Relative enhancement of photosynthesis and growth at elevated CO_2 is greater under sunflecks than uniform irradiance in a tropical rain forest tree seedling

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ABSTRACT

The survivorship of dipterocarp seedlings in the deeply shaded understorey of South-east Asian rain forests is limited by their ability to maintain a positive carbon balance. Photosynthesis during sunflecks is an important component of carbon gain. To investigate the effect of elevated CO_2 upon photosynthesis and growth under sunflecks, seedlings of *Shorea leprosula* were grown in controlled environment conditions at ambient or elevated CO_2. Equal total daily photon flux density (PFD) (~7·7 mol m^{-2} d^{-1}) was supplied as either uniform irradiance (~170 μmol m^{-2} s^{-1}) or shade/fleck sequences (~30 μmol m^{-2} s^{-1}/~525 μmol m^{-2} s^{-1}). Photosynthesis and growth were enhanced by elevated CO_2 treatments but lower under flecked irradiance treatments. Acclimation of photosynthetic capacity occurred in response to elevated CO_2 but not flecked irradiance. Importantly, the relative enhancement effects of elevated CO_2 were greater under sunflecks (growth 60%, carbon gain 89%) compared with uniform irradiance (growth 25%, carbon gain 59%). This was driven by two factors: (1) greater efficiency of dynamic photosynthesis (photosynthetic induction gain and loss, post-irradiance gas exchange); and (2) photosynthetic enhancement being greatest at very low PFD. This allowed improved carbon gain during both clusters of lightflecks (73%) and intervening periods of deep shade (99%). The relatively greater enhancement of growth and photosynthesis at elevated CO_2 under sunflecks has important potential consequences for seedling regeneration processes and hence forest structure and composition.

Key-words: *Shorea leprosula*; Dipterocarpaceae; dynamic photosynthesis; forest understorey; photosynthetic induction; stomatal conductance.

INTRODUCTION

Global climate models predict that atmospheric CO_2 concentrations will increase to between 540 and 970 μmol mol^{-1} by 2100 (IPCC 2001). Tropical forests contribute approximately 30% of net terrestrial photosynthesis (Field *et al*. 1998) and thus, as a major component of the global carbon cycle, any changes in ecosystem functioning will be important. The responses of forests will be strongly influenced by the effect of elevated CO_2 upon dynamic photosynthesis because the light environment is highly heterogeneous (Pearcy 1987; Chazdon 1988). Very little is known about the interactions between fluctuating light and elevated CO_2, the impacts of which could exert important effects on forest structure and function (Saxe, Ellsworth & Heath 1998).

The Dipterocarpaceae are the dominant climax trees of lowland rain forest in South-east Asia and a major source of hardwood timber (Whitmore 1984). Approximately 500 species are found over wide geographical ranges and in diverse species assemblages (Symington 1943; Ashton 1988). As such they are the primary determinants of forest structure and function. The trees fruit gregariously, typically every 3–8 years and produce recalcitrant seeds that are dispersed close to the parent tree, resulting in overlapping seedling banks in the deeply shaded understorey. The regeneration and composition of forests are strongly influenced by seedling competition in the understorey, as it determines which individuals later form the canopy and fulfil their reproductive potential (Watt 1947; Grubb 1977; Whitmore 1984; Still 1996).

The growth and long-term survival of most individuals, and thus the outcomes of competitive interactions, depend at least in part on carbon balance. This is primarily limited by photosynthetic carbon gain under low light supply (Press *et al*. 1996), along with damage caused by pathogens and herbivores. If elevated CO_2 changes seedling photosynthetic carbon gain and growth under sunflecks it will potentially impact upon forest biodiversity. Variation between dipterocarp species in dynamic photosynthetic characteristics increases the potential for species-specific CO_2 effects (Zipperlen & Press 1997; Cao & Booth 2001; Bungard *et al*. 2002).

This article reports the photosynthesis and growth of seedlings of *Shorea leprosula*, a model dipterocarp species, under controlled environment conditions of ambient or elevated CO_2. Seedlings were supplied with either uniform or flecked irradiance, and total daily photon flux density

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(PFD) in each treatment was equal, and typical of an understorey site with a patchy canopy (Leakey, unpublished results). Three specific hypotheses were tested, which are elucidated and justified below.

Many studies have reported the independent effects of sunfleck and elevated CO_2 treatments upon understorey vegetation. Photosynthesis during sunflecks can yield the majority of daily carbon gain (Pearcy & Calkin 1983; Pearcy 1987; Pfitsch & Pearcy 1989) and determine rates of growth in understorey vegetation (Pearcy 1983; Oberbauer *et al*. 1988; Watling, Ball & Woodrow 1997). However, it is well understood that, when compared with uniform irradiance, photosynthesis during sunflecks is restricted by additional physiological limitations (Pearcy *et al*. 1994). Therefore, first, it was hypothesized that photosynthetic carbon gain and growth would be lower under flecked irradiance.

Enhanced rates of steady-state photosynthesis and growth have been reported in understorey plants in response to elevated CO_2 (Osborne *et al*. 1997; Wurth, Winter & Körner 1998; Hattenschwiler & Körner 2000; DeLucia & Thomas 2000). Short-term increases in photosynthesis under shade conditions are due to reduced photorespiration and greater CO_2 saturation of Rubisco (Stitt 1991). Enhancement is seen even if down-regulation of the photosynthetic machinery occurs in the long term (Osborne *et al*. 1997). Therefore, second, it was hypothesized that photosynthetic carbon gain and growth would be greater at elevated CO_2.

However, very little is known about the interactions between elevated CO_2 and photosynthesis during sunflecks. No studies have reported the steady-state and dynamic components of photosynthesis together, in order to explain patterns of total daily carbon gain and growth at double the ambient CO_2 under dynamic light environments.

Physiological responses to elevated CO_2 often include reductions in stomatal conductance (Drake, Gonzalez-Meler & Long 1997; Saxe *et al*. 1998) and the expression (or activity) of the carboxylating enzyme Rubisco (Sage, Sharkey & Seemann 1989; Sage 1994; Drake *et al*. 1997). At elevated CO_2 reductions in stomatal conductance led to faster photosynthetic induction gain (Knapp, Fahnestock & Owensby 1994). Slower induction loss after flecks in temperate tree seedlings may be due to reduced enzyme deactivation in the shade (Naumburg & Ellsworth 2000). Greater CO_2 saturation of Rubisco leading to increasing and decreasing flux through photosynthetic and photorespiratory pathways, respectively, may also increase carbon gain from post-irradiance metabolism at elevated CO_2. Together, these effects of elevated CO_2 reduce the limitations to photosynthesis during sunflecks, thus addressing the main aim of the experiment and the third and key hypothesis: that the CO_2 effect would be greater, on a relative basis, in plants grown under flecked irradiance.

MATERIALS AND METHODS

Plant material

Shorea leprosula Miq. seeds were collected from primary, lowland dipterocarp rain forest close to the Danum Valley Field Centre, Sabah, E. Malaysia, Borneo (4°58′ N, 117°48′ E). They were germinated and maintained for 1 year in a forest nursery (total daily PFD ~9·0 mol m^{-2} d^{-1}) in polythene pots containing forest soil, prior to transfer to a controlled environment glasshouse in the UK. The seedlings were then transplanted into 2·1 L pots containing 2 : 2 : 1 (v/v) vermiculite, perlite and seed compost, with slow release N : P : K (14 : 13 : 13) fertilizer containing micronutrients (3 g L^{-1}; Osmocote plus; Scotts, Ohio, USA). The seedlings were maintained for 2 months with maximum and minimum temperatures of 35 and 21 °C, respectively, and a constant relative humidity of ~80%.

Forty seedlings of similar height (300–400 mm) were selected for the experiment, ranked on the basis of the product of total leaf area and height and then divided into groups of four seedlings. Then, within each successive group of four seedlings, the individuals were randomly allocated to the four treatments (see below). This minimized any impact of the small variation in plant size. The four treatments comprised a randomized block, 2 × 2 factorial design: AU = ambient CO_2 + uniform irradiance; AF = ambient CO_2 + flecked irradiance; EU = elevated CO_2 + uniform irradiance; and EF = elevated CO_2 + flecked irradiance.

