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ORIGINAL ARTICLE

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Photosynthetic CO_2 uptake in seedlings of two tropical tree species exposed to oscillating elevated concentrations of CO_2

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Abstract Do short-term fluctuations in CO_2 concentrations at elevated CO_2 levels affect net CO_2 uptake rates of plants? When exposed to 600 μl CO_2 l^{-1}, net CO_2 uptake rates in shoots or leaves of seedlings of two tropical C_3 tree species, teak (*Tectona grandis* L. f.) and barrigon [*Pseudobombax septenatum* (Jacq.) Dug.], increased by 28 and 52% respectively. In the presence of oscillations with half-cycles of 20 s, amplitude of ca. 170 μl CO_2 l^{-1} and mean of 600 μl CO_2 l^{-1}, the stimulation in net CO_2 uptake by the two species was reduced to 19 and 36%, respectively, i.e. the CO_2 stimulation in photosynthesis associated with a change in exposure from 370 to 600 μl CO_2 l^{-1} was reduced by a third in both species. Similar reductions in CO_2-stimulated net CO_2 uptake were observed in *T. grandis* exposed to 40-s oscillations. Rates of CO_2 efflux in the dark by whole shoots of *T. grandis* decreased by 4.8% upon exposure of plants grown at 370 μl CO_2 l^{-1} to 600 μl CO_2 l^{-1}. The potential implications of the observations on CO_2 oscillations and dark respiration are discussed in the context of free-air CO_2 enrichment (FACE) systems in which short-term fluctuations of CO_2 concentration are a common feature.

Keywords CO_2 oscillations · Elevated CO_2 concentrations · Free-air CO_2 enrichment (FACE) systems · Photosynthesis · Tropical trees

Owing to an unfortunate misunderstanding, the uncorrected version of this paper was published.

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Abbreviations FACE: free-air CO_2 enrichment · IRGA: infra-red gas analyser · Rubisco: ribulose 1,5-bisphosphate carboxylase/oxygenase

Introduction

Free-air carbon dioxide enrichment (FACE) facilities are presently considered the best method for manipulating atmospheric CO_2 concentrations around plants growing under otherwise natural field conditions (Hendrey et al. 1999; McLeod and Long 1999). Such systems have become an integral tool for studying, in the context of global climate change, the effects of increasing CO_2 concentrations on the growth and development of uncontained plants in situ (Miglietta et al. 2001).

All FACE systems impose two CO_2 treatments — an increase in the average CO_2 concentration and a fluctuating, often oscillating, CO_2 treatment. The amplitude and frequency of the variations in CO_2 concentration common in FACE systems are usually much greater than would ever be experienced under natural conditions, even near tropical forest floors (Holtum and Winter 2001). Changes in CO_2 partial pressures of 200–300 μl CO_2 l^{-1} over periods of 5–20 s are not uncommon in FACE systems but fluctuations of 300 μl CO_2 l^{-1} for periods of 30 s or longer are rare (Evans and Hendrey 1992). Such estimates of fluctuations may be underestimates as long sampling lines may dampen the signals and some fluctuations may be faster than monitor response times.

The CO_2 concentrations fluctuate because the CO_2 injection mechanisms overshoot or undershoot as they continually adjust to counteract variations in wind speed and direction. For example, CO_2 concentrations based on over one million 1-s measurements (each an integral of 3 s) over a 2-year period at the University of Arizona's Maricopa Agricultural Center FACE facility were more than 110 μl l^{-1} higher or lower than the set target of 550 μl CO_2 l^{-1} for 9.3% of the time (Nagy et al. 1994). For 23.9% of the time they differed by between

± 55 and ± 110 µl l^{-1}. When averaged over 1-min intervals, a common measure of FACE performance, the CO_2 concentration was within ± 55 µl l^{-1} of the set point for 95% of the time and between ± 55 and ± 110 µl l^{-1} for 6.7% of the time. The CO_2 fluctuations induced in Brookhaven-type FACE facilities that inject diluted CO_2 (e.g. Nagy et al. 1994; Hendrey et al. 1999; Jordan et al. 1999) and in comparably sized facilities that inject pure CO_2 (Miglietta et al. 2001; Okada et al. 2001; Pepin and Körner 2002) are broadly similar although FACE systems enclosing trees and natural communities tend to exhibit greater fluctuations than those enclosing crops. Open-top systems can exhibit comparable fluctuations (Cardon et al. 1995; Winter et al. 2000).

