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The relative impacts of daytime and night-time warming on photosynthetic capacity in *Populus deltoides*

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ABSTRACT

In order to investigate the relative impacts of increases in day and night temperature on tree carbon relations, we measured night-time respiration and daytime photosynthesis of leaves in canopies of 4-m-tall cottonwood (*Populus deltoides* Bartr. ex Marsh) trees experiencing three daytime temperatures (25, 28 or 31 °C) and either (i) a constant nocturnal temperature of 20 °C or (ii) increasing nocturnal temperatures (15, 20 or 25 °C). In the first (day warming only) experiment, rates of night-time leaf dark respiration (R_{dark}) remained constant and leaves displayed a modest increase (11%) in light-saturated photosynthetic capacity (A_{max}) during the day (1000–1300 h) over the 6 °C range. In the second (dual night and day warming) experiment, R_{dark} increased by 77% when nocturnal temperatures were increased from 15 °C (0·36 μmol m^{-2} s^{-1}) to 25 °C (0·64 μmol m^{-2} s^{-1}). A_{max} responded positively to the additional nocturnal warming, and increased by 38 and 64% in the 20/28 and 25/31 °C treatments, respectively, compared with the 15/25 °C treatment. These increases in photosynthetic capacity were associated with strong increases in the maximum carboxylation rate of rubisco (V_{cmax}) and ribulose-1,5-bisphosphate (RuBP) regeneration capacity mediated by maximum electron transport rate (J_{max}). Leaf soluble sugar and starch concentration, measured at sunrise, declined significantly as nocturnal temperature increased. The nocturnal temperature manipulation resulted in a significant inverse relationship between A_{max} and pre-dawn leaf carbohydrate status. Independent measurements of the temperature response of photosynthesis indicated that the optimum temperature (T_{opt}) acclimated fully to the 6 °C range of temperature imposed in the daytime warming. Our findings are consistent with the hypothesis that elevated night-time temperature increases photosynthetic capacity during the following light period through a respiratory-driven reduction in leaf carbohydrate concentration. These responses indicate that predicted increases in night-time minimum temperatures may have a significant influence on net plant carbon uptake.

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INTRODUCTION

It is now predicted that global temperatures will be 1–6 °C warmer by the year 2100 as a result of the impacts of human population growth (Hansen *et al*. 1999; IPCC 2001). In terrestrial environments this warming is more pronounced at night than during the day (Easterling *et al*. 1997; Alward, Detling & Milchunas 1999; IPCC 2001). Between 1950 and 1993, the night-time daily minimum air temperature over land increased by about 0·2 °C per decade, about twice the rate of increase in daytime maximum air temperature (IPCC 2001). Nocturnal warming has been suggested to have a number of ecologically important consequences which could have dramatic effects on ecosystem level carbon, nitrogen and water cycling, and therefore carbon storage (Dewar, Medlyn & McMurtrie 1999; Melillo 1999; Saxe *et al*. 2001). These impacts include altering plant–insect interactions (Yang & Stamp 1995), prolonging the growing season (Keeling, Chin & Whorf 1996; Myneni *et al*. 1997; Menzel & Fabian 1999), changing species abundance (Alward *et al*. 1999) and influencing net primary productivity and carbon sequestration (Coughenour & Chen 1997; Myneni *et al*. 1997; Alward *et al*. 1999).

Environmental warming is likely to have significant direct effects on plant carbon relations, particularly through its effect on dark respiration. At the leaf-level, roughly half of daily net photosynthetic carbon fixation is re-released via plant respiration in the following evening (Amthor 1989; Ryan 1991). Typically, for each successive 10 °C increase in temperature, metabolic rates, and hence respiration rates, are considered to double (Ryan 1991). However, the response of leaf respiration to temperature is highly variable, with Q_{10} values (the proportional change in respiration rate with a 10 °C increase in temperature) ranging from 1·4 to 4·0 (Azcon-Bieto 1992). In addition to this, respiration characteristics under field conditions vary with canopy position (Griffin, Turnbull & Murthy 2002a) and are also a function of temperature and physiological history (Amthor 1984; Atkin, Holly & Ball 2000; Griffin *et al*. 2002b). The processes of photosynthesis and respiration respond independently to temperature and are linked

mechanistically, through the impacts that each process has on leaf carbohydrate status (Azcon-Bieto 1992). Their responses to temperature will thus have non-linear effects on plant carbon gain (Dewar *et al*. 1999; Gunderson, Norby & Wullschleger 2000). Photosynthesis is particularly sensitive to the rate of utilization or export of its products. If this is low (e.g. with reduced sink strength or at low temperatures which limit respiration), the rate of photosynthesis may become restricted by 'feedback inhibition' (Stitt, Huber & Kerr 1987). Carbohydrates are frequently found to accumulate in leaves when photosynthesis is inhibited by low sink demand (Azcon-Beito & Osmond 1983), although the precise biochemical mechanism of feedback inhibition, and the role of carbohydrates in it, is the subject of debate (Stitt 1991; Goldschmidt & Huber 1992; Jang & Sheen 1994; Moore *et al*. 1999).

