$$

The temperature responses of different parameters were estimated using the secant method (NLIN Procedure, SAS Institute, 2004).

The optimal temperature (T_{opt}) was computed from the above parameters by solving the equation as described in Medlyn *et al*. (2002*b*):

$$T_{opt} = \frac{-\Delta H_d}{\frac{-\Delta H_a}{-\Delta H_a - \Delta H_d}} \quad (3)$$

where ΔS, the entropy term, is related to T_{opt} by the expression:

$$\Delta s = -R\ln T_{opt} = -R\ln\left[\frac{-\Delta H_d}{\frac{-\Delta H_a}{-\Delta H_d - \Delta H_a}}\right] \quad (4)$$

Temperature response parameter comparison

The temperature response of V_{cmax} from Equation (2) was compared with the Rubisco-limited photosynthesis calculation developed by Bernacchi *et al*. (2001):

$$V_{cmax},T = V_{cmax}(25)\times\exp^{(26.35-65.33/(R\times(T_l)))} \quad (5)$$

where V_{cmax},T is the value of V_{cmax} corrected to temperature, and $V_{cmax}(25)$ is the value of V_{cmax} at 25 °C. The temperature response of J_{max} from Equation (2) was compared with either acclimated or non-acclimated growth temperature using the calculations developed by Bernacchi *et al*. (2003):

$$J_{max},T = J_{max}(25)\times\exp^{(17.57-43.54/(R\times(T_l)))} \quad (6)$$

$$J_{max},T = J_{max}(25)\times 1.92\times\exp^{(-0.5\times(((T_g-26.42)/17.97)^2+((T_l-47.04/19.38)^2))} \quad (7)$$

where Equation (6) represents the J_{max} response to unacclimated temperature conditions and Equation (7) accounts for temperature acclimation. In both equations, J_{max},T is the value of J_{max} corrected to temperature, and $J_{max}(25)$ is the value of J_{max} at 25 °C. In Equation (7), T_g is the growth temperature in °C. For the present purposes, growth temperature acclimation was assumed in the 50 d crown section growth temperature-controlled treatments and unacclimated in the case of short-term temperature control only during the gas exchange measurements.

Leaf mass:area ratio, and total nitrogen

After each gas exchange measurement, the leaf was excised and measured for leaf area (L_A) (LI-3000 Li-Cor, Inc., Lincoln, NE, USA) and fresh weight. The leaf was then dried (70 °C for 72 h) and weighed. Nitrogen concentration on a dry mass basis (N_M) was determined with a LECO model FP528 nitrogen combustion analyser (LECO Corporation, St Joseph, MI, USA). Total leaf nitrogen was expressed per unit mass.

Modelled carbon uptake

Photosynthetic temperature response parameters were used to parameterize a carbon exchange model (MAESTRA). MAESTRA is an updated version of MAESTRO (Wang and Jarvis, 1990*a*) and is available on-line at www.bio.mq.edu.au/maestra/. The primary purpose of using MAESTRA in this study was to scale up to the crown and describe the effects of temperature acclimation gradients on carbon flux with depth in the canopy. MAESTRA's application in this study is grounded in the work of others where the model has been described in detail, validated, and used to estimate species-specific photosynthetic production; interested readers are referred to Wang and Jarvis (1990*a*, *b*), Kruijt *et al*. (1999), Luo *et al*. (2001), Medlyn (1998, 2004), and Bauerle *et al*. (2002, 2004*a*, 2006) for MAESTRA applications and detailed descriptions.

For this study, the model's three-dimensional spatially explicit nature was critical. MAESTRA simulated the spatial distribution of daily integrated leaf temperature within the crown, and the photosynthetic response of a 'target crown' was dependent on the distribution of microclimate within the crown. To integrate PAR absorption over temporal and spatial distributions of irradiance, each crown layer was treated as unifacial and the assimilating leaf area was defined as one-sided. The positions and dimensions of the trees surrounding the target crown were used to calculate the sunlit and shaded fractions of leaf area after passing through the neighbouring tree canopies, where the canopy is represented by an array of ellipsoidal tree crowns. In the present study, the intercepted and absorbed radiation was calculated for each crown. The model was run in two different configurations. In the spatially explicit layered configuration, the crown of a tree was divided into three layers, resulting in 12 sectors of 30° with each layer forming 36 equal subvolumes. In the big leaf configuration, the spatial explicitness was removed and the properties of the whole canopy were mapped onto a single leaf (Sellers *et al*., 1996). Other than genotype-specific temperature response parameters listed in Tables 1, 2, and 4, model parameterization followed Bauerle *et al*. (2002). In a previous study, values of leaf PAR absorption simulated by the model were validated against fibre-optic micro-quantum sensors and line-quantum sensors (Bauerle and Bowden, 2004). The model was found to simulate adequately the spatial and temporal trends of crown microclimate gradients within deciduous red maple trees (Bauerle *et al*., 2004*a*).

Another critical aspect of the model is the ability to parameterize in detail the physiological and genetic response on a genotype by genotype and crown layer basis (WL Bauerle *et al*., unpublished

Table 1. *Gas exchange characteristics calculated from the response of short-term temperature-controlled assimilation*

Mean growth temperature was 25 °C during the study. Values shown are the means and standard errors (in parentheses) for parameters of leaves of red maple genotype Summer Red (SR) and October Glory (OG). ANOVA table abbreviations are G, genotype, T, temperature, *P*, *P*-value; NS, not significant.

