Canopy position affects the temperature response of leaf respiration in *Populus deltoides*

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Summary

- Leaf respiration and its temperature response were measured in 4-m-tall, 1-yr-old *Populus deltoides* trees to assess the effect of within-canopy distribution of respiratory physiology on total foliar C exchange of a model ecosystem at Biosphere 2.
- Over the course of five nights, air temperature was varied over a 10°C range and the steady-state rate of leaf respiration was measured. These data were then modeled to calculate the temperature response of leaf and canopy respiration.
- Results indicate that there is considerable within-canopy variation in both the rate of respiration and its temperature response and that these variables are most strongly related to leaf carbohydrate and leaf N. Scaling these results to the ecosystem level demonstrates the importance of quantifying the vertical distribution of respiratory physiology, particularly at lower temperatures.
- Simplifying assumptions regarding the variation in respiration and its temperature response with canopy height tend to result in an underestimation of the actual C loss if the assumptions are based on lower- or mid-canopy leaf physiology, but overestimate C loss if the model assumptions are based on upper-canopy physiology.

Key words: respiration, Q_{10}, temperature, *Populus deltoides* (cottonwood), Biosphere 2, nitrogen, carbohydrates, scaling.

Introduction

Photosynthesis and respiration are the two primary biological processes regulating the exchange of carbon between the atmosphere and the terrestrial biosphere. Furthermore, it is the relatively small difference between these two large fluxes (net photosynthesis *c.* 122 GT C year^{-1} and respiration *c.* 64 GT C year^{-1} (autotrophic) + 58 GT year^{-1} (heterotrophic)) that defines the carbon balance of an ecosystem (Schimel, 1995; Amthor, 1997; Field, 2001). While both of these enzymatic processes are known to respond to a variety of environmental variables, the specific responses of photosynthesis and respiration can be independent of each other and therefore can lead to nonlinear effects on the overall rate of carbon gain (Ryan, 1991a; Dewar *et al.*, 1999; Gunderson *et al.*, 2000). Understanding the responses of photosynthesis and respiration to environmental variation is therefore of the utmost importance as human activities are resulting in unmitigated environmental change.

Of the environmental variables affecting respiration, by far the most often studied and therefore most well understood is temperature. As ambient temperature increases, enzymatic reactions proceed more quickly. Typically, for each successive 10°C increase in temperature, reaction rates double. The actual change in the rate of a reaction with a 10°C increase is known as the Q_{10} or the temperature coefficient of that reaction, and the Q_{10} of leaf respiration has been demonstrated to vary between 1.1 and 4.2 (Azcón-Bieto & Osmond, 1983; Tjoelker *et al.*, 2001) and itself can be influenced by a range of environmental conditions (Turnbull *et al.*, 2001). In order to interpret the existing experimental results at the global scale correctly, and to apply this knowledge to predictive models correctly, the relationship between leaf- and system-level measurements of Q_{10} must be known.

While the distribution of both respiratory activity (Bolstad *et al.*, 1999; Carswell *et al.*, 2000; Griffin *et al.*, 2002; Meir *et al.*, 2001), and photosynthetic capacity (Field, 1983; Hirose & Werger, 1987; Hollinger, 1989; Field, 1991; Evans,

1993; Anten *et al.*, 1995; Hollinger, 1996) have been shown to vary through the canopy, the temperature response of these processes at different canopy depths has not been well studied. It has been suggested that soluble carbohydrate levels regulate the temperature response. These carbohydrates are known to vary through the canopy as light extinction increases and carbon gain decreases, limiting the formation of these respiratory substrates (Atkin *et al.*, 2000; Griffin *et al.*, 2002). Similarly, leaf N tends to decrease with canopy depth (Field, 1983; Hirose & Werger, 1987; Evans, 1989; Hirose *et al.*, 1989; Hollinger, 1989; Leuning *et al.*, 1991a,b; Ellsworth & Reich, 1993; Hollinger, 1996), and a general relationship between leaf N and respiration has been reported (Ryan, 1991a; Ryan, 1995; Reich *et al.*, 1996, 1998a,b; Ryan *et al.*, 1996). Hence, the temperature response of respiration may vary through the canopy of large trees.

Methodologically, manipulating canopy temperature is quite difficult and thus there have been few experiments designed to examine the variation in leaf temperature responses within a canopy. While portable gas exchange systems are commonly equipped with temperature control modules, recent work has shown that the temperature response of respiration measured during whole-shoot or ecosystem temperature manipulations are significantly larger than those measured by changing air temperature inside a leaf cuvette independently from the air temperature around the tree (Atkin *et al.*, 2000; Griffin *et al.*, 2002). Experiments that manipulate air temperatures on short-time scales provide useful input for ecosystem models of plant growth and carbon sequestration since temperature fluctuations of several to 10 s of °C regularly occur on diel, nightly, weekly, monthly and seasonal time scales.

Here we report the results of an ecosystem warming experiment at the Biosphere 2 plant growth and global climate change facility near Tucson, Arizona. By taking advantage of the technical innovations and size of the facility we were able to manipulate night-time temperatures of an intact model ecosystem containing 77, 4 m tall, 1-yr-old *Populus deltoides* Bartr. trees. During the course of the 5-d experiment the night-time temperature was adjusted by 5°C each night (15.5, 10.5, 15.5, 20.5 and 15.5°C on the five consecutive nights). Each night, leaf respiration was measured at three canopy heights, and significant gradients were found in the rate of respiration. These data were analyzed to test the hypotheses that leaf respiration would decrease with canopy depth and that the temperature response would show a similar trend, with decreasing sensitivity with depth in the canopy. We further consider the impact of our results by calculating the net effect of the within-canopy distribution of respiration on the total carbon exchange during the five nights of our experiment.