Predicted patterns of global warming, the different responses of respiration and photosynthesis to temperature, and the link between respiration and photosynthesis are compelling reasons for investigations of the impacts of elevated day- and night-time temperature on plant carbon relations. In the present study we investigated the relative impacts of elevated night-time and daytime temperatures on photosynthetic capacity. We measured respiration and photosynthesis of leaves in canopies of large cottonwood (*Populus deltoides* Bartr. ex Marsh) trees that were experimentally exposed to (1) daytime temperature manipulation at constant night temperature and (2) dual night and day temperature manipulation, in two whole ecosystem experiments. The level of night-time warming in experiment 2 (a range of 10 °C) was approximately twice that of daytime warming (range of 6 °C) in order to mimic the relative changes predicted in global temperature change scenarios. Our overall aim in this research was to establish physiological relationships between leaf respiration and photosynthetic capacity that might regulate plant carbon gain in a global change environment. We hypothesized that elevated night-time temperature should have a greater impact on photosynthetic capacity during the following light period than elevated daytime temperature, as a consequence of an increase in dark respiration and a reduction in leaf carbohydrate concentration.

MATERIALS AND METHODS

Growth conditions and plant material

These experiments took advantage of the technical innovations and size of the Biosphere 2 Research Centre of Columbia University near Tucson, Arizona, USA to regulate night- and daytime temperature of an intact model ecosystem. The Biosphere 2 facility consists of several synthetic communities of plants and soils encased in a glass and metal shell. Details of the Biosphere 2 environmental control system are described elsewhere (Lin *et al*. 1998; Dempster 1999; Zabel *et al*. 1999; Griffin *et al*. 2002a, b). The structure covers 1·27 ha and is located 1200 m above sea level at 32·5°N latitude in southern Arizona. These experiments were conducted in the 2000 m^2 forestry section, which is physically isolated from the remainder of Biosphere 2 and has independent temperature and CO_2 control. The forestry section is subdivided into three roughly equal mesocosms, 41 m long (in a north–south orientation), 18 m wide and with a maximum height of 24 m. Experiments were conducted in the eastern mesocosm under ambient CO_2 partial pressure (42 Pa). Each mesocosm has three large air handlers that provide both the primary means of air circulation and the temperature control. Within each mesocosm, four additional fans help maintain the air circulation and break up the canopy boundary layer.

The glass and metal structure components of Biosphere 2 act as a neutral density filter for incoming solar radiation. Photon flux density (PFD) was more than 70% of that outside with midday levels exceeding 1600 μmol m^{-2} s^{-1}. At the time of the year that these experiments were conducted daily integrated light averaged 25 ± 1·3 mol m^{-2} d^{-1}. During daylight hours, CO_2 control within the mesocosm was maintained at 42 ± 1 Pa by adding pure CO_2, regulated with a mass flow meter (Sierra Side-Track; Sierra Instruments, Inc., Monterey, CA, USA), into the air stream entering the air handlers. Carbon dioxide was added to each mesocosm as needed to replace the carbon removed from the atmosphere via photosynthesis. At night, or at any other time when respiratory CO_2 release exceeded photosynthetic carbon uptake, a variable speed fan was used to add outside ambient air to Biosphere 2 air so that the CO_2 partial pressure was maintained at the desired set point (50 ± 1 Pa).

The cottonwood (*Populus deltoides*) tree cuttings used in these experiments were donated by Westvaco and came from a production fibre farm in Summerville, South Carolina, USA. The clone (S7c8) is adapted to the lower Brazos River, Texas and is day neutral. The trees were coppiced prior to the commencement of the growing season and at the initiation of the experiments, the 77 trees were 2 years old and 4–5 m tall. The physiological measurements presented here were undertaken during the most active time of the year in summer (July 2001).

Experimental design

The purpose of the experimental manipulations was to determine the impacts of changes in both night- and daytime temperature on tree photosynthesis and respiration. The investigations took the form of two separate experiments, each lasting 9 d. In experiment 1, trees were exposed to three different daytime temperatures (25 ± 0·2, 28 ± 0·14 and 31 ± 0·57 °C) and a constant night temperature of 20 ± 0·25 °C. In experiment 2, trees were exposed to a dual manipulation of day and night temperature, with night/day temperatures of 15 ± 0·70/25 ± 0·10, 20 ± 0·14/28 ± 0·23 and 25 ± 0·11/31 ± 0·20 °C. Each temperature treatment was maintained for a period of 3 d. These night and day temperatures were selected to bracket the long-term minimum and maximum temperature set-points that the trees expe-

rience in the controlled mesocosm. During this period outside weather conditions were generally constant (partially cloudy but sunny with maximum air temperatures similar to those within Biosphere 2). Although there was day-to-day variation in light levels, daily integrated light averages did not differ significantly between the 3 d treatment periods. Root-zone volumetric water content was maintained at $0{\cdot}46 \pm 0{\cdot}02$ m^3 m^{-3} using an automated irrigation system.