Controlled environment growth conditions

Seedlings were grown in controlled environment cabinets (model SGC097, Fitotron; Sanyo-Gallenkamp, UK, internal volume 920 L) at either ambient CO_2 or elevated CO_2, with uniform or flecked irradiance (10 seedlings per treatment, 20 per chamber). Although only one cabinet was used at each CO_2 concentration previous experiments established that plant growth was not significantly affected by chamber characteristics (Watling, Press & Quick 2000). Weekly, 24 h measurements of CO_2 concentration [LCA4 infrared gas analyser (IRGA); ADC, Hoddesdon, UK], temperature and relative humidity (combined relative humidity/temperature sensors and datalogger; Delta-T Devices Ltd, Cambridge, UK) confirmed that there were no significant differences in microclimate between treatments (Appendix 1).

The experiment was run over 216 d. It was terminated before any self-shading occurred. Plants were watered three times each day, with filtered tap water, using an automatic drip-irrigation system. Additional slow release N : P : K (14 : 13 : 13) plus micronutrients fertilizer (6 g per pot; Osmocote plus) was added after 110 d.

The CO_2 concentrations within cabinets were monitored by IRGA (ADC2000; ADC,). The IRGA in the elevated CO_2 cabinet determined the rate of pure CO_2 influx, from an external cylinder, via a solenoid valve. The mean CO_2

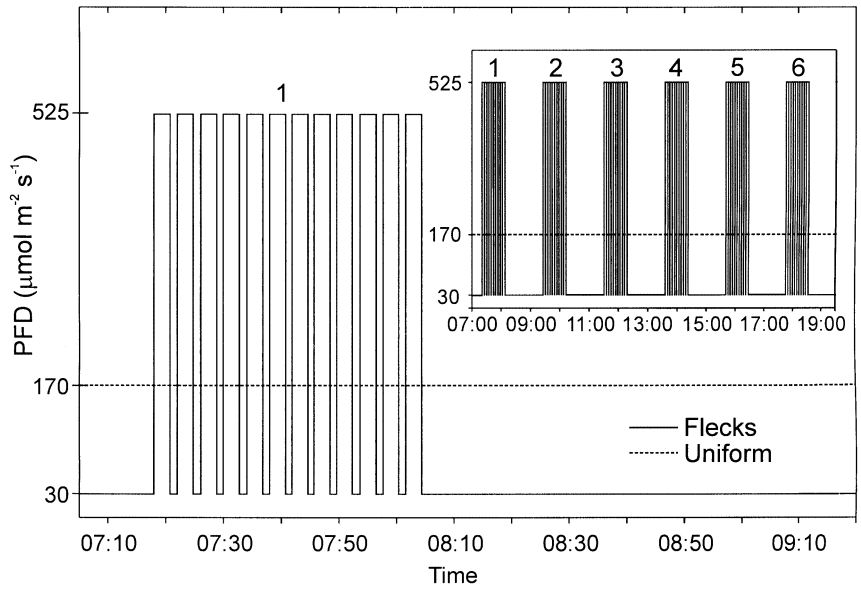

Figure 1. A schematic representation of PFD profiles during the photoperiod. The main graph illustrates the PFD pattern under flecked irradiance during the first cluster of flecks (1). The distribution of clusters through the photoperiod is shown in the inset (2–6). The dashed line in both graphs represents the light supplied under the uniform treatment.

concentrations in the elevated CO_2 and ambient treatments were 711 and 376 μmol mol^{-1} CO_2, respectively (Appendix 1).

Two irradiance regimes (uniform or flecked irradiance) were generated within each growth cabinet. The total irradiance received by the plants in each treatment was equal (7·7 mol m^{-2}d^{-1}, Appendix 1) when monitored on a monthly basis at four points 1–2 cm below each neutral density filter (leaf level – see below), using quantum sensors and a data logger (Delta-T Devices Ltd). There was little horizontal heterogeneity in light (7·4–7·8 mol m^{-2} d^{-1}), based on measurements at 10 points below the filters. In addition, the plants were randomized weekly within each growing area.

In the continuous irradiance treatment a disc of neutral density filter (Lee Filters, Andover, UK) reduced the PFD at plant height, from ~525 μmol m^{-2} s^{-1}, produced by the combination of fluorescent tubes (58 W, PLL-type; Phillips, The Netherlands) and incandescent (tungsten) lamps in the cabinet, to ~170 μmol m^{-2} s^{-1} (Fig. 1).

The pattern of PFD in the fleck treatment was a simplified representation of field conditions with repeated clusters of flecks interspersed with continuous low background PFD (Pearcy 1987; Chazdon 1988; Leakey unpubl. results). This was generated using a disc of low transmission neutral density filter above the seedlings. Radial segments were dissected in two groups of 12, from opposite sides of the disc. During each photoperiod an electrical motor turned the disc through three complete revolutions, producing six clusters of flecks (Fig. 1). Each cluster consisted of 12, 3 min flecks of ~525 μmol m^{-2} s^{-1}, separated by 1 min shade periods of ~30 μmol m^{-2} s^{-1} (Fig. 1). Between successive clusters there was a 78 min shade period of ~30 μmol m^{-2} s^{-1}, allowing photosynthetic induction to relax to steady state before the next fleck. The plants were grown on movable platforms that were gradually lowered over the course of the experiment to keep the upper leaves at the same distance from the light source throughout the experiment. In both treatments black-out material hanging from the circumference of the neutral density filter disc ensured irradiance was only incident upon leaves from above.

Growth analysis

At the end of the growth period (216 d) seedling height and leaf number were measured and then a destructive harvest was carried out. Samples were divided into leaves, stems and roots before being dried at 80 °C for 13 d. Leaf area was measured using a leaf area meter (Delta-T Devices Ltd). The total biomass and allometric relationships [leaf area ratio (LAR), leaf weight ratio (LWR), specific leaf area (SLA) and root : shoot ratio (R : S)] were calculated, according to Hunt (1990).

Steady-state gas exchange

All gas exchange measurements were made during the final month of the growth period. Measurements of steady-state photosynthesis under constant light (no induction limitation) were made, on the youngest fully expanded leaf of six individuals randomly selected from each treatment, using an open system IRGA (LCA-4; ADC) with a modified 6·25 cm^2 clamp-on leaf chamber (PLC-3; ADC). Gas exchange parameters were calculated using the equations of von Caemmerer & Farquhar (1981). The input gas was produced by using mass flow controllers (AFC 260; ASM, Bithoven, The Netherlands) to mix O_2 (21%) and N_2 (79%), which before the addition of CO_2 (0–0·15%), was bubbled through water and dried to a set humidity (65%) by a condenser coil in a temperature-controlled water bath (Julabo F40; Baird and Tatlock Ltd, London, UK). Measurements were made at leaf temperatures of 29–31 °C, controlled by the leaf chamber water jacket connected to a

heating circulator (Circulator C-400; Techne Ltd, Cambridge, UK) and heating/cooling water bath (Julabo F40; Baird and Tatlock Ltd). Actinic light was provided, via a fibre optic bundle, by halogen lamps (KL 1500; Schott, Mainz, Germany).

To assess the degree of acclimation to elevated CO_2, A–c_i curves were constructed at the saturating PFD of 800 μmol m^{-2} s^{-1} (Zipperlen & Press 1996). CO_2 supply to the leaf chamber was reduced in a stepwise manner from 1400 to 5 μmol mol^{-1}, with gas exchange parameters recorded once steady rates of photosynthesis and stomatal conductance were achieved, at each of 16 CO_2 concentrations. Analysis of the A–c_i curve for each plant was carried out using the mechanistic model of Farquhar, von Caemmerer & Berry (1980). Estimates of apparent maximum carboxylation capacity (V_{cmax}) and apparent maximum electron transport capacity (J_{max}) were calculated using the non-linear regression technique of Wullschleger (1993). The temperature corrections of Bernacchi *et al*. (2001) and von Caemmerer (2000) were incorporated for V_{cmax} and J_{max}, respectively.