Parameter	SR			OG			ANOVA
	25 °C	33 °C	38 °C	25 °C	33 °C	38 °C	
V_{cmax} (μmol CO_2 m^{-2} s^{-1})	79.8 (3.2)	125.4 (3.8)	158.8 (7.7)	70.1 (3.4)	118.1 (3.5)	151.4 (5.7)	G *P*=0.04 T *P*=0.001 G×T=NS
J_{max} (μmol electrons m^{-2} s^{-1})	140.8 (6.0)	217.5 (8.9)	225.9 (9.2)	125.9 (1.8)	200.1 (1.7)	222.9 (1.5)	G *P*=NS T *P*=0.001 G×T=NS
J_{max}/V_{cmax}	1.76 (0.28)	1.73 (0.33)	1.42 (0.17)	1.79 (0.24)	1.69 (0.36)	1.47 (0.22)	G *P*=NS T *P* <0.001 G×T=NS
A_{max} (μmol CO_2 m^{-2} s^{-1})	21.7 (0.8)	30.5 (1.1)	28.6 (1.0)	20.4 (0.8)	29.1 (1.0)	28.3 (1.2)	G *P*=NS T *P* <0.001 G×T=NS
R_d (μmol CO_2 m^{-2} s^{-1})	3.2 (0.2)	5.6 (0.3)	7.2 (0.4)	3.5 (0.2)	5.2 (0.2)	6.2 (0.2)	G *P*=NS T *P* <0.001 G×T=NS
TPU (μmol CO_2 m^{-2} s^{-1})	10.91 (0.4)	11.1 (0.3)	11.1 (0.5)	11.6 (0.7)	10.7 (0.8)	10.6 (0.6)	G *P*=NS T *P*=NS G×T=NS
Γ (μmol CO_2 m^{-2} s^{-1})	88.9 (3.0)	103.1 (3.1)	119.3 (3.3)	102.1 (3.9)	102.2 (3.5)	116.4 (3.4)	G *P*=NS T *P* <0.001 G×T=0.04

Table 2. *Gas exchange characteristics calculated from the response to long-term temperature-acclimated assimilation*

Values shown are the means and standard errors (in parentheses) for parameters of leaves of red maple genotype Summer Red (SR) and October Glory (OG). Crown sections were acclimated to 25, 33, or 38 °C for 50 d and measured bi-weekly at the acclimation temperature. ANOVA table abbreviations are G, genotype, T, temperature, *P*, *P*-value; NS, not significant.

Parameter	SR			OG			ANOVA
	25 °C	33 °C	38 °C	25 °C	33 °C	38 °C	
V_{cmax} (μmol CO_2 m^{-2} s^{-1})	69.7 (3.1)	125.6 (5.1)	158.2 (6.3)	60.2 (2.9)	104.9 (2.7)	103.3 (2.0)	G *P* <0.001 T *P* <0.001 G×T <0.001
J_{max} (μmol electrons m^{-2} s^{-1})	132.4 (7.2)	226.5 (10.7)	262.9 (10.6)	103.7 (4.9)	210.3 (7.8)	201.3 (6.7)	G *P* <0.001 T *P* <0.001 G×T=0.016
J_{max}/V_{cmax}	1.89 (0.34)	1.8 (0.3)	1.66 (0.24)	1.72 (0.22)	2.0 (0.4)	1.95 (0.44)	G *P*=NS T *P*=0.001 G×T <0.001
A_{max} (μmol CO_2 m^{-2} s^{-1})	19.4 (0.9)	25.8 (1.1)	27.6 (1.1)	16.8 (0.7)	25.3 (0.8)	24.1 (0.8)	G *P*=0.003 T *P* <0.001 G×T=NS
R_d (μmol CO_2 m^{-2} s^{-1})	2.8 (0.1)	4.3 (0.2)	5.4 (0.2)	2.9 (0.1)	4.6 (0.2)	5.6 (0.2)	G *P*=NS T *P* <0.001 G×T=NS
TPU (μmol CO_2 m^{-2} s^{-1})	9.9 (0.4)	11.1 (0.4)	10.7 (0.5)	10.41 (0.2)	11.5 (0.3)	11.4 (0.3)	G *P*=NS T *P*=NS G×T=NS
Γ (μmol CO_2 m^{-2} s^{-1})	99.3 (4.9)	103.4 (3.0)	117.6 (4.4)	85.8 (2.8)	91.9 (1.9)	107.7 (3.5)	G <0.001 T *P* <0.001 G×T=NS

results). Thus, the sampling structure and measurements allowed each genotype's genetic difference to be described with genotype-specific parameters and control equations to predict the carbon uptake of each genotype. The response of a genotype to temperature was thus represented by spatially distributing the physiological temperature response along an upper, mid, and lower canopy vertical temperature distribution. All calculations were made on a 15 min time scale.

MAESTRA was used to calculate net daily carbon gain (C_{dg}; g d^{-1}), which is a direct estimate of growth rate:

$$C_{dg} = \left(P_{n,light} - R_{d,dark}\right) \times 12 \quad (8)$$

where $P_{n,light}$ is the total net photosynthesis during the light period, $R_{d,dark}$ is the total respiration during the dark period, and 12 is the molecular mass of C. Two different temperature response modelling approaches were undertaken with MAESTRA—a spatially explicit distributed physiology layered canopy model and a big leaf distributed physiology model. In the spatially explicit model, the vertical spatial distributions of all parameter temperature acclimation responses were explicitly considered to predict carbon uptake rates on a m^2 leaf area basis per crown layer. In the big leaf model, the spatial explicitness was removed and all leaves throughout the canopy were assumed to have a constant temperature response on a m^2 leaf area basis from that measured in either the lower, mid, or upper canopy.