Materials and Methods

The basic experimental design and system components are described in detail elsewhere (Griffin *et al.*, 2002). The Biosphere 2 plant growth and climate change research facility is located 1200 m above sea level at 32.5° N latitude in southern Arizona. This experiment was conducted exclusively in the 2000-m^2 Intensive Forestry Biome, which is isolated physically from the remainder of Biosphere 2 and has independent environmental control. The total volume of this section, estimated by the injection of SF6 as a trace gas, is 35 222 m^3 (J. Van Haren, pers. comm.). The forestry biome of Biosphere 2 is further subdivided into three roughly equal-sized mesocosms with large plastic curtains. Each of the three mesocosms is roughly 41 m long (in a north–south orientation) and 18 m wide with a maximum height of 24 m. Each mesocosm has three large air handlers, each capable of moving 566 m^3 min^{-1}. These air handlers provide both the primary means of air circulation and temperature control. Within each mesocosm, four additional fans help to maintain the air circulation and break up the canopy boundary layer.

Biosphere 2 is subject to the light regimes of a temperate desert region. The glass and metal structural components of Biosphere 2 act as a neutral density filter for incoming solar radiation of wavelengths longer than 380 nm (virtually all UV radiation is blocked). Photon flux density (PFD) is more than 70% of outside incident PFD with midday levels exceeding 1600 μmol m^{-2} s^{-1}. Due to the low-latitude location of this facility, mean daily PFD levels inside the forest biome are approximately 15 mol m^{-2} d^{-1} in the winter, and 25 mol m^{-2} d^{-1} in the summer. During daylight hours, [CO_2] control within each mesocosm is maintained by adding pure CO_2 with a mass flow meter (Sierra Side-Track, Sierra Instruments, Inc., Monterey, CA, USA) into the air stream entering the air handlers to mass balance for the carbon removed from the atmosphere via photosynthesis. At night, or at any other time when respiratory CO_2 release exceeds photosynthetic carbon uptake, a variable speed fan brings in outside ambient air to flush out the CO_2 released from the system and maintain the [CO_2] at the desired set point. The CO_2 partial pressure averaged 42 Pa during the experiment. The Biosphere 2 environmental control system is described elsewhere (Lin *et al.*, 1998; Dempster, 1999; Zabel *et al.*, 1999).

Cuttings of the cottonwood (*Populus deltoides* Bartr.) clone (S7c8) used in this experiment came from a production fiber farm in Summerville, South Carolina. This particular clone is adapted to the lower Brazos River, Texas, and is day neutral. Two hundred and eighty-two cottonwood cuttings, each 50 cm long and 2 cm in diameter were planted on 2.5 m centers on May 20, 1998. The cuttings were placed into the artificial soil system, which had been in place for the previous 10 yr and has been fully described elsewhere (Marino & Odum, 1999). All trees were coppiced to a height of 20 cm in December of 1998, they resprouted in March of 1999 and were subsequently pruned to have a single leader in May 1999. This experiment began on June 3, 1999, using the 77 trees growing in the west mesocosm of the forestry section of Biosphere 2. The six trees used were 3.99 ± 0.05 m tall, 30 cm

in stem diameter at the soil surface and possessed symmetrical crowns with a width of 2 m at the base. There were no statistical differences between these experimental trees and the other 71 trees in the mesocosm.

Gas-exchange

Leaf-level gas-exchange measurements were made with several cross-calibrated, portable open-flow gas-exchange systems incorporating CO_2 control (Li-6400, Li-Cor Inc., Lincoln NE, USA). Environmental conditions within the cuvette were controlled to match the ambient conditions within the mesocosm. Respiration measurements were recorded only after a visual inspection of a graph of respiration and stomatal conductance as a function of time was stable and the total coefficient of variation was less than 1% (measured as the variation in the $[CO_2]$, $[H_2O]$ and flow rate over a 1-min period). Although every effort was made to use the same leaves each night, there was an unavoidable loss of leaves from through damage or inadvertent removal (*c.* 20%). When this occurred the next appropriate leaf on the same branch was used to replace the damaged or lost leaf. All leaves were fully expanded and of similar age.

Experimental protocol

On day one of the experiment (June 3, 1999) the temperature inside the forestry section of Biosphere 2 was maintained at its long-term set point of 28/15.5°C (day/night). Respiration measurements were initiated no sooner than 2300 h, and thus were made during the most stable part of the night (Azcón-Bieto & Osmond, 1983). Leaf-level measurements were made at the same time of night on each of the 5 d of the experiment. The steady-state respiration rate of two leaves from the lower, mid- and upper-canopy (*c.* 0.75, 1.75 & 3.25 m, respectively) of six individual trees was measured under ambient environmental conditions (n = 12 leaves). On day two of the experiment, the daytime conditions remained at the long-term set point (28°C). The air temperature of the mesocosm was set 5°C lower for the entire night-time period, and the respiration measurements described above were repeated. On day three of the experiment the day and night-time temperatures were returned to the long-term set point and the above measurements were repeated. The night-time temperature was increased by 5°C compared with the long-term set point on day four of the experiment, and measurement protocol was repeated. On day five of the experiment, all conditions were again returned to the long-term set points and all measurements were repeated.