Respiration measurements (as described below) were made over a 2 h period at least 1 h after sunset and at least 2 h after the mesocosm reached its night temperature set point (between 2100 and 2300 h) on the third night of each temperature treatment. This gave a 3 d period to allow the trees to acclimate to each temperature set point. Our observations indicated that this period was sufficient to allow for acclimation of photosynthesis to temperature (unpublished results). Continuous measurements of leaf respiration indicated that respiration rate did not change significantly throughout the night (K.L. Griffin, unpubl. results). Photosynthetic measurements were made between 1000 and 1300 h on the day immediately following respiration measurements.

Respiration and photosynthesis measurements

Measurements of leaf dark respiration and photosynthesis were made on fully expanded leaves from the mid-canopy of each experimental tree (at approximately 2–3 m height). These leaves were fully sunlit during the day. At least 12 leaves (from at least two separate branches on six individual trees) were measured. Leaf dark respiration and photosynthesis measurements were made using infra-red gas analysis systems (Li-Cor model 6400; Li-Cor, Lincoln, NE, USA) equipped with CO_2 control modules. *In situ* rates of leaf dark respiration were measured with leaf temperatures controlled at the appropriate night-time set-point (i.e. 20 °C in experiment 1 and 15, 20 or 25 °C in experiment 2). External CO_2 partial pressure (C_a) was maintained at ambient atmospheric levels (50 Pa). Measurements were taken when respiratory gas exchange had equilibrated (taken to be when the coefficient of variation (CV) for the CO_2 partial pressure differential between the sample and reference analysers was below 1%). This condition was typically achieved within 1 min after enclosing the leaf in the cuvette. Each respiration measurement was the average of three values logged at 30 s intervals.

The A–C_i response curves were determined following measurements of steady-state responses of assimilation (A) to internal leaf CO_2 partial pressures (C_i). External CO_2 partial pressure (C_a) was supplied in 10 steps from 150 to 0 Pa. Measurements were made at each C_a setpoint when photosynthetic gas exchange had equilibrated (CV below 1%). This condition was typically achieved in 1–2 min after a stable C_a set-point had been reached. Leaf temperatures were maintained at the appropriate daytime set-point using thermoelectric coolers and water vapour pressure deficit was generally held between 1·0 and 1·5 kPa. A constant PFD of 2000 μmol m^{-2} s^{-1} was provided by blue–red light emitting diodes mounted above the leaf cuvette.

Analysis of A–C_i response curves involved calculation of parameters potentially limiting to photosynthesis: V_{cmax} [maximum carboxylation rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)], J_{max} [ribulose-1,5-bisphosphate (RuBP) regeneration capacity mediated by maximum electron transport rate] and the rate of triose phosphate utilization (TPU, which indicates the availability of inorganic P for the Calvin cycle). This was achieved using Photosynthesis Assistant (v1·1, Dundee Scientific, Dundee, UK) which uses a biochemical model describing A (Farquhar, von Caemmerer & Berry 1980) and relies on the concept that it is the minimum of any of the above three factors that limit CO_2 assimilation. R_{day} (the release of CO_2 in the light by processes other than photorespiration) is also calculated from the A–C_i response data. Temperature response functions used in the model calculations were calculated for the appropriate operational temperatures (Bernacchi *et al*. 2001). Photosynthetic capacity was determined under saturating PFD and ambient C_a (42 Pa, A_{max}) and saturating C_a (A_{sat}).

In order to confirm the rate of acclimation of photosynthesis the temperature response of photosynthesis was determined for leaves in each temperature treatment in experiment 2. This involved measurements of steady-state responses of assimilation (A) to leaf temperature measured using a thermocouple in direct contact with the leaf. Leaf temperatures were manipulated by changing cuvette temperature in 10–12 steps over a range ± 8 °C from the ambient mesocosm set-point. A constant saturating PFD of 2000 μmol m^{-2} s^{-1} was provided by blue–red light-emitting diodes mounted above the leaf cuvette.

Leaf analyses

Carbohydrate analyses were performed on leaf discs taken from leaves adjacent to those used for gas exchange measurements. Samples were taken just prior to sunrise and at sunset. Soluble sugar content and starch content of leaves were determined colorimetrically using an ethanol extraction technique (Hendrix 1983; Griffin, Sims & Seemann 1999). Total non-structural carbohydrate (TNC) content was calculated as the sum of soluble sugar and starch. Daily turnover of soluble sugars, starch and TNC was calculated by

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

Other leaf analyses were determined on material harvested directly following gas exchange measurements. Specific leaf area (SLA) was calculated following determination of individual leaf area and dry weight. Leaf nitrogen content (N_{area}) was determined on dried and ground material using a CNS autoanalyzer (Carlo Erba Na 1500, Milan, Italy).

Neither SLA (range 13·2–14·2 $m^2\ kg^{-1}$) nor N_{area} (range 175–215 mmol N m^{-2}) differed significantly between trees in either experiment.