To assess maximum apparent quantum yield (ϕ), light-saturated photosynthesis and dark respiration, light response curves were measured for all treatments at growth CO_2 concentration, as well as at 350 μmol mol^{-1} CO_2 for seedlings from the elevated CO_2, uniform irradiance (EU) treatment. Gas exchange parameters were recorded once steady-state values (100% induction state) were reached at each of 16 PFD values, starting at 900 μmol m^{-2} s^{-1} and decreasing stepwise to 0 μmol m^{-2} s^{-1}. Light response curve parameters (with the exception of ϕ) were estimated, as in Zipperlen & Press (1996), by fitting a non-rectangular hyperbola. Maximum apparent quantum yield was calculated by linear regression of data points on the light-limited part of the light response curve.

Dynamic gas exchange

Measurements of dynamic photosynthesis were made using the same apparatus as for steady-state photosynthesis except with a different IRGA. The gas exchange apparatus had a quicker response time (4·5 s at a flow rate of 470 mL min^{-1}) and a faster measurement cycle, logging at 1 s intervals (ADC 2250; ADC). The response time was estimated by introducing a burst of high CO_2 (1–5%) into the closed chamber, via a syringe, and tracking the output response of the IRGA. Estimates of photosynthetic induction processes and post-irradiance gas exchange were calculated after raw output was corrected for the system lag time. Dynamic photosynthesis in the flecked irradiance treatments was assessed by measuring gas exchange during a simulation of the fleck sequence experienced by the plants in the controlled environment cabinets. Leaves of six individuals were randomly selected from flecked irradiance treatments and exposed to a PFD of 30 μmol m^{-2} s^{-1}, under growth CO_2 concentrations, until stable rates of A and stomatal conductance (g_S, including negligable cuticular conductance) were achieved. They were then subjected to 12, 3 min flecks of 525 μmol m^{-2} s^{-1} separated by 1 min low light periods of 30 μmol m^{-2} s^{-1}.

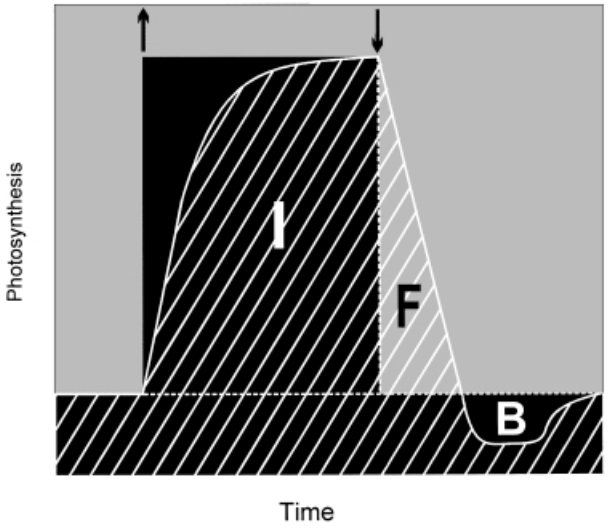

Figure 2. Model of photosynthetic carbon gain (hatched area) during a sunfleck compared with instantaneous induction gain and loss (black area). The transitions from shade to high light to shade are indicated by up and down arrows, respectively. F and B indicate areas representing integrated, net carbon gain and loss during post-irradiance CO_2 fixation and burst, respectively. I equals integrated carbon gain during the period of irradiance.

These data were used to calculate parameters describing the progression of photosynthetic induction gain and increasing stomatal conductance. Maximum stomatal conductance ($g_{Smax\text{-}fleck}$) and the times to reach 50 and 90% of the maxima ($T_{50\%gS}$ and $T_{90\%gS}$) were estimated after fitting a sigmoidal function to the data (Zipperlen & Press 1996). Due to the highly dynamic signal, the maximum rate of photosynthesis ($A_{max\text{-}fleck}$) was calculated using the same statistical method, but fitting the curve to data from minutes 0–6, 8–10, 12–14, 16–18 and so on, of the sequence of flecks. The times to 50 and 90% of the maximum photosynthetic rate ($T_{50\%A}$ and $T_{90\%A}$) were identified as the period between the start of the first fleck and the first data point of the measured time-course that exceeded each of the values in turn.

Photosynthetic induction processes incur a lag period after the rise in PFD and photosynthetic CO_2 fixation continues into the shade period after the fleck, before dropping below $A_{initial}$ and finally returning to steady-state rates (Fig. 2). Comparing the real response with an instantaneous square wave response allows two periods of net gas exchange to be defined: post-irradiance CO_2 fixation and post-irradiance CO_2 burst. For each fleck, these variables were calculated by integrating the CO_2 fixation rates, either exceeding $A_{initial}$ (post-irradiance CO_2 fixation) or below $A_{initial}$ (post-irradiance CO_2 burst), in the 1 min shade period between flecks (Vines *et al*. 1983; Laisk, Kurats & Oja 1984; see Fig. 2). This value was then expressed as a percentage of the integrated carbon gain during the pro-

ceeding fleck period and averaged across the sequence of flecks.

Lightfleck utilization efficiency and induction state (*IS*%) during the fleck sequence were calculated by the method of Chazdon & Pearcy (1986a, b). Rates of induction loss were measured by the method of Zipperlen & Press (1997) over shade periods of 1, 5, 10, 20 and 40 min length, leading to the calculation of *IS*% and g_S.

Daily carbon gain

Daily carbon gain under uniform irradiance treatments was calculated for each CO_2 concentration on an individual plant basis by integrating the steady-state photosynthetic rate at a PFD of 170 μmol m^{-2} s^{-1} (from the light response curve) across the photoperiod. Daily carbon gain of plants under the fleck irradiance regime was calculated as the sum of two components: (1) the total assimilation measured during a single, simulated sunfleck sequence, multiplied by six (the number of clusters per day); and (2) the steady-state gas exchange rate at a PFD of 30 μmol m^{-2} s^{-1} (from the light response curve) integrated across the combined interspersing shade periods (see Fig. 1). Estimates of total daily carbon gain were not confounded by afternoon depression of photosynthesis or stomatal closure (data not shown).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and Western blotting analysis of Rubisco content

Leaf discs were taken 10 d before the destructive harvest and stored in liquid N_2 prior to extraction with 25 mg of polyvinylpolypyrrolidone and 500 μL of extraction buffer optimized for this leaf material [50 mM Hepes (*N*-2-hydroxyethyl piperazine-*N*′-2-ethanesulphonic acid)-HCl, pH 7·0, 200 mM glycine, 30 mM dithiothreitol, 20% (v/v) glycerol, 1·0% (w/v) sodium dodecyl sulphate (SDS) and 1 mM ethylenediaminetetraacetic acid]. The resulting extract was analysed using SDS-polyacrylamide gel electrophoresis gel separation of proteins, immunoblotting of Rubisco and enhanced chemiluminescence for visualization before digital imaging, as described by Watling *et al*. (2000).

Chlorophyll, nitrogen and carbohydrate determination

For all analyses the youngest fully expanded leaves of 10 seedlings were sampled. Leaf discs were collected 10 d before the destructive harvest, weighed and ground in 2 mL buffered 80% aqueous acetone (2·5 mM $NaPO_4$, pH 7·8). Chlorophyll *a* and *b* concentrations were determined using the method of Porra, Thompson & Kriedemann (1989).

Foliar nitrogen concentrations were determined, after a micro-Kjeldahl digestion procedure, by a colorimetric assay using a flow injection analysis system (Tecator 5042 detector and 5012 analyser) as described in Bungard *et al*. (1999).

Leaf discs were taken immediately pre-dawn and at 11 h into the photoperiod, weighed and immediately stored under liquid nitrogen. Concentrations of soluble sugars and starch were determined as described in Scholes *et al*. (1994).