Statistical analysis

For each photosynthetic parameter reported in Tables 1 and 2, a two-way analysis of variance (ANOVA; SPSS 14.01, SPSS Inc., Chicago, IL, USA) was performed as a 2×3 factorial with genotype and temperature as independent variables. Error terms were pooled and reported as standard error of the difference of the means. For each photosynthetic parameter reported in Table 3, a two-way ANOVA was performed to compare the long-term versus short-term acclimation responses with genotype and temperature as independent variables.

Results

General environmental conditions

Under cloud-free conditions, all crown sections received full sun >1800 μmol m^{-2} s^{-1} incident irradiance, a level well above the light saturation point of red maple (Bauerle *et al*., 2003). Under either overcast conditions or full sun, the PFD variation within a crown was <10% along the vertical gradient (minimal within-crown shading similar to open grown crowns).

Crown position versus microclimate temperature gradient within the crown

Independent effects of the canopy layer were not significant and, regardless of the crown position (top, middle, or bottom layer), long-term exposure to different daytime growth temperatures resulted in leaf acclimation to a specific temperature (25, 33, or 38 °C). With respect to long-term temperature, gas exchange parameters J_{max}, A_{max}, and R_d had a significant temperature acclimation in both genotypes, whereas after 50 d of controlled growth temperature V_{cmax} only appeared to acclimate in the OG genotype (Table 1 versus 2). The significance of the acclimation responses becomes more pronounced as temperatures steadily increase above 25 °C (Table 1 versus 2), where Table 3 compares these acclimation percentage changes at the highest controlled temperature of 38 °C. The manipulation of crown section temperature did not result in a difference among photosynthetic characteristics at identical controlled temperatures among replicates; however, regardless of crown position, temperature had a significant effect on photosynthetic characteristics (Tables 1 and 2 with standard errors). The spatial influence of long- and short-term microclimate temperature gradients was shown to be a vertical gradient temperature

Table 3. *Percentage change at 25, 33, and 38 °C long-term daytime growth temperature-acclimated gas exchange characteristics relative to short-term acclimation for leaves of red maple genotypes Summer Red (SR) and October Glory (OG)*

The *P*-values are for the significance of the two-way ANOVA of the comparison between long- and short-term temperature acclimation with genotype and temperature as independent variables. ANOVA table abbreviations are G, genotype, T, temperature, *P*, *P*-value; NS, not significant.

Parameter	SR			OG			ANOVA
	25 °C	33 °C	38 °C	25 °C	33 °C	38 °C	
V_{cmax}	−12.6	+1	−0.4	−14.1	−11.1	−31.8	G *P* <0.001 T *P* <0.001 G×T=0.03
J_{max}	−6	+4	+16.4	−18.7	−4.9	−9.7	G *P*=NS T *P* <0.001 G×T=NS
J_{max}/V_{cmax}	+6.9	+3.9	+16.9	−3.9	+15.5	+32.6	G *P*=0.149 T *P*=0.006 G×T=0.045
A_{max}	−10.6	−15.4	−3.5	−17.6	−13.1	−14.8	G *P*=NS T *P* <0.001 G×T=0.022
R_d	−12.5	−23	−25	−17.1	−11.5	−9.7	G *P*=NS T *P* <0.001 G×T=NS
TPU	−9.3	0	−3.6	−10.3	+6.9	+7.5	G *P*=NS T *P*=NS G×T=NS
Γ	+10.5	+0.3	−1.4	−15.9	−10.1	−7.5	G <0.010 T *P* <0.001 G×T=NS

factor rather than other microclimate variables that vary with canopy position. Variation in the calculated temperature-dependent parameters describing leaf photosynthetic and respiration responses are described in the short-term versus long-term temperature exposure section below, and values of temperature-dependent parameters of the leaf photosynthetic temperature response are reported in Table 4.

The influence of short-term versus long-term temperature manipulation on photosynthetic parameters

In all but one case, photosynthetic parameters derived from A_{net}–C_i response curves increased in response to temperature from 25 °C to 38 °C under conditions where the crown section temperature was only controlled during leaf gas exchange measurement (Table 1). Triose phosphate utilization (TPU), on the other hand, was relatively unchanged in response to this temperature alteration. In addition, the two genotypes did not present a significant difference in their response to the short time scale temperature change, nor did they have a significant alteration in their photosynthetic capacity up to 38 °C (Table 1). Furthermore, regardless of canopy position, the thermodynamic temperature response was similar in both genotypes. No differences in nitrogen concentration of leaves used for gas exchange measurements was found among genotypes, canopy position, or growth temperature, where the SR and OG genotype mean N_M was 1368±19 μmol g^{-1} and 1333±17 μmol g^{-1} dry weight. In addition, leaf mass per L_A was not different either where the SR and OG genotype mean was 0.06±0.003 g cm^{-2} and

Table 4. *Values of the temperature-dependent parameters describing leaf photosynthetic temperature response in long- and short-term acclimated leaves of red maple genotypes Summer Red (SR) and October Glory (OG)*

Values are followed by the standard error (in parentheses) when statistical analysis was possible. Parameters were calculated from Harley and Sharkey (1991), Harley *et al*. (1992), and Medlyn *et al*. (2002*b*).