Statistical analysis

Analysis of variance (ANOVA) was used to test for the effect of temperature regime on respiration, carbohydrate concentration and photosynthesis (S-Plus v3·3, MathSoft Inc., Seattle, WA, USA). A nested model (individual leaves nested within trees) was used to account for tree versus leaf-level variation in measured parameters (Underwood 1981). Differences were considered significant if probabilities (P) were less than 0·05. Treatment means were compared by least significant difference to determine whether means of the dependent variable were significantly different at the 0·05 probability level (Sokal & Rohlf 1981).

RESULTS

Experiment 1 – daytime temperature manipulation

In experiment 1 (day warming only), rates of night-time leaf dark respiration (R_{dark}) remained constant over the three temperature treatments and the leaves displayed a modest increase in light-saturated photosynthetic capacity (A_{max}) during the day (1000–1300 h) of 11% over the 6 °C range (Fig. 1). Photosynthetic parameters derived from A–C_i response curves varied in their response to temperature regime (Table 1). Whereas V_{cmax} increased from 111 μmol CO_2 m^{-2} s^{-1} in the 20/25 °C treatment to 147 μmol CO_2 m^{-2} s^{-1} in the 20/31 treatment, J_{max} and TPU decreased slightly. Photosynthetic rate at saturating PFD and C_a (A_{sat}) and C_i/C_a were both unresponsive to daytime temperature.

Table 1. Gas exchange characteristics calculated from the response of assimilation, A, to internal CO_2 partial pressure, C_i, for leaves of *Populus deltoides* trees experiencing three daytime temperature treatments and a constant night temperature of 20 °C

	Night/day temperature (°C)			ANOVA statistics (T)
	20/25	20/28	20/31	
R_{day} (μmol CO_2 m^{-2} s^{-1})	2·01 (0·62)[a]	1·81 (0·19)[a]	1·48 (0·13)[a]	NS
V_{cmax} (μmol CO_2 m^{-2} s^{-1})	111 (6·2)[a]	126 (2·3)[b]	147 (4·0)[c]	<0·001
J_{max} (μmol m^{-2} s^{-1})	230 (19·1)[a]	213 (4·0)[a]	217 (4·1)[a]	NS
TPU (μmol $m^{-2}s^{-1}$)	10·64 (0·16)[b]	10·84 (0·29)[b]	9·86 (0·13)[a]	<0·05
J_{max}/V_{cmax}	2·06 (0·06)[c]	1·69 (0·03)[b]	1·48 (0·02)[a]	<0·001
A_{sat} (μmol CO_2 m^{-2} s^{-1})	39·3 (2·04)[a]	37·0 (0·68)[a]	36·8 (0·51)[a]	NS
C_i/C_a	0·74 (0·03)[a]	0·84 (0·02)[b]	0·78 (0·02)[a]	NS

Values shown are means (± SEM) where $n = 12$. Significance of treatment effect for daytime temperature (T) is indicated as the value of P or as non-significant (NS). Different letters within rows indicate statistically different values at $P < 0·05$ using least significant difference test of treatment means.

Experiment 2 – Dual night and day temperature manipulation

In experiment 2 (dual night and day warming), R_{dark} increased by 77% when nocturnal temperatures were increased from 15 °C (0·36 μmol m^{-2} s^{-1}) to 25 °C (0·64 μmol m^{-2} s^{-1}) (Fig. 1). This corresponded to a Q_{10} of