Temperature response

The response of leaf respiration to temperature was described two ways, first by calculating Q_{10}:

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{10/(T_2 - T_1)} \qquad \text{Eqn 1}$$

where T_1 is the lower measurement temperature, T_2 is the higher measurement temperature, k_1 is respiration rate at the lower temperature and k_2 is the respiration rate at the higher temperature (Salisbury & Ross, 1985). While Q_{10} is a commonly used formulation of the temperature response of enzymatic reactions, it has limitations and should be used with caution since the exponential nature of the Q_{10} relationship results in the value changing depending on the temperature range it is calculated over. For this reason we used a second formulation, a modification of a basic Arrhenius equation that builds on the work of Lloyd & Taylor (1994) and was previously described Turnbull *et al.* (2001):

$$R = R_{10} e^{\left(\frac{E_o}{Rg} * \left(\frac{1}{T_o} - \frac{1}{T_a}\right)\right)} \qquad \text{Eqn 2}$$

Where R is the respiration rate, R_{10} is the respiration rate at 10°C (fitted), T_o is the absolute temperature at 10°C 283 K, T_a is the measurement temperature of R (K), R_g is the ideal gas constant (8.314 J mol^{-1} K^{-1}) and E_o is a variable related to the energy of activation, also a fitted variable (J mol^{-1}). This model was fitted using a nonlinear technique (NLIN procedure, SAS Inc., 1998). An algebraic rearrangement of the equation let us solve for the predicted respiration rate at any temperature of interest.

Leaf characteristics

Carbohydrate analyses were performed on leaf disks taken from leaves adjacent to those used for gas exchange measurements. Samples were taken just before sunrise and at sunset. Soluble sugar and starch content of leaves were determined colorimetrically using the ethanol extraction technique of Hendrix (1983) as described by Griffin *et al.* (1999). All samples were analyzed in triplicate and reported as the mean value. Total nonstructural carbohydrate (TNC) content was calculated as the sum of soluble sugar and starch, and the daily turnover of TNC was calculated by

$$\text{Carbohydrate turnover} = \frac{\text{value at sunset} - \text{value at sunrise}}{\text{value at sunset}} \times 100 \qquad \text{Eqn 3}$$

Following the experiment, fresh material was collected from each measurement leaf with a cork borer and immediately analyzed for chlorophyll. Chlorophyll was extracted in ethanol and determined spectrophotometrically (Wintermans & De Mots, 1965). The remainder of the measurement leaf was dried and weighed to determine leaf mass per area (LMA). Each sample was ground to a fine powder in a ball mill

(Cianflone Scientific Instrumental Corporation, Pittsburgh, PA, USA) and leaf carbon and nitrogen concentrations were determined using an elemental analyzer (Ce440 Elemental analyzer, Leeman Laboratories, Hudson, New Hampshire, USA).

Light environment, canopy light extinction and vertical leaf area distribution

Two silicon quantum sensors (Li-190sb, Li-Cor Inc., Lincoln NE, USA) were placed inside the experimental chamber above the canopy to record the ambient light environment. After the completion of the experiment a sun fleck ceptometer (SF-80, Decagon, Pullman Washington, USA), was used to measure light extinction through the canopy. Measurements were made at noon on clear days, each day for a week. For each of the six trees measured the center of the ceptometer was near the main stem of the tree first in a north–south orientation and then in an east–west orientation. Measurements were made at 0.33 m increments from the ground to the top of the tree and then normalized to the maximum amount of light above the canopy.

The leaf area index of the individual experimental trees was determined allometrically from the tree height and diameter at the soil surface (R. Murthy, unpublished data). The maximum LAI and light extinction data were then used to model the vertical distribution of leaf area within the canopy based on Beer's Law.

Modeled Carbon Loss The calculated temperature response (Eqn 2) was used to predict the leaf respiration from the mean air temperature during the five nights of the experiment. The instantaneous rates were scaled to an m^2 ground surface area level by multiplying by the leaf area index (3.1 m^2 m^{-2}) of the western mesocosm of the Biosphere 2 cottonwood plantation. Two separate model calculations were made. In the first, all leaves were assumed to have the respiration rate and temperature response measured in the lower, mid or upper canopy. This model will henceforth be referred to as the 'constant physiology model' (with the additional designation of lower, mid or upper canopy). In the second the vertical distribution of both the respiration rate and the temperature response of respiration were explicitly considered along with the calculated vertical distribution of leaf area. For this second calculation the total leaf area from the upper 1 m of the canopy (0.82 m^2 m^{-2}) was assumed to have the respiratory characteristics of the upper canopy leaves (Fig. 2 and Table 3). Similarly the lower 1.3 m of the canopy (1.28 m^2 m^{-2}) was assumed to have the respiratory characteristics of the lower canopy leaves and the remainder of the canopy (0.98 m^2 m^{-2}) was assumed to have the characteristics of the mid-canopy leaves. This model will henceforth be referred to as the 'distributed physiology model'. All calculations were made on a 15-min time scale and then were summed for the duration of the night-time period (2200 h−0600 h). A similar set of calculations was made from the mass-based respiration.

Statistical analysis

Analysis of variance for the temperature response of respiration rates obtained using the two protocols described above (Eqns 1 and 2), was performed using general linear models (GLM procedures, SAS Inc., 1998) and the means were compared using Tukey's test. All the tests of significance were made at the 0.05 level. All other statistical analysis was performed using Data Desk statistical software (version 6.0, Data Description Inc, Ithaca, NY, USA). The main effects of temperature and canopy position were determined with a 2-way ANOVA and predetermined means separation was accomplished with a protected LSD test. A nested model (individual leaves nested within trees) was used to account for pseudoreplication (Underwood, 1981).

Results

The mean night-time temperature (2200 h–0600 h) on nights 1, 3 and 5 of the experiment was 15.3 ± 0.1°C (Fig. 1). There were no significant temperature differences between these three 'ambient' nights. The cold night (night 2, target temperature of 10.5°C) had a mean night-time temperature of 11.5 ± 0.2°C and the warm night (night 4, target temperature of 20.5°C) had a mean temperature of 19.7 ± 0.1°C. Ambient air temperature had always reached its steady state set-point temperature before the initiation of the leaf level gas-exchange measurements. No significant fluctuations were observed in soil temperature measured at either 50 or 80 cm from the surface (averaging 21.5 ± 0.01°C at 50 cm depth and 20.9 ± 0.01°C at 80 cm depth). The 20 cm measurements reflected changes in air temperature, fluctuating between 19 and 24.5°C with a five-night average of 21.9 ± 0.1°C.