Statistical analysis

Data from measurements of cabinet environmental conditions using ANOVA and Tukey tests (Minitab 12·0 software, Minitab Inc., Pennsylvania, USA). Although the light treatments were nested within the CO_2 concentrations, no significant differences between cabinets in variables other than CO_2 concentration were observed (see Appendix 1). ANOVA and Tukey tests were used to analyse seedling allometry and architecture, steady-state gas exchange, leaf biochemical analysis and calculated daily carbon gain. Daily carbon gain data were log transformed before analysis, in order to determine the significance of treatment effects on a relative basis. Total biomass data were analysed using ANCOVA with values of seedling size from the start of the experimental period as covariates. Data of ϕ at 350 μmol mol^{-1} CO_2 in ambient and elevated CO_2-grown plants were analysed by two sample, two-tailed *t*-tests. Data from dynamic gas exchange measurements and were analysed by two sample, two-tailed *t*-tests, except for the responses of $g_{Sinitial}$ and $g_{Smax\text{-}fleck}$, which were analysed by two sample, one-tailed *t*-tests after hypothesizing decreases in response to elevated CO_2.

RESULTS

Growth analysis

Growth at elevated CO_2 significantly stimulated biomass accumulation in plants grown under both uniform and flecked irradiance (Table 1). At both CO_2 concentrations biomass accumulation was greater in plants supplied with uniform irradiance rather than flecked irradiance. There was a greater relative stimulation of growth with elevated CO_2 under the flecked light regime (60%) compared with uniform irradiance (25%).

There was a significant reduction in SLA at elevated CO_2. However, there was no change in biomass allocation between roots, shoots and leaves at elevated CO_2. Furthermore, there were no significant changes in biomass allocation in response to the irradiance treatment (Table 1). Mean seedling height and leaf number are shown in Table 1.

A–c_i curves

Analysis of *A*–c_i response curves revealed that growth at elevated CO_2 resulted in acclimation of the photosynthetic apparatus (Fig. 3). At elevated CO_2 there was an approximately equal decrease in V_{cmax} and increase in the apparent maximum whole chain electron transport capacity (J_{max})

Table 1. Total biomass, root : shoot ratio (R : S), leaf weight ratio (LWR), leaf area ratio (LAR), specific leaf area (SLA), seedling height and seedling leaf number of *S. leprosula* at final harvest

	AU	AF	EU	EF	CO_2	Fleck	Interaction
Total biomass (g DW)	$15{\cdot}4 \pm 0{\cdot}9^{b}$	$10{\cdot}3 \pm 0{\cdot}7^{a}$	$19{\cdot}3 \pm 1{\cdot}0^{c}$	$16{\cdot}5 \pm 0{\cdot}9^{b}$	***	***	*
R : S	$0{\cdot}29 \pm 0{\cdot}02$	$0{\cdot}28 \pm 0{\cdot}03$	$0{\cdot}33 \pm 0{\cdot}03$	$0{\cdot}33 \pm 0{\cdot}03$	NS	NS	NS
LWR	$0{\cdot}42 \pm 0{\cdot}02$	$0{\cdot}39 \pm 0{\cdot}03$	$0{\cdot}41 \pm 0{\cdot}01$	$0{\cdot}41 \pm 0{\cdot}02$	NS	NS	NS
LAR ($m^2\ kg^{-1}$)	$11{\cdot}0 \pm 0{\cdot}9$	$10{\cdot}3 \pm 0{\cdot}8$	$9{\cdot}9 \pm 0{\cdot}6$	$9{\cdot}4 \pm 0{\cdot}7$	NS	NS	NS
SLA ($m^2\ kg^{-1}$)	$26{\cdot}7 \pm 0{\cdot}6^{a}$	$26{\cdot}1 \pm 0{\cdot}7^{ab}$	$24{\cdot}3 \pm 0{\cdot}7^{bc}$	$23{\cdot}6 \pm 0{\cdot}7^{b}$	***	NS	NS
Height (cm)	$65{\cdot}0 \pm 1{\cdot}5^{b}$	$58{\cdot}6 \pm 1{\cdot}5^{a}$	$70{\cdot}8 \pm 1{\cdot}7^{c}$	$63{\cdot}7 \pm 1{\cdot}5^{ab}$	**	***	NS
Leaf number	22 ± 1^{b}	18 ± 1^{a}	27 ± 1^{c}	21 ± 1^{b}	***	***	NS

Treatments were: AU (ambient CO_2 + uniform irradiance), AF (ambient CO_2 + flecked irradiance), EU (elevated CO_2 + uniform irradiance) and EF (elevated CO_2 + flecked irradiance). Values are means (± SE), $n = 10$. Total biomass data were analysed by ANCOVA with seedling size at the start of experimental period as covariates. Allometric, height and leaf number data were analysed by ANOVA. The level of significance of treatments and any interaction are indicated by asterisks: $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$; NS, not significant. Where statistical differences occur, means sharing a common superscript letter do not differ significantly (Tukey test $P < 0{\cdot}05$).

(Table 2). As a consequence, light-saturated photosynthetic rates at growth CO_2 concentration were 65 and 61% greater for elevated CO_2 plants grown under uniform and flecked irradiance, respectively. There was no acclimation of photosynthesis in response to irradiance treatment (Table 2).

Light response curves

The values of ϕ and light-saturated rate of photosynthesis (A_{max}) of leaves grown and measured at elevated CO_2 were significantly greater than those of leaves grown and measured under ambient CO_2 (Table 3). This resulted in greater rates of photosynthesis at all PFDs in elevated CO_2-grown plants compared with ambient CO_2-grown plants (Fig. 4). The relative enhancement was greatest at low PFDs. Consequently, the enhancement of steady-state photosynthetic rates also differed under uniform irradiance (59% at 170 μmol $m^{-2}\ s^{-1}$) compared with flecked irradiance (99% at 30 μmol $m^{-2}\ s^{-1}$ and 62% at 525 μmol $m^{-2}\ s^{-1}$). There were no significant differences in dark respiration (R_d). However, the light compensation point (Q_{lcp}) at elevated CO_2 was 49 and 33% lower under uniform and flecked irradiance, respectively (Table 3).

When measured at a common CO_2 concentration of 350 μmol mol^{-1}, ϕ did not differ significantly between plants grown at ambient CO_2 ($0{\cdot}036 \pm 0{\cdot}001$ mol mol^{-1}) and elevated CO_2 ($0{\cdot}036 \pm 0{\cdot}002$ mol mol^{-1}).

No significant differences were seen in the light-limited rates of photosynthesis under uniform versus flecked irradiance (Fig. 4).

Leaf nitrogen, chlorophyll, Rubisco and carbohydrate contents

Leaves of plants grown at elevated CO_2 had significantly lower SLA (Table 1) and 25% less Rubisco on a leaf area basis compared with ambient CO_2 plants (Table 4). Also, soluble sugar and starch concentrations were significantly higher in leaves of plants grown at elevated CO_2 (Table 4). Growth at elevated CO_2 also resulted in lower leaf nitrogen