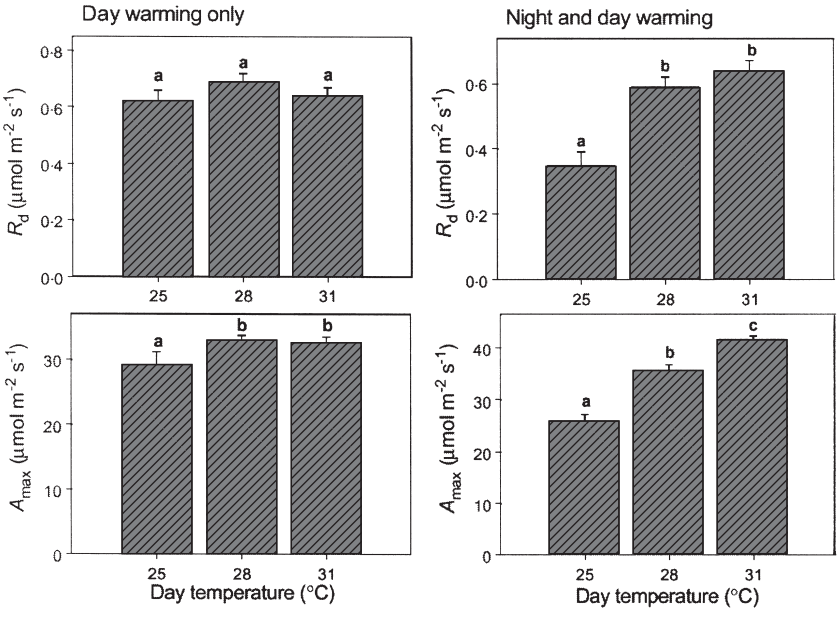

Figure 1. Night respiration, R_d, and maximum assimilation rate, A_{max}, in the subsequent light period (measured between 1000 and 1300 h) for leaves of *Populus deltoides* trees experiencing different temperature regimes. Left panels: Experiment 1 – trees were subjected to an increase in day temperature only (night temperature constant at 20 °C). Right panels: Experiment 2 – trees experienced an increase in both day and night temperature (i.e. night/day temperatures of 15/25, 20/28 and 25/31 °C). Each bar is the mean (± SEM) of 12 determinations of each parameter. Different letters above bars indicate statistically different values at $P < 0·05$ using least significant difference test of treatment means.

Table 2. Gas exchange characteristics calculated from the response of assimilation, A, to internal CO_2 partial pressure, C_i, for leaves of *Populus deltoides* trees experiencing three temperature treatments involving both day and night warming

	Night/day temperature (°C)			ANOVA statistics (T)
	15/25	20/28	25/31	
R_{day} (μmol CO_2 m^{-2} s^{-1})	1·23 (0·08)^{ab}	1·76 (0·61)^{b}	0·33 (0·10)^{a}	NS
V_{cmax} (μmol CO_2 m^{-2} s^{-1})	93·2 (3·6)^{a}	133 (3·3)^{b}	176 (2·0)^{c}	<0·0001
J_{max} (μmol m^{-2} s^{-1})	177 (6·3)^{a}	220 (7·1)^{b}	256 (3·8)^{c}	<0·0001
TPU (μmol m^{-2} s^{-1})	9·73 (0·27)^{a}	10·68 (0·28)^{b}	10·35 (0·18)^{b}	<0·05
J_{max}/V_{cmax}	1·91 (0·04)^{c}	1·65 (0·03)^{b}	1·45 (0·01)^{a}	<0·0001
A_{sat} (μmol CO_2 m^{-2} s^{-1})	32·3 (1·00)^{a}	40·0 (1·29)^{b}	42·9 (0·56)^{c}	<0·0001
C_i/C_a	0·79 (0·02)^{a}	0·87 (0·01)^{b}	0·86 (0·01)^{b}	<0·001

Values shown are means (± SEM) where $n = 12$. Significance of treatment effect for daytime temperature (T) is indicated as the value of P or as non-significant (NS). Different letters within rows indicate statistically different values at $P < 0·05$ using least significant difference test of treatment means.

1·78. A_{max} responded positively to the additional nocturnal warming, and increased by 38 and 64% in the 20/28 and 25/31 °C treatments, respectively, compared with the 15/25 °C treatment. These increases in photosynthetic capacity were associated with strong increases in V_{cmax} (from 93·2 μmol CO_2 m^{-2} s^{-1} in the 15/25 °C treatment to 176 μmol CO_2 m^{-2} s^{-1} in the 25/31 °C treatment) and J_{max} (from 177 μmol m^{-2} s^{-1} in the 15/25 °C treatment to 256 μmol m^{-2} s^{-1} in the 25/31 °C treatment) (Table 2). TPU displayed only a very modest increase over the same temperature range. In contrast to its response to day warming only, photosynthetic rate at saturating PFD and C_a (A_{sat}) increased significantly with the addition of nocturnal warming (from 32·3 μmol m^{-2} s^{-1} at 15/25 °C to 42·9 μmol m^{-2} s^{-1} at 25/31 °C). Measurements of the temperature response of photosynthesis indicated that it responded rapidly (~ 48 h) to the 6 °C range of temperature imposed in the daytime warming (Fig. 2). The optimum temperature for photosynthesis (T_{opt}) tracked the actual mesocosm daytime temperature set point very closely (T_{opt} of 24·6 ± 0·72 °C at 25 °C, 28·7 ± 0·64 °C at 28 °C and 31·2 ± 1·10 °C at 31 °C).

Leaf soluble sugar content, measured at sunrise following respiration measurements, declined significantly from 6·13 to 4·26 g m^{-2} as nocturnal temperature increased (Table 3). Leaf starch concentration at sunrise responded similarly to nocturnal warming, and was significantly lower following the warmest night (0·82 g m^{-2}) than the coolest night (2·01 g m^{-2}). By sunset, soluble sugar had increased in all leaves, but more substantially so in leaves previously subjected to the higher night-time temperature treatments. Soluble sugar content increased by 41% between sunrise and sunset in leaves following the 25/31 °C night-time treatment but by only 13% following the 15/25 °C treatment. The increase in starch content was less pronounced, with an increase of 94% during the day in leaves following the 25/31 °C temperature treatment but only 84% in leaves following the 15/25 °C treatment. This pattern of response resulted in significantly greater daily soluble sugar and starch (and hence TNC) turnover under the higher night-time temperature regimes (Table 3). The nocturnal temperature manipulation resulted in a significant inverse relationship between A_{max} and leaf carbohydrate status prior to the light period (Fig. 3). This relationship was slightly stronger for leaf soluble sugar content ($r^2 = 0·47$; Fig. 3a) than for leaf starch content ($r^2 = 0·36$; Fig. 3b).