	SR	SR	OG	OG	Units
	Short-term	Long-term	Short-term	Long-term	
Values for V_{cmax}					
T_{opt}	39.4	40.9	35.2	35.5	°C
ΔH_a	46.3 (10.9)	61.6 (12.2)	52.2 (10.7)	76.7(10.8)	kJ mol^{-1}
c	22.3 (4.4)	29.2 (4.9)	25.4 (4.1)	35.1 (4.3)	–
ΔS	0.63 (0.02)	0.63 (0.01)	0.64 (0.01)	0.644 (0.003)	J K^{-1} mol^{-1}
V_{cmax} at T_{opt}	153.3	169.7	171.1	114.8	μmol m^{-2} s^{-1}
Values for J_{max}					
T_{opt}	36.3	37.8	35.2	32.8	°C
ΔH_a	53.4 (11.8)	64.8 (14)	52.3 (12.1)	136.3 (12.1)	kJ mol^{-1}
c	26.6 (4.7)	31 (5.6)	25.9 (4.9)	59.8 (4.8)	–
ΔS	0.638 (0.004)	0.637 (0.005)	0.64 (0.01)	0.66 (0.006)	J K^{-1} mol^{-1}
J_{max} at T_{opt}	239	274.4	231.2	227.6	μmol m^{-2} s^{-1}
Values for A_{max}					
T_{opt}	37.2	37.4	35.1	34.7	°C
ΔH_a	48.3 (10.7)	31.2 (9.6)	47.2 (11.2)	56.5	(10.5)
kJ mol^{-1}					
c	22.62 (4.3)	15.6 (3.8)	22.1 (4.5)	25.7 (4.2)	–
ΔS	0.635 (0.003)	0.63 (0.007)	0.64 (0.003)	0.639 (0.003)	J K^{-1} mol^{-1}
A_{max} at T_{opt}	30.9	28.3	30.9	26.8	μmol m^{-2} s^{-1}
Values for R_d					
ΔH_a	46.2 (6)	38.9 (4.5)	33.7 (3.5)	37.8 (3.8)	kJ mol^{-1}
c	19.9 (2.4)	16.8 (1.8)	14.9 (1.4)	16.4 (1.5)	–
Values for Γ					
ΔH_a	17.7 (2.7)	9.8 (3.4)	7.5 (2.9)	13.4 (2.6)	kJ mol^{-1}
c	11.6 (1)	8.6 (1.3)	7.6 (1.1)	9.8 (1)	–
Values for k_c					
k_c at 25 °C	434.48	434.48	434.48	434.48	μmol mol^{-1}
ΔH_a	79.3 (0.06)	79.3 (0.06)	79.3 (0.06)	79.3 (0.06)	kJ mol^{-1}
c	38.1 (0.02)	38.1 (0.02	38.1 (0.02)	38.1 (0.02)	–
Values for k_o					
k_o at 25 °C	287.8	287.8	287.8	287.8	μmol mol^{-1}
ΔH_a	36.3 (0.03)	36.3 (0.03)	36.3 (0.03)	36.3 (0.03)	kJ mol^{-1}
c	20.3 (0.01)	20.3 (0.01)	20.3 (0.01)	20.3 (0.01)	–
Values for τ					
τ at 25 °C	2.3	2.3	2.3	2.3	μmol mol^{-1}
ΔH_a	–28.9 (0.04)	–28.9 (0.04)	–28.9 (0.04)	–28.9 (0.04)	kJ mol^{-1}
c	–10.9 (0.02)	–10.9 (0.02)	–10.9 (0.02)	–10.9 (0.02)	–

0.07 ± 0.004 g cm^{-2}. Values of the temperature optimum for V_{cmax}, J_{max}, and A_{max}, however, ranged from 1 °C to 5 °C lower for the OG as opposed to the SR genotype. The V_{cmax} ΔH_a was slightly higher in the OG genotype, whereas the ΔH_a values for J_{max} and A_{max} were similar.

In contrast to values in ambient temperature growth conditions, photosynthetic parameters derived from A_{net}–C_i response curves after long-term exposure to various controlled temperature treatment conditions did change as a function of genotype (Table 2). Within the canopy, growth temperature had a different effect on the two genotypes. Whereas A_{max}, V_{cmax}, and Γ increased in response to temperature, J_{max} and R_d not only increased, but acclimated to growth temperature (Table 3). This resulted in an overall decrease in R_d for both genotypes and a down-regulation of J_{max} for OG and up-regulation for SR at both 33 °C and 38 °C. Among the two thermally contrasting genotype habitats, the genotype from the warmer environment (SR) up-regulated J_{max} by 4% and 16.4%, and down-regulated R_d by 23% and 25% at 33 °C and 38 °C, respectively. In contrast, the genotype from the cooler climate (OG) decreased J_{max} by 4.9% and 9.7% and R_d by 11.5% and 9.7% at 33 °C and 38 °C, respectively. In addition, V_{cmax} and A_{max} were relatively unchanged in comparison with short-term temperature-controlled leaves in the SR genotype, but were substantially down-regulated by 31.8% and 14.8 % in the OG genotype (Table 3). Another contrast among the genotypes was the J_{max}:V_{cmax} ratio. Both genotypes had a change in the ratio of J_{max} to V_{cmax} in response to growth temperature; however, the ratio shift was in the opposite direction (Tables 2, 3). At a long-term acclimation temperature of 38 °C, the SR genotype up-regulated J_{max}, whereas the OG genotype value was lower when compared with short-term temperature-controlled leaves. Moreover, the long-term growth temperature was very significant in relation to the photosynthetic parameter values. Acclimation to long-term growth temperature resulted in a higher optimum temperature for V_{cmax}, J_{max}, and A_{max} for the SR compared with the OG genotype (Table 4). Furthermore, the sensitivity to changes in temperature was more pronounced in the genotype from the cooler climate. The differences in temperature sensitivity can be seen in the estimated energy of the activation parameter (ΔH_a). Reactions with higher activation energy such as those observed in the OG genotype are more temperature sensitive compared with the SR genotype (Table 4). Values of k_c, k_o, and τ, on the other hand, were similar to published values (Bernacchi *et al*., 2001).