The average leaf area index of the six trees used in this experiment was 3.1 ± 0.44 m^2 m^{-2}. Within the canopy of these trees, the upper canopy leaves were significantly different in several respects from the lower canopy leaves (Table 1). Upper canopy leaves were 68% larger (total area per leaf), had a 42% higher LMA, 13% more carbon and 17.5% less nitrogen per unit leaf mass but 16% more nitrogen per unit leaf area, a 37% higher C:N and a 15% higher Chl_a : Chl_b ratio than lower canopy leaves. Leaf nitrogen concentrations were quite high, particularly in the lower and mid-canopy. By comparison mid-canopy leaves collected from cuttings of the same genetic stock, planted at the same time in a fiber farm in South Carolina had 2.7% nitrogen compared with the 3.8% measured here (K. L. Griffin, unpublished data). It should be noted that the upper canopy leaves were growing from the main stem, rather than from sylleptic branches. As the primary leader was still expanding, there were no branches in the upper half meter of the canopy. As a result, some of the

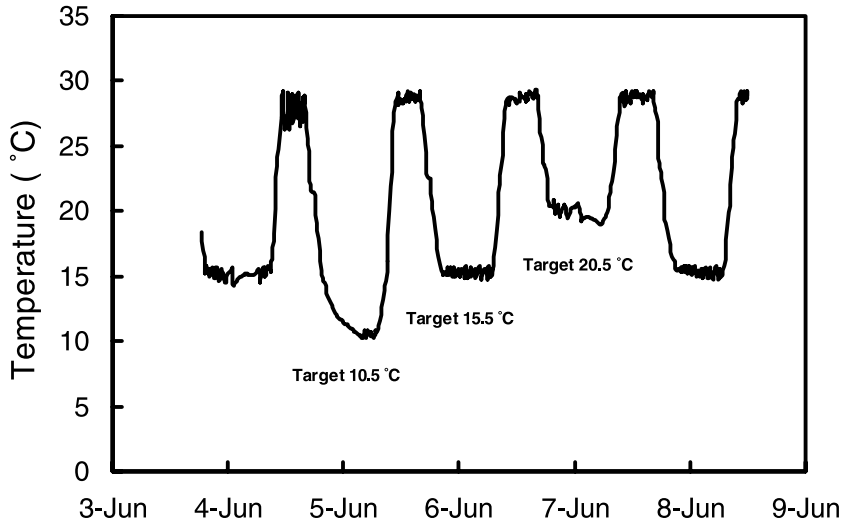

Fig. 1 Measured air temperature in the experimental mesocosm of the cottonwood plantation of Biosphere 2 from June 3–8 1999.

Table 1 Leaf properties as a function of canopy height in *Populus deltoides* trees growing in the experimental mesocosm of Biosphere 2. Values presented are means of 12 individual measurements (± SEM)

Leaf property	Lower canopy	Mid-canopy	Upper canopy
Leaf Area (cm^2)	170.4 ± 8.91a	194.7 ± 15.50a	286.1 ± 15.30b
Leaf Mass per Area ($g\ m^{-2}$)	63.5 ± 2.32a	69.9 ± 2.62a	90.2 ± 1.78b
% C	42.4 ± 0.51a	46.1 ± 0.22b	47.9 ± 1.44b
% N	4.0 ± 0.12a	3.8 ± 0.017a	3.3 ± 0.13b
N ($g\ m^{-2}$)	2.6 ± 0.06a	2.7 ± 0.06a	3.0 ± 0.05b
C : N	10.5 ± 0.28a	12.2 ± 0.54b	14.4 ± 0.52c
Chlorophyll a : b	5.2 ± 0.22a	5.6 ± 0.11b	6.0 ± 0.11b

Statistically significant differences among canopy positions are indicated by values followed by different letters.

differences detected in leaf physical properties may be related to predetermined leaf morphological traits in addition to environmental variation.

Total nonstructural carbohydrate content per unit leaf area was highest in the upper canopy leaves and decreased by 12% in the mid-canopy leaves and 36% in the lower canopy leaves (Table 2). The rate of carbohydrate turnover increased with night time temperature from 5.1% at 10°C to 20.4% at 15°C and 29.0% at 20°C across all canopy positions. In the upper canopy leaves the temperature treatment and the time of measurement both had a significant effect on TNC and there was a significant interaction between temperature and time. Only the time of sampling had a significant effect on the TNC of the lower canopy leaves.

Although somewhat variable, there were no significant differences in leaf respiration between the three nights when the ambient air temperature was 15°C (with the exception of a significant decrease in respiration between day one and day three in the lower canopy leaves, Table 3). Leaf respiration did vary as a function of canopy height (Fig. 2). Upper canopy leaves consistently had higher rates of respiration than leaves from the lower canopy at all measurement temperatures, with the increase varying from 123% at 10.5°C to 48% at 20.5°C. Respiration in the mid-canopy leaves was similar to, although slightly higher than, the lower canopy leaves. The temperature response of respiration varied with canopy depth (Fig 2 and Table 4). Upper canopy leaf respiration was least sensitive to changes in ambient air temperature while lower canopy leaf respiration was most sensitive. These differences in temperature sensitivity can be seen in the estimated E_o values (Table 4). This model parameter, which is related to the energy of activation, was 105% higher for the lower canopy leaves than it was in the upper canopy leaves. Expressed as a Q_{10} value over the 10–20°C measurement range, the lower canopy leaves had a 53% higher temperature coefficient than the upper canopy leaves.