Comparison of the relative impacts of temperature treatments in experiment 1 and 2 is facilitated by calculation of the percentage changes in photosynthetic parameters over the temperature range experienced (Table 4). These comparisons clearly indicate that the addition of nocturnal warming resulted in a significant increase in photosynthetic capacity (V_{cmax}, J_{max} and A_{max}) over that which was displayed in the daytime warming only treatment.

DISCUSSION

Our findings show that nocturnal temperature may have a significant effect on subsequent photosynthetic capacity in leaves during the day, through its impact on leaf respiration and carbohydrate status. It is particularly noteworthy that these experiments were conducted by manipulating the whole ecosystem air temperature of large trees. This is an important distinction given that the scale of temperature

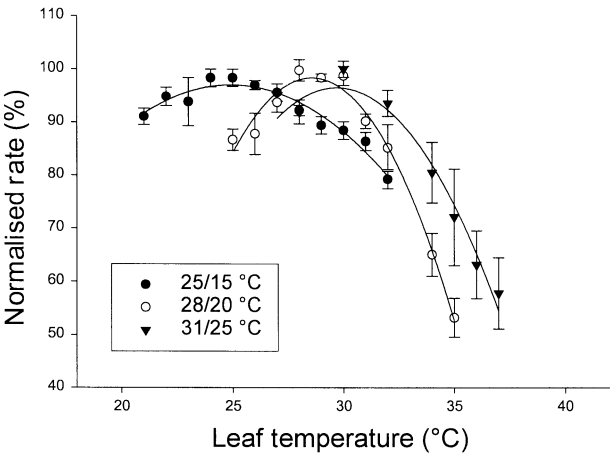

Figure 2. The response of photosynthesis to instantaneous temperature for leaves of *Populus deltoides* experiencing three different day/night temperature regimes. Each curve is the mean (± SEM) of the same six leaves measured at each temperature regime. Trees received 48 h pre-treatment at each temperature set-point before measurements were made. Normalized photosynthetic rate is expressed as a percentage of the maximum achieved at the optimum temperature (T_{opt}).

Table 3. Carbohydrate contents for leaves of *Populus deltoides* trees experiencing three temperature treatments involving both day and night warming

Time of day	Characteristic	15/25 °C	20/28 °C	25/31 °C	ANOVA statistics (*T*)
Sunrise	Sugars (g m^{-2})	6·13 (0·15)^{b}	4·65 (0·37)^{a}	4·26 (0·15)^{a}	<0·0001
	Starch (g m^{-2})	2·01 (0·28)^{b}	1·22 (0·18)^{a}	0·82 (0·17)^{a}	<0·0001
	TNC (g m^{-2})	8·14 (0·40)^{c}	5·87 (0·25)^{b}	5·09 (0·25)^{a}	<0·0001
Sunset	Sugars (g m^{-2})	6·93 (0·12)^{b}	6·17 (0·17)^{a}	5·99 (0·15)^{a}	<0·001
	Starch (g m^{-2})	3·70 (0·39)^{c}	2·65 (0·26)^{b}	1·59 (0·15)^{a}	<0·0001
	TNC (g m^{-2})	10·64 (0·46)^{c}	8·83 (0·37)^{b}	7·57 (0·22)^{a}	<0·0001
Turnover	Sugars (%)	11·6 (1·3)^{a}	25·2 (5·2)^{b}	30·3 (2·9)^{b}	<0·001
	Starch (%)	47·1 (4·2)^{a}	64·7 (2·3)^{b}	59·5 (5·7)^{b}	0·032
	TNC (%)	23·6 (1·5)^{a}	33·3 (1·5)^{b}	37·2 (2·1)^{c}	<0·0001

Values shown are means (± SEM) where $n = 10$–12. Significance of treatment effect for temperature (T) is indicated as the P-value or as non-significant (NS). Different letters within rows indicate statistically different values at $P < 0·05$ using least significant difference test of treatment means.

manipulation may yield very different plant responses (Griffin *et al*. 2002b). In these experiments, an increase in night temperature of 10 °C had a significantly greater impact on photosynthesis during the day than did a 6 °C increase in operational daytime temperature. This indicates that nocturnal temperature may be a significant, but little recognized, environmental factor regulating photosynthetic capacity in plants. Further investigations incorporating nocturnal temperature under both controlled and field conditions will be pivotal in extending our understanding of leaf level processes in responses to global environmental change, and in extrapolating from the leaf- to canopy-level in modelling efforts.