Testing the temperature response against generalized temperature functions for predicting leaf level Rubisco- and RuBP-limited photosynthesis

In comparison with recent improvements in temperature response functions for models of Rubisco-limited and RuBP-limited photosynthesis, fairly good agreement was found between the observations of V_{cmax} at 25 °C and those of the improved Rubisco-limited Bernacchi *et al*. (2001) model (Table 5). However, at temperatures of 33 °C and 38 °C, the Bernacchi *et al*. (2001) model underestimated V_{cmax} by 33–57% in comparison with what was observed in the temperature response of the genotypes in this study. Similarly, regardless of the genotype, the Bernacchi *et al*. (2003) equations for predicting both temperature-acclimated (long-term) and non-acclimated (short-term) RuBP-limited photosynthesis performed well at 25 °C. However, at temperatures of 33 °C and 38 °C, the Bernacchi *et al*. (2003) model of short-term temperature-acclimated J_{max} underestimated by 36–54% on the OG genotype and performed only slightly better on the SR genotype (36–38% underestimation). The long-term temperature-acclimated J_{max} values were either over- or underpredicted, depending on temperature. In the case of the SR genotype, J_{max} was overestimated by 14% at

Table 5. *Comparison of percentage deviation of actual versus predicted gas exchange parameters for red maple genotypes Summer Red (SR) and October Glory (OG)*

Short-term temperature-acclimated conditions are designated (sta) and long-term temperature-acclimated (lta). Predicted values of the maximal rate of Rubisco carboxylation (V_{cmax}) were calculated according to Bernacchi *et al*. (2001). Predicted values of the maximum rate of electron transport (J_{max}) for both short- and long-term temperature-acclimated conditions were calculated according to Bernacchi *et al*. (2003).

Parameter	Temperature (°C)	SR		OG	
		Predicted value	% Difference	Predicted value	% Difference
V_{cmax} (lta)	25	68.6	–2	69	+13
J_{max} (sta)	25	139.3	+1	102.6	–18
J_{max} (lta)	25	141.2	+0	103.9	+0
V_{cmax} (lta)	33	68.6	–45	69	–34
J_{max} (sta)	33	139.4	–36	102.6	–49
J_{max} (lta)	33	194.5	+14	143.2	–32
V_{cmax} (lta)	38	68.7	–57	69.1	–33
J_{max} (sta)	38	139.4	–38	102.7	–54
J_{max} (lta)	38	197	–25	145.1	–28

33 °C and underpredicted by 25% at 38 °C. In the OG genotype, J_{max} was underestimated by 32% and 28% at 33 °C and 38 °C, respectively.

Comparison of carbon uptake estimate differences between a genotype-specific spatially explicit layered canopy model versus a big leaf model

First, regardless of model spatial explicitness, a clear difference was observed in the carbon uptake among climatically divergent genotypes of red maple (Figs 2, 3). Next, this inherent climate adaptation resulted in quite different modelled net carbon uptake of long-term (acclimated) temperature-controlled red maple canopies versus short-term (unacclimated) genotypes in response to elevated temperatures above 25 °C (Fig. 2 versus Fig. 3). At temperatures above 25 °C, the net carbon uptake distinctly separated, with the OG genotype curtailing carbon uptake at 38 °C in acclimated temperature conditions. Up to 38 °C, the SR genotype, however, did not appear to limit carbon uptake to the extent of the OG genotype. Figure 3 illustrates that short-term temperature-controlled leaves (unacclimated) responded to temperature increases; however, genotypes were similar in their temperature response. In fact, intraspecific differences within the species were not evident under the short-term temperature-controlled conditions (Fig. 3).

Table 3 indicates that the difference between the two genotype carbon uptake responses to long-term elevated temperature was primarily attributed to three temperature-dependent physiological factors. The most significant factor was the V_{cmax} difference at both 33 °C and 38 °C, where the SR genotype V_{cmax} hardly changed but the OG carboxylation efficiency went down by 11.1% and 31.8% at 33 °C and 38 °C, respectvely. In addition, among the genotypes, elevated temperature had an opposite effect on J_{max}. The SR genotype increased J_{max} by 16.4% at 38 °C, but the OG electron transport rate declined by 9.7%. The combination of the V_{cmax} and J_{max} changes also resulted in an altered J_{max}/V_{cmax} ratio (Table 3). Lastly, regardless of genotype, R_d declined at elevated temperature; however, the SR genotype had 15.3% less of a R_d decrease than the OG at 38 °C (Table 3).