When measured at 15°C, leaf respiration per unit leaf mass varied from 12.5 ± 0.22 nmol $g^{-1}\ s^{-1}$ in the mid canopy leaves to 14.0 ± 0.19 nmol $g^{-1}\ s^{-1}$ in the lower canopy leaves (Table 5). Mass-based leaf respiration was not significantly different between the three canopy positions at any measurement temperature. Respiration per unit nitrogen (μmol C g^{-1} $N^{-1}\ s^{-1}$) ranged from 0.33 ± 0.01 in the mid-canopy leaves to 0.39 ± 0.01 in the upper canopy leaves when measured at 15°C (Table 5). The upper-canopy nitrogen-based respiration rates were significantly higher than the mid-canopy leaves

Total nonstructural carbohydrate (g m^{-2})		Canopy position		
Night-time temperature	Sample time	Lower	Mid	Upper
10.5°C	Sunset	3.8 ± 0.56a	5.4 ± 0.45b	6.5 ± 0.35b
	Sunrise	3.6 ± 0.47a	5.3 ± 0.35b	6.0 ± 0.29b
	% Turnover	5.7	2.0	7.8
15.5°C	Sunset	4.6 ± 0.49a	5.6 ± 0.42a	6.9 ± 0.31b
	Sunrise	3.4 ± 0.48a	4.9 ± 0.38b	5.4 ± 0.49b
	% Turnover	26.4	12.0	22.8
20.5°C	Sunset	4.4 ± 0.51a	4.9 ± 0.69ab	5.6 ± 0.10b
	Sunrise	2.7 ± 0.20a	4.2 ± 0.30b	3.6 ± 0.60b
	% Turnover	37.4	13.5	36.0
Within canopy postion ANOVA statistic		Temp, ns Time < 0.01 Temp*time, ns	Temp, ns Time, ns Temp*time, ns	Temp < 0.001 Time < 0.001 Temp*time < 0.001

Statistically significant differences among canopy positions are indicated by values followed by different letters. Significance of treatment effect for temperature and the sampling time within a canopy position is indicated as the *P*-value or as nonsignificant (ns).

Table 2 Total nonstructural carbohydrate contents from leaves of *Populus deltoides* trees experiencing three different night-time temperature regimes. Carbohydrate samples were collected at sunset and sunrise on each of the treatment nights from three canopy heights. The average nightly turnover of nonstructural carbohydrates was calculated from these means as the percent loss. Values shown are means ((SEM) where n = 10–12)

	Leaf respiration (μmol m^{-2} s^{-1})			
Canopy position	Day 1	Day 3	Day 5	Model mean
Lower	0.98 ± 0.10b	0.62 ± 0.06a	0.76 ± 0.07ab	0.83 ± 0.06
Mid	1.09 ± 0.09a	0.83 ± 0.14a	0.80 ± 0.01a	0.95 ± 0.04
Upper	1.20 ± 0.09a	1.19 ± 0.07a	1.38 ± 0.06a	1.27 ± 0.02

Statistically significant differences among canopy positions are indicated by values followed by different letters.

Table 3 Leaf respiration a function of canopy height in *Populus deltoides* trees growing in Biosphere 2 on the three nights with ambient air temperatures of 15.5°C, and the mean estimate from the fitted temperature response model (see Eqn 2). Values presented are measurement means of 12 individual leaves (± SEM)

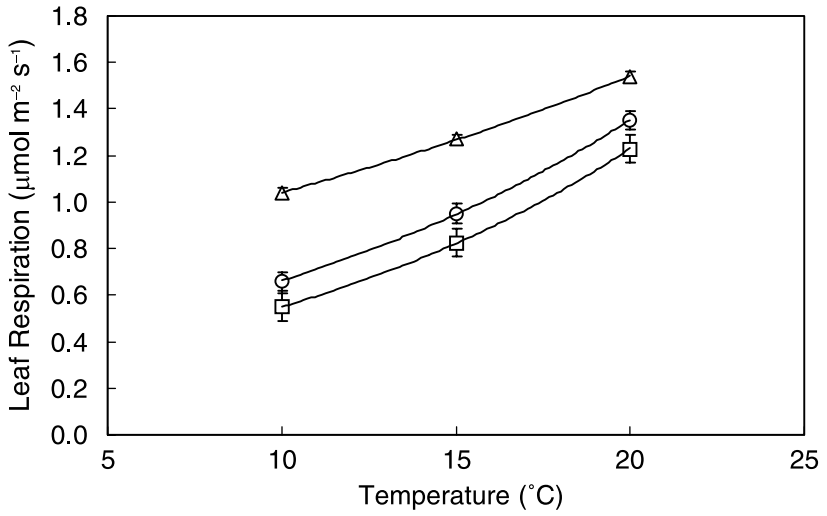

Fig. 2 Mean temperature response of dark respiration at three canopy heights in *Populus deltoides* trees growing in the experimental mesocosm of Biosphere 2. Leaf-level measurements were made each night of the five-night experiment with the cuvette temperature matching the ecosystem air temperature. Triangles = upper canopy leaves, circles = mid-canopy leaves and squares = lower canopy leaves. Each symbol represents the mean of 12 individual leaves, from six replicate trees (SEM) and the line is fit to the pooled sample using Eqn 2 from the text.

(P = 0.01) and nearly so for the lower-canopy leaves (P = 0.06). Leaf area-based respiration at 15°C was positively correlated to leaf area-based nitrogen ($R = 0.31x + 0.13$, $r^2 = 0.45$). Mass-based respiration at 15°C was similarly correlated with mass-based leaf nitrogen but the regression had significantly lower predictive power ($R = 1.48x + 7.68$, $r^2 = 0.19$).