In experiment 1 (daytime warming only), leaf respiration at night (R_{dark}) was predictably unaffected. The 6 °C increase in daytime temperature the cottonwood trees experienced resulted in a modest (11%) increase in photosynthetic capacity. This is consistent with previous studies investigating direct temperature effects on photosynthesis (Bassow & Bazzaz 1998; Saxe *et al*. 2001). In experiment 2 (dual night–day warming), the addition of 10 °C nocturnal warming had a significant impact on R_{dark}. The value of Q_{10} in this whole-ecosystem temperature manipulation (1·8) was within the range previously found for a range of plants (Azcon-Bieto 1992), including deciduous tree species (Bolstad, Mitchell & Vose 1999; Amthor 2000; Gunderson *et al*. 2000; Turnbull *et al*. 2001; Griffin *et al*. 2002a). Higher rates of respiration at elevated nocturnal temperature resulted in lower pre-dawn leaf sugar and starch concentrations. The patterns of accumulation of soluble sugars and starch in leaves experiencing the lowest night-time temperatures, particularly at sunrise, are consistent with previous findings with leaves of plants exhibiting reduced sink demand (Stitt & Quick 1989; Harley & Sharkey 1991; Stitt & Schulze 1994). Increased nocturnal temperature relative to daytime temperature also had a pronounced effect on patterns of carbohydrate turnover in leaves. These are consistent with our previous measurements involving nocturnal warming (Griffin *et al*. 2002a). Daily turnover of TNC, a measure of total carbohydrate utilization, increased by nearly 60% in the 25/31 °C treatment in comparison with the baseline treatment of 15/25 °C.

The dual day–night warming experiment indicates clearly that a respiration-driven reduction in carbohydrates at higher night temperatures has important implications for plant photosynthetic capacity. The addition of a nocturnal temperature rise to the daytime increase resulted in the temperature enhancement of A_{max} increasing from 11 to 64%. That greater photosynthetic capacity was associated with lower leaf carbohydrate concentration following warmer nights (i.e. greater sink demand) is consistent with previous findings (Azcon-Bieto & Osmond 1983; Stitt & Quick 1989; Harley & Sharkey 1991; Azcon-Bieto 1993;

Table 4. Percentage changes in gas exchange characteristics for leaves of *Populus deltoides* trees experiencing either (1) a 6 °C daytime warming only or (2) both a 6 °C daytime warming and a 10 °C night-time warming

	(1) Daytime temperature increase	(2) Night-time and daytime temperature increase	ANOVA statistics (*T*)
V_{cmax} (%)	+37·8 (8·7)	+89·2 (7·4)	<0·001
J_{max} (%)	0 (6·7)	+44·0 (4·5)	<0·0001
J_{max}/V_{cmax} (%)	–27·5 (2·7)	–23·5 (2·1)	NS
TPU (%)	–7·2 (2·0)	+6·3 (3·3)	<0·01
A_{max} (%)	+12·7 (9·8)	+64·2 (8·5)	<0·001
C_i/C_a (%)	+8·1 (5·5)	+9·6 (2·6)	NS
R_{dark} (%)	+6·6 (7·9)	+89·2 (17·3)	<0·001

Values shown are means (± SEM) where $n = 12$. Significance of treatment effect for the night-time temperature increase (T) is indicated as the value of P or as non-significant (NS).