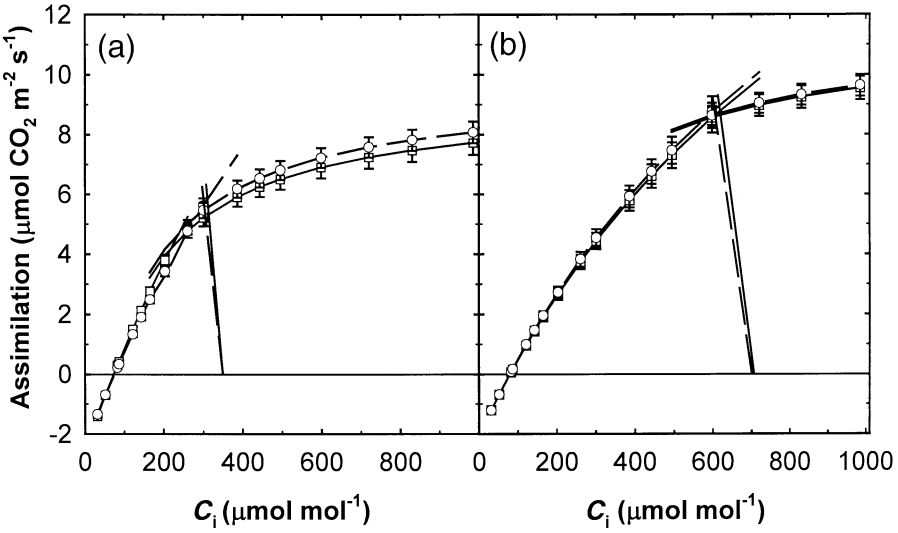

Figure 3. Mean fitted A–c_i response curves for *S. leprosula* grown at either (a) ambient or (b) elevated CO_2 concentrations, with uniform (solid line) or flecked (dashed line) irradiance. Curves were derived from mean estimates ($n = 6$) of photosynthetic model parameters V_{cmax} (below inflexion) and J_{max} (above inflexion) (Farquhar *et al.* 1980). Open circles (flecked irradiance) and open squares (uniform irradiance) are means of original data points (± SE) $n = 6$. Lines show the linear supply function for each curve, the intersection between line and curve indicating photosynthetic rates at growth CO_2 concentration.

Table 2. Mean estimates of photosynthetic model parameters (Farquhar *et al*. 1980)

	AU	AF	EU	EF
V_{cmax} (μmol m^{-2} s^{-1})	25·3 ± 1·1^a	23·2 ± 0·9^a	18·8 ± 1·0^b	19·2 ± 1·1^b
J_{max} (μmol m^{-2} s^{-1})	54·1 ± 2·7^a	56·4 ± 2·5^a	66·4 ± 2·6^b	67·0 ± 2·4^b

Maximum Rubisco carboxlation capacity (V_{cmax}) and maximum electron transport capacity (J_{max}), derived from A–c_i response curves. Treatments as in Table 1. Values are means (± SE), $n = 6$. Means sharing a common superscript letter do not differ significantly (Tukey multiple comparison test $P < 0{\cdot}05$).

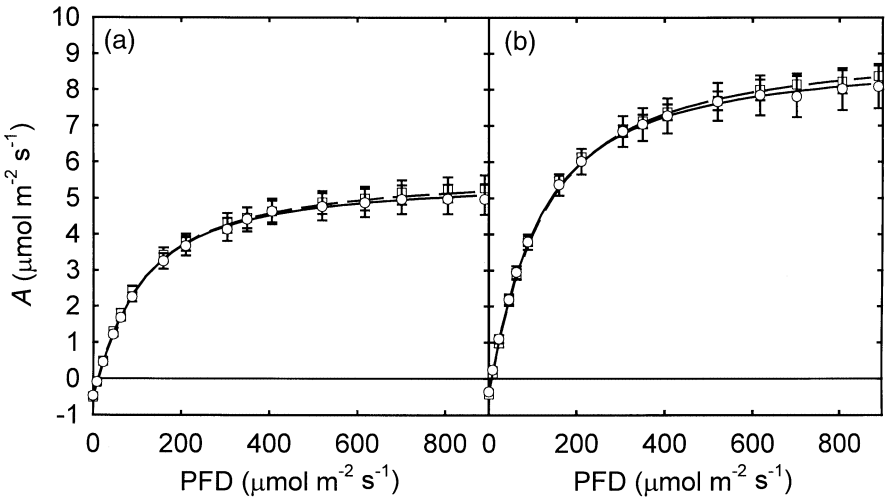

Figure 4. Mean fitted light response curves for *S. leprosula* measured and grown at either (a) ambient or (b) elevated CO_2 concentrations, with uniform (solid line) or flecked (dashed line) irradiance. Curves fitted to a nonrectangular hyperbola ($n = 6$) (Thornley 1976). Open circles (flecked irradiance) and open squares (uniform irradiance) are means of original data points (± SE) $n = 6$.

and chlorophyll contents on a leaf mass basis, but there were no differences on a leaf area basis (Table 4).

There were no significant differences in nitrogen, chlorophyll or Rubisco, expressed on either an area or mass basis, with respect to irradiance treatments.

Dynamic photosynthesis

Both steady-state and dynamic components of photosynthesis during a sequence of lightflecks changed significantly at elevated CO_2. The initial steady-state photosynthetic rate ($A_{initial}$) in the shade (30 μmol m^{-2} s^{-1}) was 109% greater under elevated CO_2 compared with ambient CO_2 (Table 5). The maximum steady-state rate of photosynthesis ($A_{max\text{-}fleck}$) achieved during the fleck sequence was 66% greater in elevated CO_2. Stomatal conductance was significantly lower at elevated CO_2, both initially (–21%) and after complete induction (–22%).

The induction response at elevated CO_2 followed a sigmoidal shape compared with a more typical hyperbolic increase under ambient CO_2 (e.g. Fig. 5d). As a consequence, the time required for photosynthetic induction to reach 50% of completion ($T_{50\%A}$) was slower at elevated CO_2, whereas the time to reach 90% of completion ($T_{90\%A}$) was faster at elevated CO_2 (Fig. 5a & b, Table 5). Similarly, although stomatal opening was initially slower ($T_{50\%gS}$), 90% of maximum stomatal conductance was attained significantly faster ($T_{90\%gS}$, Table 5).

Loss of photosynthetic induction during shade periods of between 1 and 10 min duration was slower in elevated CO_2,

Table 3 Mean estimates of light saturated rate of photosynthesis (A_{max}), dark respiration (R_d), light compensation point (Q_{lcp}) and apparent quantum yield (ϕ), derived from light response curves

	AU	AF	EU	EF
Measurement CO_2 (μmol mol^{-1})	350	350	700	700
A_{max} (μmol m^{-2} s^{-1})	6·08 ± 0·33^a	6·22 ± 0·36^a	9·41 ± 0·64^b	9·73 ± 0·52^b
R_d (μmol m^{-2} s^{-1})	–0·51 ± 0·08	–0·47 ± 0·07	–0·39 ± 0·07	–0·41 ± 0·08
Q_{lcp} (μmol m^{-2} s^{-1})	11·1 ± 1·4^a	10·0 ± 1·4^a	5·7 ± 1·0^b	6·7 ± 1·1^b
ϕ (mol mol^{-1})	0·036 ± 0·001^a	0·037 ± 0·002^a	0·061 ± 0·003^b	0·059 ± 0·002^b

Treatments as in Table 1. Values are means (± SE), $n = 6$. Where statistical differences occur, means sharing a common superscript letter do not differ significantly (Tukey multiple comparison test $P < 0{\cdot}05$).

Table 4. N content per unit leaf mass (N-mass) and per unit leaf area (N-area), chlorophyll content per unit leaf fresh mass (Chl-mass) and per unit leaf area (Chl-area), chlorophyll $a:b$ ratio (Chl $a:b$) Rubisco content per unit leaf area (Rubisco-area, AU value = 100% standard), soluble sugars content per unit leaf area and starch content per unit leaf area of youngest fully expanded leaf of *S. leprosula*

	AU	AF	EU	EF
N-mass (mg g^{-1})	19·3 ± 1·0[a]	19·3 ± 0·6[a]	16·4 ± 0·8[b]	16·6 ± 0·7[b]
N-area (g m^{-2})	0·71 ± 0·04	0·74 ± 0·04	0·70 ± 0·05	0·73 ± 0·04
Chl-mass (mg g^{-1})	4·09 ± 0·31[a]	4·12 ± 0·30[a]	3·24 ± 0·17[b]	3·24 ± 0·22[b]
Chl-area (mg m^{-2})	297 ± 11	311 ± 13	300 ± 13	316 ± 13
Chl $a:b$	2·91 ± 0·05	2·98 ± 0·10	2·93 ± 0·06	3·03 ± 0·07
Rubisco-area (%)	100 ±7[a]	100 ±6[a]	75 ±7[b]	76 ±6[b]
Soluble sugars (mmol m^{-2})	10·6 ± 0·8[a]	9·3 ± 0·8[a]	17·0 ± 1·3[b]	16·2 ± 1·2[b]
Starch (glucose mmol m^{-2})	20·7 ± 1·3[a]	19·6 ± 1·6[a]	34·4 ± 2·6[b]	31·6 ± 2·4[b]

Carbohydrate measurements were made 11 h into the photoperiod. Treatments as in Table 1. Values are means (±SE), $n = 10$. Where statistical differences occur, means sharing a common superscript letter do not differ significantly (Tukey multiple comparison test $P < 0·05$).

but this was significant only at 5 min of shade (Fig. 6). This was accompanied by no significant difference in stomatal conductance when expressed relative to g_{Smax}.