If all leaves in the cottonwood canopy are assumed to have constant physiological characteristics, then total night-time

Table 4 Modeled response of respiration to changing temperature as a function of canopy height in *Populus deltoides* trees growing in Biosphere 2. Values presented are modeled from measurement means of 12 individual leaves (± SEM), each measured on five consecutive nights

	Lower canopy	Mid-canopy	Upper canopy
R_{10} (μmol m^{-2} s^{-1})	0.55 ± 0.06a	0.66 ± 0.04ab	1.04 ± 0.02b
E_o (J $mole^{-1}$)	55608 ± 9512b	49354 ± 5839b	27092 ± 3060a
Q_{10}	2.3	2.1	1.5

Statistically significant differences among canopy positions are indicated by values followed by different letters.

Table 5 Mass-(R_m) and nitrogen-based (R_N) leaf respiration a function of canopy height and ambient air temperature in *Populus deltoides* trees growing in Biosphere 2. Values presented are measurement means of 12 individual leaves (± SEM)

	Night-time temperature °C	Lower canopy	Mid-canopy	Upper canopy
R_{m10} (nmol g^{-1} s^{-1})	10.5	8.3 ± 0.58a	9.4 ± 0.71a	9.8 ± 0.98a
R_{m15} (nmol g^{-1} s^{-1})	15.5	14.0 ± 0.19a	12.5 ± 0.22a	13.1 ± 0.32a
R_{m20} (nmol g^{-1} s^{-1})	20.5	15.9 ± 1.09a	19.8 ± 0.62a	18.2 ± 0.66a
R_{N10} (mmol g^{-1} Ns^{-1})	10.5	0.20 ± 0.01a	0.24 ± 0.02a	0.30 ± 0.03a
R_{N15} (mmol g^{-1} Ns^{-1})	15.5	0.35 ± 0.01ab	0.33 ± 0.01a	0.39 ± 0.01b
R_{N20} (mmol g^{-1} Ns^{-1})	20.5	0.40 ± 0.03a	0.52 ± 0.02ab	0.55 ± 0.02b

Statistically significant differences among canopy positions are indicated by values followed by different letters.

Table 6 Modeled respiratory carbon release from *Populus deltoides* foliage in Biosphere 2. The constant temperature response models assumed all leaves have the physiological properties of either the lower, mid or upper canopy leaves, respectively. In the distributed response model the upper 1 m of the tree was assumed to have the response of the 'upper canopy leaves', the lower 1.3 m of the canopy was assumed to have the temperature response of the 'lower canopy leaves' and the remainder was assumed to have the temperature response of the 'mid canopy leaves' (Fig. 1 and Table 3). Area and mass-based total respiration estimates are shown

Night-time temperature °C	Constant temperature response (mmol C m^{-2})			Distributed temperature response (mmol C m^{-2})
	Lower canopy	Mid-canopy	Upper canopy	
10.5	69.5	81.8	118.6	86.4
15.5	92.3	104.5	136.8	107.3
20.5	127.3	140.0	160.5	140.0
5 night total	474.1	540.0	688.6	551.8
	Constant temperature response (nmol C g^{-1})			Distributed temperature response (nmol C g^{-1})
10.5	1165.2	1245.7	1392.2	853.1
15.5	1455.1	1510.3	1518.3	1067.4
20.5	2005.5	2005.7	1766.9	1410.9
5 night total	4625.9	4761.6	4677.4	3331.3

leaf carbon loss varied from 69.5 mmol m^{-2} assuming the properties of the lower canopy leaves on the 10.5°C night to 160.5 mmol m^{-2} assuming the properties of the upper canopy leaves on the 20.5°C night (Table 6). If the canopy was assumed to be made entirely of mid-canopy leaves, the nightly estimates of total leaf carbon loss were not significantly different from those estimates that explicitly consider the vertical distribution of respiration and the temperature response of respiration (Table 6). However, if the canopy were assumed to be made entirely of lower canopy leaves, the constant model would underestimate the distributed model by 19.5% on the 10.5°C night and 9.1% on the 20.5°C night. By contrast, if the canopy were assumed to be made entirely of upper canopy leaves the constant model would consistently overestimate the total amount of respiration by 37.4% on the 10.5°C night and 14.6% on the 20.5°C night. Over the five nights of the experiment, an estimated 474.1 mmol C m^{-2} ground area was respired assuming the canopy was made entirely of leaves with lower canopy physiological properties. Assuming the canopy was made entirely of leaves with upper canopy physiological properties would increase this estimate of total respiration by 45% to 688.6 mmol C m^{-2}. The constant-lower canopy model underestimates carbon loss by 77.7 mmol m^{-2} or 16.4% while the constant-upper canopy model overestimates carbon loss by 136.8 mmol m^{-2} or 24.8% of the distributed model flux over the duration of the experiment. The distributed physiology model estimates the total carbon loss will increase by 62% when the experimental treatment was increased from 10.5°C to 20.5°C. The constant physiology model estimates this response to be a 83, 71 or 35% increase over the same temperature range assuming the canopy is made entirely of lower, mid- or upper-canopy leaves, respectively.