The differences in the temperature responses of the two modelling approaches (spatially explicit layered canopy model versus a big leaf model) are illustrated in each panel of Figs 2 and 3. If all the leaves in the canopy are assumed to have a constant physiological response as in a big leaf model, the prediction would overestimate the net carbon uptake at temperatures above 25 °C, regardless of whether the leaves were acclimated to canopy microclimate gradients or not (Figs 2, 3). In fact, the variation among the two modelling approaches increased as the temperature increased beyond 25 °C in both long- and short-term temperature-controlled leaves. For genotype OG, there was a 24% short-term and 22% long-term difference between the big leaf versus spatially explicit layered canopy model estimate at 38 °C. For SR, the results were similar, where there was a 24% and 25% difference between the two modelling methods at 38 °C, respectively. Thus, as temperature increased above 25 °C, there was in increase in the divergence among the two carbon uptake modelling approaches (Figs 2, 3).

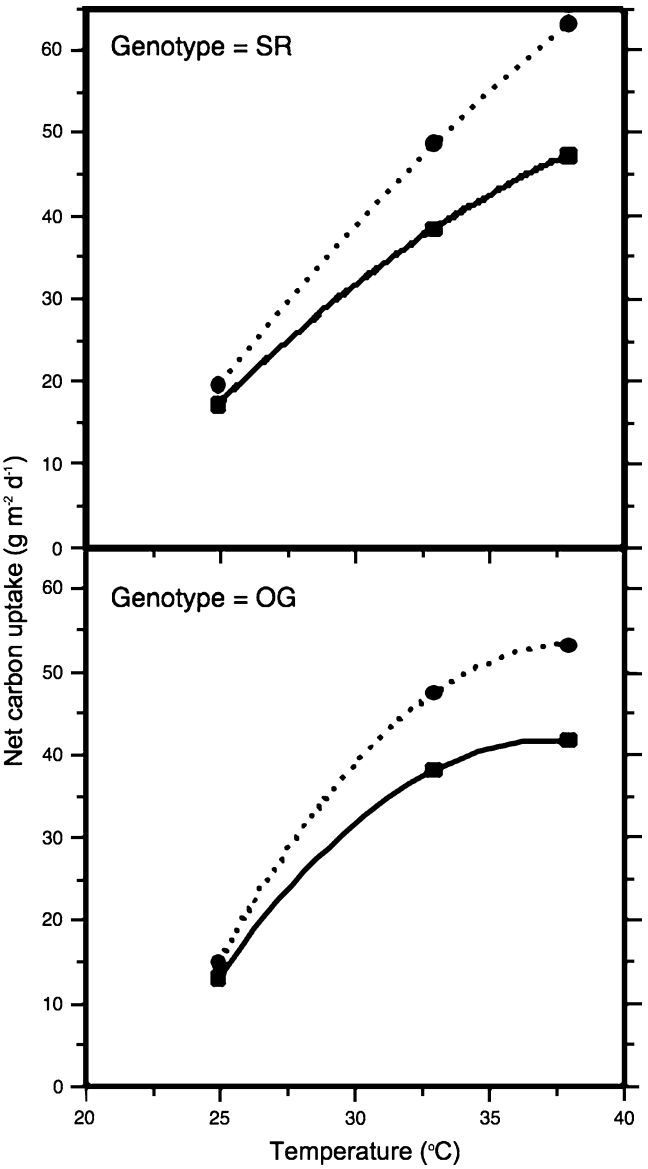

Fig. 2. Modelled long-term temperature-acclimated response of net carbon uptake at an atmospheric CO_2 concentration of 370 μmol m^{-2} s^{-1}, vapour pressure deficit of 1.25 kPa, and incident radiation of 1500 μmol m^{-2} s^{-1} for red maple genotypes Summer Red (SR) and October Glory (OG). The spatially explicit layered canopy model estimates (filled squares; solid line) and the big leaf model estimates (filled circles; dashed line) are illustrated in each panel.

Discussion

Elevated global temperatures raised numerous concerns regarding ecosystem function and the influence on carbon