Elevated CO_2 influenced post-irradiance gas exchange, causing a significant increase (14%) in post-irradiance CO_2 fixation (Table 5), as well as decreasing the post-irradiance CO_2 burst to almost zero. Lightfleck utilization efficiency (LUE), a measure of the overall efficiency of dynamic photosynthesis during the fleck sequence, was significantly greater (5%) at elevated CO_2 (Table 5). The combined effects of changes in steady-state and dynamic photosynthesis, described above, resulted in significantly greater carbon gain during a fleck cluster at elevated CO_2 (73%, Table 5).

Calculated daily carbon gain

Elevated CO_2 significantly stimulated calculated daily carbon gain in plants grown under both uniform and flecked irradiance (Fig. 7). At both CO_2 concentrations, daily carbon gain was greater in plants supplied with uniform irradiance rather than flecked irradiance. The enhancement of daily carbon gain at elevated CO_2 under the flecked irradiance treatment (0·08 mol m^{-2} d^{-1}) was significantly lower, on an absolute basis, compared with uniform irradiance (0·09 mol m^{-2} d^{-1}). Crucially, however, the enhancement of daily carbon gain at elevated CO_2 was significantly greater, on a relative basis, under the flecked irradiance treatment (89%) compared with uniform irradiance (59%).

DISCUSSION

Growth responses to the experimental treatments supported all three hypotheses: (1) growth was greater at elevated CO_2; (2) growth was lower under flecked irradiance; and (3) the relative enhancement of growth at elevated CO_2 was greater in plants grown under flecked irradiance. There were no significant changes in allometry associated with the growth responses. The absence of a reduction in R : S ratio confirmed observations that root growth was not restricted by pot size and that photosynthetic acclimation was not therefore an artefact of root confinement (Arp 1991; Thomas & Strain 1991).

Photosynthetic capacity

Photosynthetic acclimation to growth at elevated CO_2 was characterized by a decrease in V_{cmax} and an increase in J_{max}. The photosynthetic acclimation response to elevated CO_2 can be modified by total irradiance (Curtis & Wang 1998). However, there was no interaction effect of flecked irradiance with elevated CO_2 upon photosynthetic acclimation. As reported previously there was also no significant acclimation to flecked irradiance (Wayne & Bazzaz 1993; Watling *et al*. 1997).

Table 5. Photosynthetic rates at steady state in shade ($A_{initial}$), maximum photosynthetic rate attained during flecks ($A_{max\text{-}fleck}$), stomatal conductance at steady state in shade ($g_{Sinitial}$) maximum stomatal conductance attained during flecks ($g_{Smax\text{-}fleck}$), time to 50 and 90% of $A_{max\text{-}fleck}$ ($T_{50\%A}$ and $T_{90\%A}$), time to 50 and 90% of $g_{Smax\text{-}fleck}$ ($T_{50\%gS}$ and $T_{90\%gS}$), post-irradiance CO_2 fixation, post-irradiance CO_2 burst, lightfleck utilization efficiency (LUE) and carbon gain during a fleck cluster (carbon gain) of *S. leprosula* measured and grown at either ambient or elevated CO_2 concentrations in response to a sequence of sunflecks simulating the growth irradiance regime

	Ambient CO_2	Elevated CO_2	%
$A_{initial}$ (μmol m^{-2} s^{-1})	0·69 ± 0·15	1·44 ± 0·31	+109*
$A_{max\text{-}fleck}$ (μmol m^{-2} s^{-1})	4·91 ± 0·41	8·15 ± 0·93	+66*
$g_{Sinitial}$ (mmol m^{-2} s^{-1})	43·6 ± 3·5	34·5 ± 2·7	–21*
$g_{Smax\text{-}fleck}$ (mmol m^{-2} s^{-1})	122·9 ± 9·3	96·3 ± 10·0	–22*
$T_{50\%A}$ (minutes)	1·2 ± 0·1	2·4 ± 0·2	+104**
$T_{50\%gS}$ (minutes)	2·5 ± 0·2	3·8 ± 0·3	+49**
$T_{90\%A}$ (minutes)	13·6 ± 1·2	9·8 ± 0·8	–28*
$T_{90\%gS}$ (minutes)	13·1 ± 1·1	9·8 ± 0·9	–26*
Post-irradiance CO_2 fixation (%)	18·1 ± 0·7	20·6 ± 0·6	+14*
Post-irradiance CO_2 burst (%)	0·56 ± 0·05	0·07 ± 0·03	–88**
LUE (%)	90·5 ± 1·5	95·1 ± 1·2	+5*
Carbon gain (mol m^{-2})	0·011 ± 0·001	0·019 ± 0·002	+73**

Values are means (± SE), $n = 6$. Significant differences between means indicated by (t-test) $*P < 0·05$, $**P < 0·01$.% difference in parameter value between ambient and elevated CO_2 as percentage of mean value at ambient CO_2.