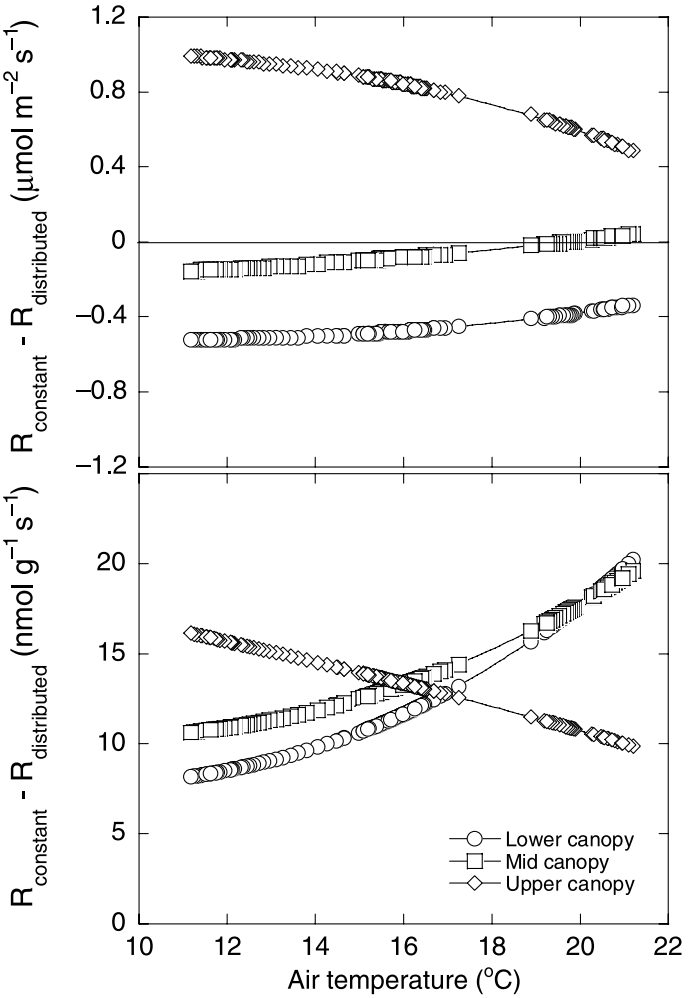

Fig. 3 Difference in the rate of canopy respiration estimated from a fixed physiology model and a distributed physiology model of the cottonwood plantation of Biosphere 2 as a function of air temperature. The constant physiology models assumed all leaves have the physiological properties of either the lower (circles), mid-(squares) or upper (diamonds) canopy leaves, respectively. In the distributed physiology model the upper 1 m of the tree was assumed to have the response of the 'upper canopy leaves', the lower 1.3 m of the canopy was assumed to have the temperature response of the 'lower canopy leaves' and the remainder was assumed to have the temperature response of the 'mid canopy leaves' (Fig. 1 and Table 3). Area (top panel) and mass-based (bottom panel) estimates are shown.

Less variation exists between the three mass-based constant physiology models at any temperature, but the relationship among these three constant physiology models and the distributed physiology model is more variable. For example the upper canopy constant physiology model overestimates total carbon loss compared with the distributed canopy model by 63% at 10.5°C, 42% at 15.5°C and 63% at 20.5°C (Table 6). By comparison both the mid- and lower canopy constant physiology models are less variable.

The relationship between the constant physiology mid-canopy model (area based) and the distributed physiology model is curvilinear such that at lower temperatures the distributed model predicts higher carbon loss than the constant model, but at higher temperatures the distributed model predicts lower carbon loss than the constant model (Fig. 3). When the air temperature is equal to 18.8°C the two models give the same estimate. The lower canopy constant physiology model consistently underestimates the distributed physiology model by as much as 0.6 mmol s^{-1}, while the upper canopy constant physiology model consistently overestimates the distributed physiology model by as much as 1 mmol s^{-1}. Similar to the leaf level response, the upper canopy constant physiology model shows the strongest temperature sensitivity. By contrast, all three constant physiology models based on mass-based respiration rates consistently overestimate the distributed physiology model by 8.1–20.2 nmol g^{-1} s^{-1} (Fig. 3). The amount of overestimation from the upper canopy constant physiology model compared to the distributed physiology model decreases with increasing temperature while the relationship between both the mid and lower canopy constant physiology models and the distributed physiology model increases with increasing temperature.

Discussion

Leaf respiration per unit leaf area in *P. deltoides* trees growing in Biosphere 2 decreased with depth in the canopy and the magnitude of this decrease was dependent on the ambient air temperature. When measured at the long-term temperature set point of 15.5°C, leaf respiration decrease by 35% from the upper to lower canopy, similar to trends reported for other tree species (Reich *et al.*, 1998a; Bolstad *et al.*, 1999; Carswell

et al., 2000; Griffin *et al.*, 2002; Meir *et al.*, 2001). Since the upper canopy leaves received the most direct sunlight and therefore would have the largest daily total carbon gain compared with the leaves in the mid- or lower canopy, we suggest the respiratory response is driven by the ambient light environment. As a result, upper canopy leaves contained more soluble sugars and starch, which in turn provide the substrate to support the increased respiration rates (Azcón-Bieto & Osmond, 1983).

Leaf mass per unit area also declined with depth in the canopy by 30%. Therefore, when respiration was expressed per unit leaf mass rather than per unit leaf area, no significant trends in respiration with canopy depth were observed, suggesting the area-based decrease is the result of less leaf mass per unit leaf area in the lower canopy leaves. A similar dependence of leaf area-based respiration on LMA (as influenced by canopy position) was observed in tropical lowland rainforest trees (Meir *et al.*, 2001) but not in the semidecidous species *Nothofagus fusca* (Griffin *et al.*, 2002). While other studies have suggested that a general relationship exists between LMA, leaf N, and R (Ryan, 1995; Reich *et al.*, 1996; Reich *et al.*, 1998a,b), we found these relationships did not hold for leaves within a *P. deltoides* canopy grown in high soil N. While the lack of statistically significant variation in mass-based respiration rates with canopy depth may suggest that mass-based estimates of respiration may be more easily scaled to the canopy or ecosystem level, the temperature response is independent of the means of data expression (mass vs area-based respiration) and thus suggests canopy position must be explicitly considered regardless.