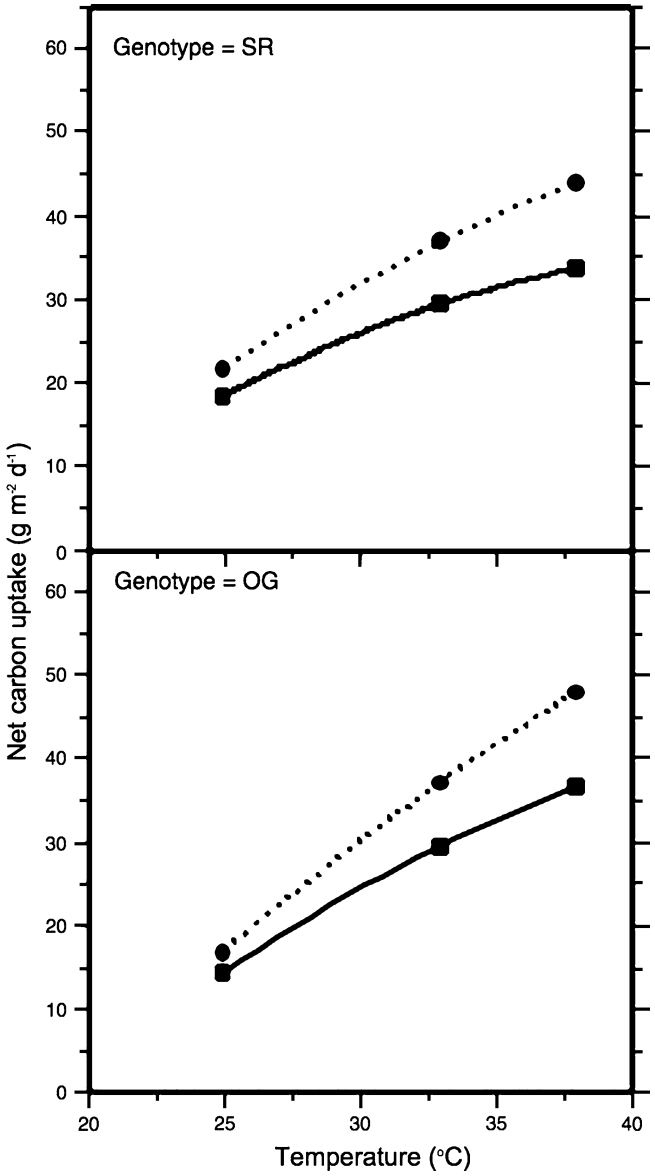

Fig. 3. Modelled short-term temperature-unacclimated response of net carbon uptake at an atmospheric CO_2 concentration of 370 μmol m^{-2} s^{-1}, vapour pressure deficit of 1.25 kPa, and incident radiation of 1500 μmol m^{-2} s^{-1} for red maple genotypes Summer Red (SR) and October Glory (OG). The spatially explicit layered canopy model estimates (filled squares; solid line) and the big leaf model estimates (filled circles; dashed line) are illustrated in each panel.

exchange (IPCC, 2007). Trees comprise the majority of the carbon-sequestering biomass in terrestrial ecosystems; thus their responses to environment and climate change are a key determinant of global net primary production and carbon sequestration (Valentini *et al*., 2000; Barford *et al*., 2001; Breshears *et al*., 2005). Concern about the gradual rise in atmospheric temperature, predicted to range from 1 °C to 7 °C by 2100, has brought about several temperature-related studies that investigate the physiological responses of trees (Roden and Ball, 1996; Teskey and Will, 1999; Medlyn *et al*., 2002*a*; Haldimann and Feller, 2004). Although it is widely known that photosynthesis is affected by temperature on a short-term basis, the direct relationship between temperature and global forest productivity requires an understanding of long-term acclimatory photosynthetic responses that help explain variation in forest carbon exchange across climates and within canopies—two spatial temperature entities that are likely to diverge.

Because of the high autocorrelation between light and temperature along a canopy height gradient, it is very difficult to ascertain whether the decrease in photosynthetic response at lower heights is due to light or temperature acclimation. Recently, however, Zhang *et al*. (2006) found that temperature was the dominant factor controlling seasonal variation in ecosystem apparent quantum yield and maximum photosynthetic rates in a temperate mixed forest. Zhang *et al*. (2006) also found that ecosystem respiration of a temperate mixed forest is more sensitive to temperature than that of either a subtropical evergreen coniferous plantation or a subtropical evergreen broad-leaved forest. Our findings on one of the most abundant and widely distributed forest tree species in eastern North America (Hutnick and Yawney, 1961) support the hypothesis that temperature gradients within a canopy drive physiological acclimation of leaf photosynthetic capacity. Even though temperature was not controlled in an independent study by Mäkelä *et al*. (2004), they too found temperature to affect seasonal changes in photosynthetic capacity. Alternatively, Rayment *et al*. (2002) found a difference between J_{max} values at the upper versus lower canopy of *Picea mariana* (Mill.) B.S.P.; however, that study did not attribute the large seasonal variation in V_{cmax} and J_{max} to temperature (V_{cmax} and J_{max}) or microclimate (V_{cmax}). Thus, the relative importance of leaf temperature on photosynthetic acclimation within plant canopies has not arrived at a consensus, probably due to the major challenge of controlling tree crown temperature under outdoor conditions.

It is particularly noteworthy that the present experiment specifically manipulated the temperature of whole crown sections while attempting to minimize variation in other microclimate variables. Since there has been an absence of studies that have been conducted across a controlled canopy temperature gradient, it allowed investigation of an unknown—namely if temperature microclimates within a canopy influence photosynthetic parameters due to acclimation. The current analyses of the temperature response of both photosynthetic and respiratory characteristics indicate that crown sections do indeed acclimate to temperature. Furthermore, the results demonstrate that the temperature-dependent variation in the acclimation of photosynthetic parameters can occur along a height (temperature) gradient, implying that the forest canopy has a response gradient that influences local carbon gain

from the upper to lower canopy position. As a result, the variation in photosynthesis and respiration responses along temperature gradients within a canopy need to be accounted for in canopy and ecosystem-level flux models—especially in tall trees.