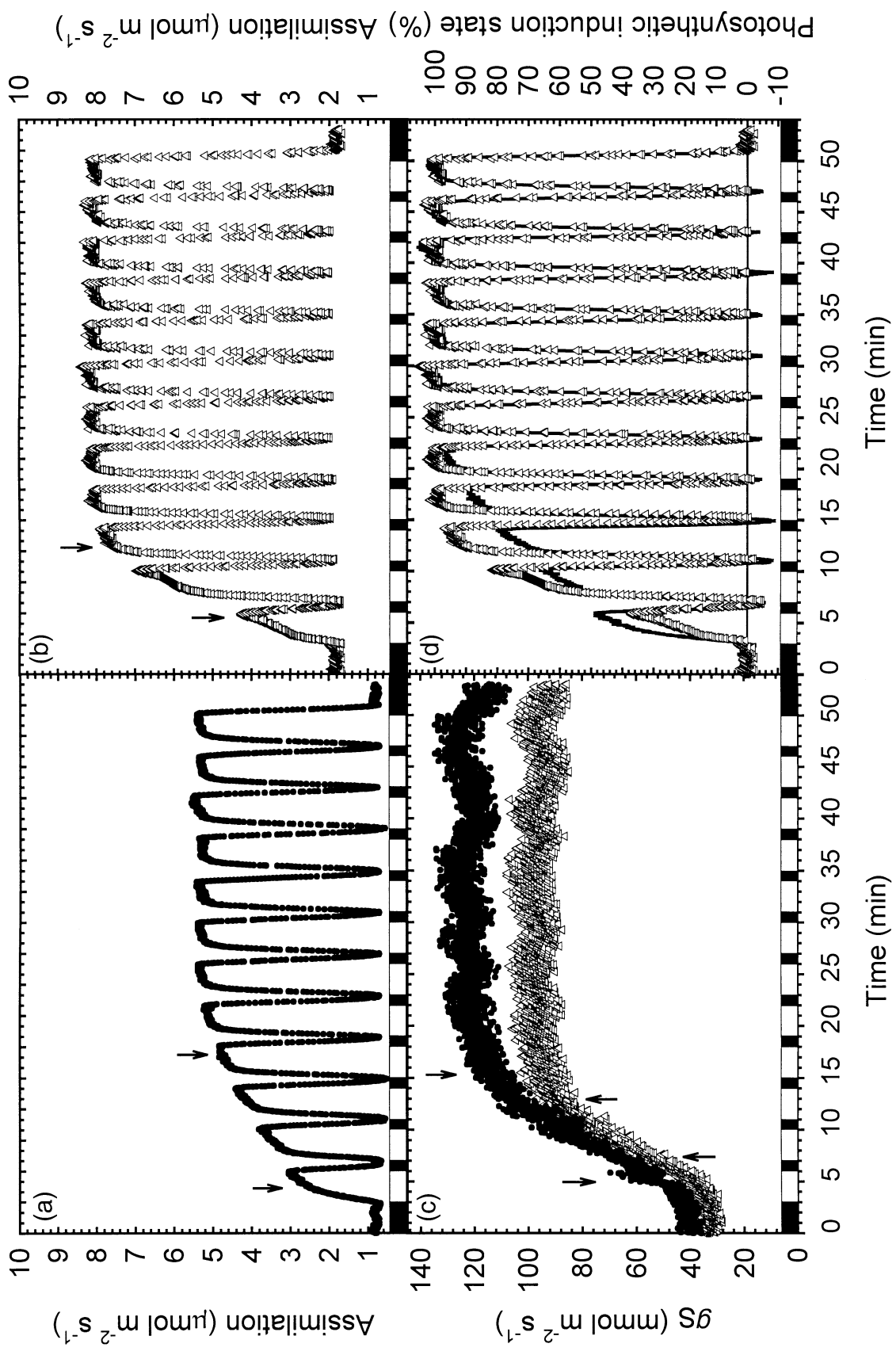

Figure 5. Representative time courses of net photosynthetic rates (a, b), stomatal conductance (g_S) (c) and photosynthetic induction state (d) of *S. leprosula* grown and measured in ambient (closed circles) and elevated (open triangles) CO_2, during a simulated sequence of sunflecks. Flecks are 3 min in duration (white bands) separated by 1 min shade periods (dark bands). Arrows indicate time for induction to 50 or 90% of parameter maximum.

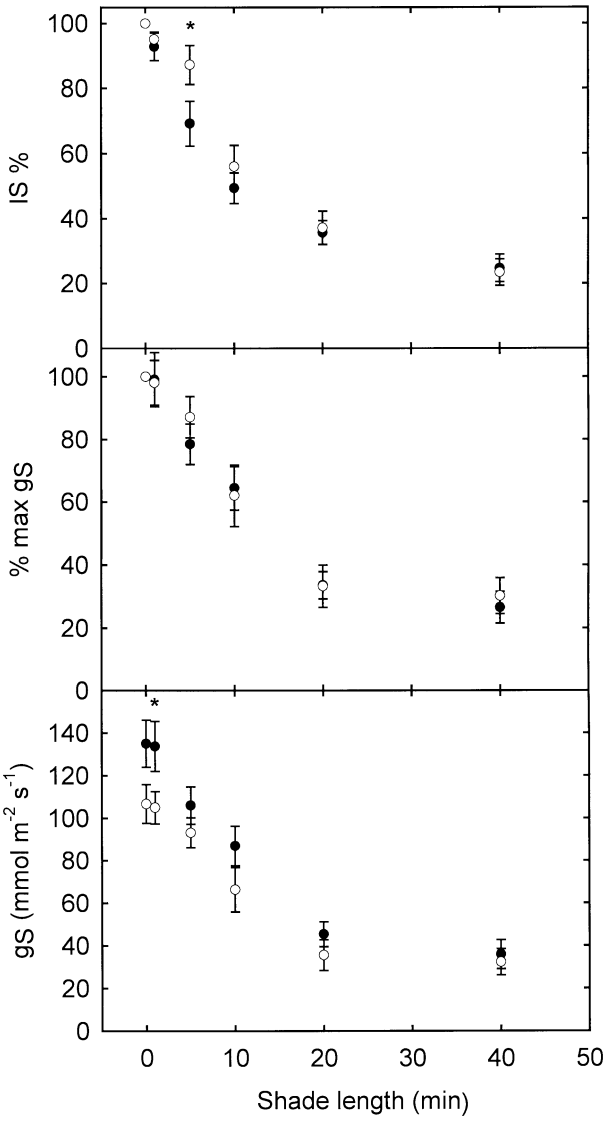

Figure 6. Induction state (IS,%), stomatal conductance relative to g_{Smax} (% max g_S) and stomatal conductance (g_S, mmol m^{-2} s^{-1}) after shade periods of differing lengths in *S. leprosula* grown and measured at ambient (closed circles) and elevated CO_2 (open circles). Values at zero shade length represent steady-state rates under saturating light. Asterisks indicates significant difference between values at $P < 0{\cdot}05$ (*t*-test).

The patterns of V_{cmax} and J_{max} observed are indicative of re-allocation of resources between the biochemical components of the photosynthetic machinery, in order to optimize photosynthesis at elevated CO_2 under shade conditions (Sage *et al*. 1989; Sage 1994). There are few reports of the response of understorey plants to elevated CO_2 under low light. Those studies that exist have consistently shown reductions in V_{cmax} (Osborne *et al*. 1997; Osborne *et al*. 1998; DeLucia & Thomas 2000), but only in the latter did photosynthetic acclimation involve greater J_{max}. However, J_{max} is not always measured and the lower leaves of a wheat crop had greater light harvesting complex content at elevated CO_2 (Osborne *et al*. 1998). Fully understanding the mechanism controlling photosynthetic acclimation to elevated CO_2 will require further studies.

Despite the lower Rubisco content there was no change in nitrogen or chlorophyll concentration on a leaf area basis. This suggests that nitrogen re-allocated from Rubisco was retained in the photosynthetic machinery. The decrease in SLA at elevated CO_2 did not appear to result from changes in leaf morphology, as typically found in a sun–shade acclimation response (Gunderson & Wullschleger 1994; Luo, Field & Mooney 1994). Lower N-mass, chlorophyll-mass and SLA all appear to have resulted from the greater total non-structural carbohydrate content of leaves at elevated CO_2 on a leaf area basis. This is consistent, even under flecked irradiance, with the suite of responses seen in recent meta-analyses (Curtis 1996; Curtis & Wang 1998) and modelling exercises (Peterson *et al*. 1999).

Light-limited photosynthesis

Although photosynthetic rates were greater at all PFDs at elevated CO_2, the greatest enhancement occurred at the very low PFD. Since deep shade predominated in the flecked experimental treatment (as it does in many forest understories) even a small change in absolute rates of photosynthesis significantly impacts upon daily carbon gain when integrated over time. The value of ϕ at 350 p.p.m. CO_2 was not significantly different in seedlings grown at ambient and elevated CO_2, importantly indicating that, as in Indiana strawberry (Osborne *et al*. 1997), this aspect of photosynthetic enhancement at elevated CO_2 was not subject to acclimation under either flecked or uniform irradiance. The reduction in the light compensation point that resulted is generally important as it will always provide a mechanism for increasing carbon gain at elevated CO_2 in the deep shade of the forest understorey (Long & Drake 1991).

Dynamic photosynthesis

At elevated CO_2 changes were seen in both steady-state and dynamic components of photosynthetic performance during a sequence of flecks. The changes in $A_{initial}$ and $A_{max\text{-}flecks}$ matched the enhancements seen at elevated CO_2 at the corresponding PFDs (30 and 525 μmol m^{-2} s^{-1}) on the light response curves. The response of g_S to elevated CO_2 in tree species is not consistent (Saxe *et al*. 1998), being typically weak in pot-grown seedlings (Curtis & Wang 1998) but stronger in long-term field studies (Medlyn *et al*. 2001). The lower absolute stomatal conductance at elevated CO_2 was importantly accompanied in *S. leprosula* by a 22% smaller differential between $g_{Sinitial}$ and $g_{Smax\text{-}fleck}$. This appears to have driven the 28% decrease in the time for photosynthetic induction gain seen at elevated CO_2. Under natural dynamic irradiance environments this would reduce the photosynthetic induction limitation upon apparent incident quantum yield that is important in determining carbon gain (Timm, Stegemann & Küppers 2002).