Clearly, FACE systems do not mimic atmospheric CO_2 conditions over time-scales of a few minutes or less (with the exception of natural CO_2 vents; Koch 1993; Miglietta et al. 1993). Although there is extensive literature on the effects of constant high CO_2 concentrations on plant growth and development, there have been few studies that compare the effects on net CO_2 uptake and plant performance of rapidly oscillating versus constant CO_2 concentrations. Do short-term oscillations in CO_2 concentration affect photosynthetic CO_2 exchange in the shorter term and plant growth in the longer term? Although it is commonly expressed that such short-term variations are unimportant in situ (Hendrey et al. 1997, 1999), particularly in tree species for which the responses of stomata are believed to be slower than in crop plants (Saxe et al. 1998), short-term CO_2 oscillations have been reported to perturb photosynthesis in leaves of the C_3 species *Gossypium hirsutum* L. (Evans and Hendrey 1992), *Triticum aestivum* L. (Hendrey et al. 1997) and *Phaseolus vulgaris* L. (Cardon et al. 1994, 1995) and in the C_4 species *Zea mays* L. (Cardon et al. 1994, 1995). However, in none of the above-mentioned examples was CO_2 uptake studied during oscillations of less than 1 min that are characteristic of FACE experiments.

Gossypium leaf tissue exposed to 1-min oscillations of between 360 and 1,090 µl CO_2 l^{-1} (mean of 700 µl l^{-1}) exhibited a mean rate of uptake of $^{14}CO_2$ that did not differ from that of leaf tissue which had been exposed to a constant concentration of 700 µl l^{-1} (Evans and Hendrey 1992). However, oscillations of 2 min and longer were associated with an increase in net CO_2 gain, reaching 27% when the oscillation was extended to 10 min. It was speculated that the mechanism responsible for the increase was related to postulated changes from ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco)-limited to inorganic phosphate- and triose phosphate-limited photosynthesis. Furthermore, it was suggested by extrapolation that oscillations of less than 1 min duration would have little effect on the rate of long-term carbon gain.

Photosynthetic CO_2 uptake in wheat was inferred from measurements of instantaneous photosystem II fluorescence (F_t) during oscillations with an amplitude of 225 µl CO_2 l^{-1} around a mean of 575 µl CO_2 l^{-1} and half-cycles between 0.1 and 64 s (Hendrey et al. 1997). Oscillations in chlorophyll fluorescence were observed for half-cycles greater than 2 s and reductions in electron transport rate (J) were observed for half-cycles of 30 s and greater. It was concluded that at least 180 s were required before F_t signals achieved a new steady state, and that a substantial decrease in CO_2 uptake would occur only if the duration of a CO_2 oscillation was greater than 1 min, or if the oscillation was not symmetric around the mean.

In *Z. mays* and *P. vulgaris* subjected to CO_2 oscillations of 100–160 µl CO_2 l^{-1} for between 2 and 20 min, stomatal conductance shifted away from the steady-state level observed under the median CO_2 concentration of 333–340 µl CO_2 l^{-1} (Cardon et al. 1994, 1995). The extent and direction of the shifts, which depended upon the species and the oscillation frequency, were related to species-specific differences in the kinetics of stomatal movement and photosynthetic characteristics. The non-steady-state conditions changed short-term water-use efficiencies in both species although photosynthetic rates remained fairly constant.

In order to dispel uncertainty on the effects of short-term fluctuations in CO_2 concentrations on carbon gain we have tested whether the responses of net CO_2 exchange by seedlings or leaves of two tropical tree species, teak (*Tectona grandis* L. f.) and *Pseudobombax septenatum* (Jacq.) Dug., to an increase in CO_2 concentration from ca. 370 to 600 µl CO_2 l^{-1} are affected by symmetric oscillations around 600 µl CO_2 l^{-1}, with half-cycles of considerably less than 1 min.