In most plants, the activation energy of V_{cmax} increases with growth temperature (Hikosaka *et al*., 2006). In addition, photosynthetic performance at elevated temperature is largely a function of Rubisco kinetics and the Rubisco activation state (Yamori *et al*., 2006; Weston *et al*., 2007). A mechanistic response to temperature was observed, where the activation energy of V_{cmax} and J_{max} increased with growth temperature from 25 °C to 38 °C. The response is consistent with the changes in temperature dependence of the photosynthetic rate due to elevated growth temperature (Yamori *et al*., 2005, 2006; Hikosaka *et al*., 2006). In fact, the optimum temperature for growth can shift towards a higher temperature (Yamori *et al*., 2005). That was found to be the case for the SR genotype but not for the OG genotype, where four temperature-dependent physiological factors diverged among genotypes. In response to temperature, the V_{cmax} was significantly reduced in the OG genotype, whereas the SR genotype tolerated the elevated temperature by not changing its carboxylation efficiency. With respect to J_{max} under acclimated elevated temperature, the genotypes diverged in the opposite direction. The divergence could explain some of the difference in carbon exchange at elevated temperature. For instance, Wilson *et al*. (2001) showed that neglecting seasonal changes in J_{max} and V_{cmax} could lead to overestimation of net ecosystem carbon exchange. The present findings support this observation and strengthen the need to examine J_{max} and V_{cmax} on both a seasonal and growth temperature basis. Thirdly, the R_d values were lower at elevated growth temperature. It has been shown that respiration is a main determinant of carbon balance in forests (Valentini *et al*., 2000). Consequently, the 11.5% and 15.3% variation in R_d at 33 °C and 38 °C could help explain why the SR genotype was capable of storing more carbon compared with the OG genotype. Together, these factors suggest that temperature acclimation is dependent on the thermal conditions of the native climate.

Extrapolating carbon dioxide and water vapour flux exchange from the leaf to the canopy can take one of two common paths: the one-layered big leaf or the multilayered spatially explicit modelling approach. The big leaf approach maps properties of the whole canopy onto a single leaf (Sellers *et al*., 1996) and the multilayered type model integrates the fluxes from each layer (Wang and Jarvis, 1990*a*). Due to the nature of non-linearity parameterization, the big leaf methodology cannot define the arithmetic mean of leaf-level parameters (McNaughton, 1994; Wang *et al*., 2001). The multilayered models, on the other hand, can use parameters that are measured and validated at the leaf level. The big leaf model prediction was found to overestimate the net carbon uptake at temperatures above 25 °C, regardless of whether the leaves were acclimated to canopy microclimate gradients or not. Comparatively, the multilayered spatially explicit model was capable of integrating the fluxes through the various temperatures of a canopy by compensating for the non-linear photosynthetic response to temperature. The outcome was a 22–25% lower estimate in the multilayered spatially explicit model due to the arithmetic mean of leaf-level temperature response parameters.

The short-term contribution of the present study is the potential improvement of both spatial and genetic parameterization for scaling carbon uptake estimates to canopies of temperate deciduous forests. In addition, the results are noteworthy because long-term temperature acclimation has not been studied in many commonly widespread species that occur across a continental temperature gradient (Bolstad *et al*., 2003). It was observed that even though photosynthesis parameters can change due to the temperature effect on enzymatic reactions, these parameters can also down-regulate at long-term daytime temperature exposure above 33 °C. Moreover, the results suggest that there is adaptive variation in the acclimation response along the disparate climatic gradient represented by the red maple genotypes and thus one should not generalize across species with wide climatic distributions in carbon flux modelling. Unlike others who did not control temperature within the crown and speculate acclimation to irradiance (Han *et al*., 2004) or who made measurements on detached shoots (Medlyn *et al*., 2002a), the present study accounted for long-term temperature acclimation and leaves were measured *in situ*. If, as is suggested, acclimation of photosynthetic parameters to temperature does not support the assumption that temperature responses are universally similar within a woody plant species, further knowledge of the mechanisms responsible for temperature acclimation would be useful for modelling carbon uptake under a range of microclimate conditions and provenance, ecotype, and/or genotypes.

Due to the fact that all carbon gain models use a temperature response function, it is imperative that inter- and intraspecific acclimatory temperature dependencies be parameterized correctly. Models that only incorporate a single temperature response curve for all leaves in a canopy may cause significant errors in model estimates of carbon exchange. Recently, Bernacchi *et al*. (2001, 2003) have attempted to re-parameterize temperature responses of V_{cmax} and J_{max} to improve modelling estimates. Their temperature functions were derived from a transformed line of wild-type tobacco (*Nicotiana tobaccum* L. cv. W38) operating under the assumption that it would predict well across terrestrial C_3 systems. The present comparison of predicted versus actual responses indicates that this is not the case, where the influence of temperature

acclimation on enzyme activity and the respective influence on photosynthetic parameters differed substantially. Thus, the development of a mechanistic understanding of these responses is still a major challenge to pursue if we hope to quantify accurately the interactions among temperature, physiological genetics, and ecosystem function.

Controlled environment chambers are useful for developing hypotheses; however, the present findings should still be tested in natural ecosystems. Although the experimental protocol was sufficient to allow investigation of the temperature response, the 13 °C growth temperature range would not be common in the size of trees in this study. The results, on the other hand, should be directly applicable to forest crowns ⩾50 m and, in part, relevant to temperature gradients in all forest crowns. The results of this study may also apply to shifts in seasonal climate, as temperature in forest canopies varies not only vertically but also daily, seasonally, and annually (Harley *et al*., 1996; Singsaas *et al*., 1999; Zweifel *et al*., 2002). In addition, the results indicate the importance of explicitly considering the spatial variation in leaf physiology within a canopy and that this spatially explicit description of canopy physiology can produce a substantially different predicted outcome when compared with a big leaf approach. Clearly, the implications of the present findings identify a weakness in current predictions of how changes in climate may affect carbon storage and release in deciduous forests worldwide and should be the subject of further experimentation.

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