The time course of IS% indicates a lag phase in induction gain during the first two flecks at elevated CO_2, which ini-

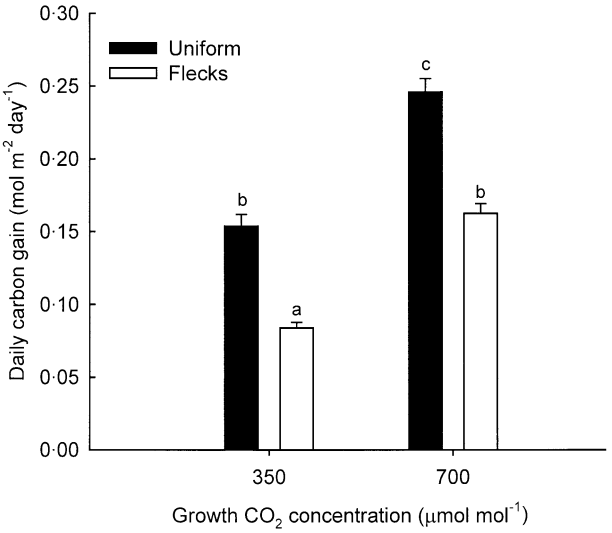

Figure 7. Calculated rates of daily photosynthetic carbon gain (mol m^{-2} d^{-1}) of *S. leprosula* grown at either ambient or elevated CO_2, with uniform or flecked irradiance. Values are means (± SE), $n = 6$. Bars not showing a common letter differ significantly (Tukey multiple comparison test $P < 0{\cdot}05$).

tially results in lower values relative to those at ambient CO_2. This sigmoidal induction function has been attributed to differences in Rubisco biochemistry (Watling & Woodrow 1993) or alternatively stomatal limitation to induction (Küppers & Schneider 1993; Valladares, Allen & Pearcy 1997). The mechanism for the initial lag in stomatal opening in response to increasing PFD at elevated CO_2 in this case is uncertain but will have the greatest impact upon photosynthetic carbon gain when sunfleck duration is shortest.

Photosynthetic induction loss was only slower at elevated CO_2 at 5 min after the transition from fleck to shade PFD. However, in natural irradiance regimes where many shade periods may be of this duration (Pearcy *et al*. 1994, Leakey unpublished results) this would increase photosynthetic carbon gain during subsequent flecks. Slower induction loss was not caused by stomatal dynamics as there was no significant difference in percentage g_{Smax} between treatments. The slower loss of induction in the short term may have resulted from decreased deactivation of photosynthetic enzymes in the shade. However, as in Naumburg & Ellsworth (2000), it is uncertain whether the limitation on deactivation could be due to enzymes responsible for ribulose-1,5-bi-phosphate regeneration or Rubisco (Sassenrath-Cole & Pearcy 1992; Ernstsen, Woodrow & Mott 1997).

Increased post-irradiance CO_2 fixation (14%) and reduced post-irradiance CO_2 burst (–88%) in seedlings growing at elevated CO_2 correspond to changes seen in response to short-term treatments of high CO_2 or low O_2 (Doehlert, Ku & Edwards 1979; Peterson 1983; Vines *et al*. 1983; Laisk *et al*. 1984). They were probably due to changes in fluxes of intermediates in the photosynthetic and photorespiratory pathways, respectively (Sharkey, Seemann & Pearcy 1986; Rawsthorne & Hylton 1991). Greater post-irradiance carbon gain is likely to be of even greater significance under natural patterns of short, high frequency flecks where post-irradiance metabolism contributes a greater proportion of net carbon gain (Pearcy 1990).

Collectively, the increase in the rate of induction gain, decrease in the rate of induction loss as well as greater post-irradiance CO_2 fixation and reduced post-irradiance CO_2 burst led to a 5% increase in the LUE of photosynthesis during the sequence of flecks. This improvement appears modest but it is important to recognize that it interacts with the greater steady-state photosynthetic rates at elevated CO_2, resulting in a large absolute increase in photosynthetic carbon gain during a cluster of flecks (73%).

Integrated daily carbon gain and implications for seedling ecology in the field

Together, the photosynthetic responses are consistent with the differences in growth of *S. leprosula* under elevated CO_2 and flecked irradiance. The increase in dynamic photosynthesis and greater light-limited, steady-state photosynthesis combine to increase calculated total daily carbon gain under flecked irradiance at elevated CO_2 by 89%. This was significantly greater than the relative enhancement with elevated CO_2 under uniform irradiance (59%). The potential for elevated CO_2 to change seedling performance is clearly stronger when the dynamic nature of their irradiance environment is considered. The study needs to be repeated under natural conditions, without the limitations imposed by controlled environment cabinets and extended to quantify how the effect will vary with total light supply and different sunfleck patterns. The result has important potential implications for the ecology of tropical rain forests.

Seedling mortality in the closed-forest understorey is driven by an inability to maintain a positive carbon balance (Chazdon 1988) and the necessity to allocate sufficient resources to defence and re-growth associated with pathogens, herbivores and non-biotic physical damage. Therefore, in an elevated CO_2 scenario greater carbon gain may lead to generally longer survivorship, with consequences for forest regeneration. In addition, differences in CO_2 responsiveness between dipterocarp species are likely, based upon the range of growth and photosynthetic rates they display along a continuum of shade tolerance (Zipperlen & Press 1996; Barker, Press & Brown 1997; Kerstiens 1998, 2001). This possibility is increased by the sensitivity of dynamic photosynthesis to elevated CO_2. Differences also occur between dipterocarp species in the limitations imposed variously by photosynthetic induction gain and loss, photosynthetic capacity and post-irradiance metabolism upon carbon gain under fleck irradiance (Zipperlin & Press 1997; Cao & Booth 2001; Bungard *et al*. 2002; Leakey *et al*. in prep.). As a consequence, individualistic responses to elevated CO_2 in tropical rain forest understories are more likely. Thus, the efficiency of dynamic photosynthesis is a second axis of variation among species,

along which carbon gain, growth and competitive interactions might change with elevated CO_2. The possibility of changes in competitive interactions are particularly significant in the context of the developing view that climax species performance under shaded conditions, along with gap regeneration dynamics, is a key determinant of forest regeneration and the maintenance of high biodiversity (Lieberman *et al*. 1995; Whitmore & Brown 1996; Hubbell *et al*. 1999; Schnitzer & Carson 2001).

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APPENDIX 1

Mean CO_2 concentration, range of CO_2 concentrations, mean day and night temperature and mean day and night relative humidity experienced by seedlings in four treatment blocks in controlled environment growth cabinets.

	AU	AF	EU	EF
CO_2 concentration (μmol mol^{-1})	376 ± 1^a	378 ± 1^a	711 ± 3^b	709 ± 2^b
Range CO_2 concentrations (μmol mol^{-1})	361–422	358–419	640–756	643–749
Day temperature (°C)	30·2 ± 0·1	30·2 ± 0·1	30·3 ± 0·1	30·3 ± 0·1
Night temperature (°C)	20·1 ± 0·1	20·1 ± 0·1	20·2 ± 0·1	20·2 ± 0·1
Day relative humidity (%)	80·6 ± 0·4	80·6 ± 0·4	80·4 ± 0·4	80·4 ± 0·4
Night relative humidity (%)	98·2 ± 0·4	98·1 ± 0·3	98·2 ± 0·3	98·1 ± 0·3
Total PFD (mol m^{-2} d^{-1})	7·7 ± 0·1	7·7 ± 0·1	7·7 ± 0·1	7·7 ± 0·1

Treatments as in Table 1. Values are means (± SE). Total PFD $n = 7$; CO_2, temperature and relative humidity $n = 19$. Data were analysed by ANOVA. Where statistical differences occur, means sharing a common superscript letter do not differ significantly (Tukey Test $P < 0{\cdot}05$).