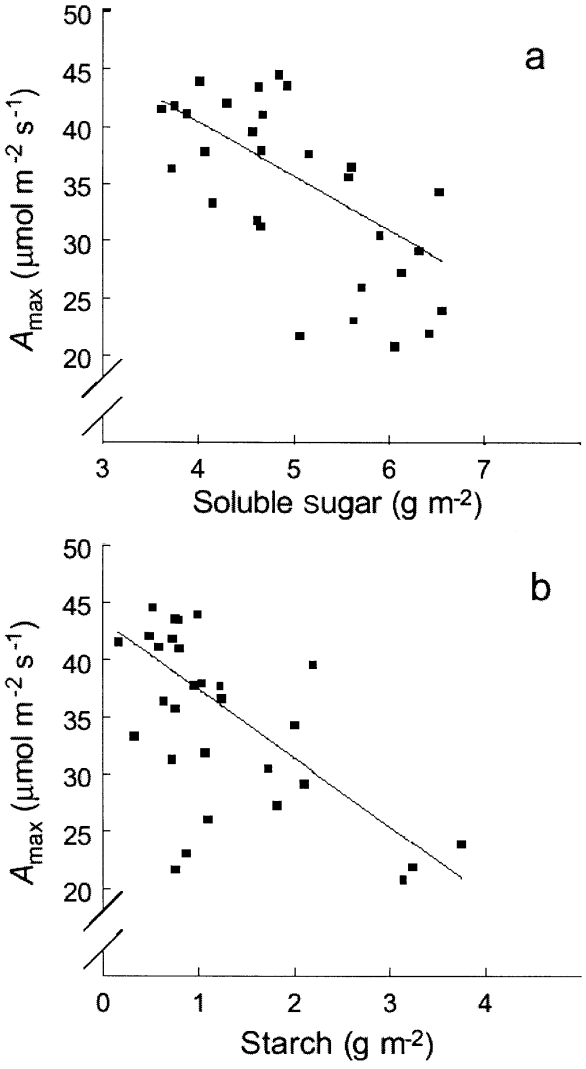

Figure 3. Relationships between (a) leaf soluble sugar concentration and (b) leaf starch content (determined at sunrise) and maximum assimilation rate, A_{max} (determined during the subsequent light period from 1000 to 1300 h), in leaves of *Populus deltoides* trees experiencing an increase in both day and night temperature (i.e. night/day temperatures of 15/25, 20/28 and 25/31 °C). The relationships are described by the equations: $A_{max} = 63{\cdot}0 - (5{\cdot}68 \times \text{soluble sugar})$, $P < 0{\cdot}0001$, $r^2 = 0{\cdot}47$. $A_{max} = 40{\cdot}9 - (5{\cdot}07 \times \text{starch})$, $P < 0{\cdot}0001$, $r^2 = 0{\cdot}36$.

Stitt & Schulze 1994). A number of direct [physical damage to the chloroplast by starch grains (Schaffer *et al.* 1996)] and indirect [down-regulation of gene expression mediated by increases in carbohydrate concentration (Krapp, Quick & Stitt 1991; Krapp *et al.* 1993); reduced levels of RuBP and cytosolic P_i (Goldschmidt & Huber 1992)] feedback mechanisms have been proposed to regulate photosynthetic capacity via low sink strength (Krapp & Stitt 1995; Paul & Foyer 2001). Given that V_{cmax} and J_{max} increased at high night temperature we are led to conclude that increased A_{max} was supported by an increase in Rubisco activity and RuBP regeneration capacity. Our results showing an increase in TPU also indicate that the increase in Rubisco activity may, in part, be the result of an alleviation of triose phosphate limitation. Intriguingly, we found that TPU decreased slightly with day warming but increased with night warming. The combined effect of the dual warming was that the increase in TPU was lower than we have observed previously in an experiment involving nocturnal warming only (unpublished results).

The photosynthetic impacts of changes in operational daytime air temperature, and their interaction with other environmental factors (e.g. elevated CO_2 partial pressure), have been previously considered (Long 1991; McMurtrie & Wang 1993; Teskey 1997; Ziska & Bunce 1997a, b; Saxe *et al.* 2001). Modest increases in day temperature within the optimal range for temperate trees (25–40 °C) are considered likely to be generally positive in terms of photosynthesis (Saxe *et al.* 2001). Here we propose that nocturnal temperature may play an important role in *indirectly* influencing photosynthetic capacity in trees under predicted global change scenarios. These predict that night temperatures will increase more rapidly than day temperatures (Easterling *et al.* 1997; Alward *et al.* 1999). Given the direct link between processes influencing carbon uptake (photosynthesis during the day) and loss (respiration at night), the 'decoupling' of environmental temperatures in future climates may be significant.

The 64% increase in A_{max} measured under the combination of higher diurnal and nocturnal temperatures has the potential to significantly affect patterns of tree carbon gain. Here we measured leaf responses in mid-canopy leaves, but it is likely that photosynthetic responses will vary with position in the canopy. Our previous measurements have shown that we might expect leaves from higher or lower in the canopy to have larger or smaller responses, respectively. However, when modelling whole ecosystem response, mid-canopy leaves are most representative (Griffin *et al.* 2002a). Further, assessment of long-term implications of these findings to net carbon uptake in a global climate change environment must take account of acclimatory responses to long-term exposure to the changing balance of night- and daytime temperatures. In these experiments, trees were given a 48 h 'acclimation' period at each experimental temperature prior to making measurements. Our measurements of the temperature response of photosynthesis indicated that it responded rapidly (within the 48 h) and acclimated fully to the 6 °C range of temperature imposed in the daytime warming. The optimum temperature for photosynthesis (T_{opt}) tracked the actual mesocosm daytime temperature set point very closely. Further, there is good evidence that respiration acclimates rapidly to temperature (Atkin *et al.* 2000). Although we can thus be confident that our experimental protocol here was sufficient to allow for short-term acclimation to temperature in both photosynthesis and respiration, longer-term studies will be required to establish patterns of response over seasonal time scales.

In conclusion, our findings indicate that predicted increases in night-time minimum temperatures may have a significant influence on net plant carbon uptake. They also

suggest that future investigations should manipulate combinations of the major variables likely to vary with future climate change (e.g. night-time and daytime temperature and CO_2 partial pressure) so that robust predictions of future plant responses to changes in global climate can be made. A major issue that arises from this study is the extent to which observed increases in respiration, carbohydrate turnover and photosynthesis contribute to increases in growth. We are mindful of the fact that an increase in photosynthesis does not necessarily translate into increased growth (Chapin & Shaver 1996; Roden & Ball 1996), although the potential for significant impacts of elevated nocturnal temperature make this possibility worthy of future study. Clearly, the implications of our findings for growth rates and total carbon sequestration can only be established in future long-term experiments.

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