Upper-canopy leaves had significantly less nitrogen per unit mass and more nitrogen per unit area than lower-canopy leaves, even though they received less than 15% of the ambient light. As a result leaf respiration was significantly correlated to leaf N as long as both respiration and nitrogen were expressed on a similar basis (area or mass). When respiration was expressed per unit leaf nitrogen, the upper-canopy leaves had the highest rates of respiration, further indicating that respiration in these leaves was related both to respiratory substrate availability or general metabolic energy demand and to leaf N.

Very little information exists on the nature of the variation in the temperature response of respiration through the canopy. However, the temperature response of respiration would be expected to be related to metabolic activities such as growth, the maintenance of ion gradients, protein turnover and cellular repair, phloem loading and in some cases excess carbohydrate consumption, all of which are likely to vary with canopy depth. Thus, while our results are novel, it should perhaps not be surprising that the temperature response of respiration varied with depth in the canopy. Our results are, however, contrary to our hypothesis that the spatial distribution of the temperature response of respiration would follow the overall distribution of respiratory activity, and thus would decrease with depth in the canopy. Instead, the lower canopy leaves were significantly more responsive to temperature than the upper canopy leaves, with respiration increasing by 131% in the lower leaves and by only 48% in the upper leaves when the ambient air temperature was increased from 10.5°C to 20.5°C. Given the short-term nature of the temperature treatments and the fact that all measurements were made on fully expanded leaves, it seems unlikely that growth processes were significantly affected and thus it is logical to expect the observed results are related to maintenance processes. Thus we conclude that both the instantaneous rate of respiration and its temperature response are related to substrate availability or metabolic activity that covaries with substrate availability, as well as to leaf nitrogen and maintenance processes.

Assessing the impact of the observed variation in leaf-level respiratory physiology requires the results be scaled to the canopy-level as is commonly done in ecosystem, regional and global models (e.g. Ryan, 1991b; Williams *et al.*, 2000; Knorr & Heimann, 2001). Doing so further allows us to examine the possibility that simplifying assumptions can be made, which have a limited impact on the estimated carbon flux. Our results demonstrate that location of individual leaf measurements, or the means of data expression (mass vs area based respiration) can have a significant effect on the model outcome. When scaling our area-based results to the canopy level, we find that simplifying assumptions regarding the variation in respiration and the temperature response of respiration with canopy height tend to result in an underestimation of the actual carbon loss if the assumptions are based on the lower or mid-canopy physiology, but overestimate the actual carbon loss if the model assumptions are based on the upper canopy physiology. Furthermore, when the effects of the observed variation in both the rates of respiration and the temperature response of respiration with canopy depth are taken into consideration, we find that our model forest ecosystem is most strongly affected at lower temperatures. Over the course of the 10.5°C night the explicit consideration of the variation in respiratory physiology results in an estimated −16.8 to +32.3 mmol C m^{-2} difference in the total carbon respired from the canopy. This range represents a 70.6% difference in the response of lower and upper canopy constant physiology models on the 10.5°C night. By comparison, when estimated at 20.5°C the response is significantly reduced, and ranges from −12.7 mmol C m^{-2} from the lower canopy model to 20.4 mmol C m^{-2} in the upper canopy model compared with the distributed physiology model. In general, the difference between the constant physiology and distributed physiology models is minimized when the constant physiology model is based on the mid-canopy leaves and thus may suggest that it is possible to use simplifying assumptions regarding respiratory carbon loss in more complex models containing ecosystem physiology if mid-canopy leaves are used to generate the empirical relationship between respiration and temperature. At the same time, the significant differences observed both

among the canopy positions and in the scaled canopy-level fluxes may have important mechanistic implications and could lead to a better understanding of whole-tree and ecosystem response to variations in night-time temperatures. Similarly, the use of mass-based leaf respiration rates simplifies scaling to the canopy since most of the variation in area-based respiration was offset by covariation in LMA. Still, since the temperature response of respiration differed among the three canopy positions, differences in the total modeled carbon loss from the canopy were found and further indicate the importance of a spatially explicit description of the canopy physiology.

Controlled environment facilities like Biosphere 2 are useful for developing hypotheses that can then be taken to natural ecosystems and tested. Clearly an explicit test of the hypothesis that respiration and the temperature response of respiration vary with depth in the canopy needs to be completed in a more natural setting. Natural ecosystems are subject to large variations in air temperature that range over diel, seasonal and interannual time scales. The short duration of the temperature treatments during this experiment makes the results most applicable to better understanding the response of forest systems to nightly and weekly changes in temperature variation. Over longer time scales, leaf respiratory response to temperature is a function of both the physiological and temperature history, and may be subject to varying degrees of acclimation and/or adaptation (Amthor, 1984; Amthor, 1989; Larigauderie & Körner, 1995; Arnone & Körner, 1997; Atkin *et al.*, 2000; Gunderson *et al.*, 2000; Tjoelker *et al.*, 2001). Clearly more study is needed to extend these results to longer-time scales relevant to global warming, yet the implications of our findings for predicting the carbon sequestration potential of natural and managed ecosystems are substantial. Given the expected impact of human population growth it is now predicted that global temperatures will continue to increase and be 1–6°C warmer by the year 2100 (Hansen *et al.*, 1999). Taken together, the predicted global warming and respiratory responses to temperature are compelling evidence of our need to be able to accurately quantify the interactions among temperature, plant respiration and ecosystem function. The development of a mechanistic understanding of these responses while explicitly considering the variation in leaf physiology within canopies is critical for accurately modeling and predicting ecosystem carbon balance and is the subject of further experimentation currently underway at Biosphere 2.

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