Exposure to enhanced and fluctuating CO_2 are not the only treatments imposed by FACE systems. A number of FACE systems impose a third CO_2 treatment: the CO_2 injectors are turned off during the dark (Pepin and Körner 2002). Apart from reducing the use and thus the cost of CO_2, switching off the CO_2 supply avoids the technical problem of controlling and maintaining constant and relatively uniform CO_2 concentrations when wind speeds are low, and reduces blower-induced canopy temperature increases (Pinter et al. 2000). There is uncertainty as to whether plant performance and development is affected by increased concentrations of CO_2 in the dark, a period when photosynthesis is not taking place and ambient concentrations of CO_2 tend to be higher. Although dark respiration by C_3 and C_4 grasses, C_3 herbaceous species and C_3 trees has been reported to be inhibited under enhanced CO_2 concentrations (e.g. Drake et al. 1999), there are many reports of little or no effect of enhanced CO_2 concentrations on dark respiration (e.g. Amthor et al. 2001; Hamilton et al. 2001; Tjoelker et al. 1999, 2001). Recently it has been suggested that some reports on the effects of high CO_2 on dark respiration may be artefacts caused by the leakage of CO_2 from plant gas-exchange chambers through gaskets or through contiguous pores which connect regions of plant mesophyll that transcend the boundaries of the chambers (Jahnke 2001; Jahnke and Krewitt 2002; Pons and Welschen 2002).

In order to quantify dark CO_2 efflux in *T. grandis* and to circumvent problems associated with the leakage of respiratory CO_2 through leaves or across gaskets we determined the effects of an increase in CO_2 concentration from 370 to 600 μl CO_2 l^{-1} on dark respiration by whole intact shoots of teak seedlings that were fully enclosed in a gas-exchange chamber.

Materials and methods

Plant material and growth conditions

Seeds of *Tectona grandis* L. f. (Verbenaceae) and *Pseudobombax septenatum* (Jacq.) Dug. (Bombacaceae) were collected locally and germinated in potting soil in a screenhouse on the roof of the Tupper Building, Smithsonian Tropical Research Institute, Panama City, Republic of Panama. After 2–3 weeks, seedlings were transplanted into half-strength Johnson's solution (Winter 1973) and grown under a 12 h light, 26 °C/12 h dark, 23 °C cycle in an environmental growth chamber (GCT-8; GEC, Chagrin Falls OH, USA) equipped with eight fluorescent light tubes (Sylvania 115 W F48T12/CW/VHO).

Gas exchange system

Net CO_2 exchange was measured for the shoots of whole plants in a through-flow gas exchange system (Walz, Effeltrich, Germany). Oscillating CO_2 concentrations were generated by mixing two air streams, one containing CO_2 and the other containing CO_2-free air. The CO_2-containing air stream was generated by mixing pure CO_2 and CO_2-free air in a custom-made mixing unit (Walz GMA-3). The CO_2-free stream was generated by passing air through soda-lime. The dew-points of the two air streams were set by electronically controlled cold-traps (Walz KF-24/6BM and KF-18/2) before passage through two mass-flow controlled pumps (Walz LD-5R and LD-10R). Air streams with oscillating CO_2 concentrations were generated by alternating the supply from each pump at appropriate intervals using a timer-controlled solenoid gas switch (Walz TG 101A and Walz GUS-8). Air was pumped through a Plexiglas cuvette with a volume of 1.21 l (11 cm × 11 cm × 10 cm). Mixing of the atmosphere inside the cuvette was facilitated by a 4-cm-diameter CPU cooler fan (12 V, 0.08 A). The airstream leaving the cuvette was dehumidified in a cold-trap at 2 °C (Walz KF-18/2) and the CO_2 concentration determined by an infra-red gas analyser (IRGA; LI-6252; LI-COR, Lincoln, NE, USA) previously calibrated using CO_2 gas standard (Scott Speciality Gases, Plumsteadville PA, USA) and a set of three gas-mixing pumps (Wösthoff, Bochum, Germany). Gas flow rates were 2.200, 2.128, 1.100 and 1.032 l min^{-1} for experiments at constant CO_2 in the light, for 20-s oscillations, for 40-s oscillations and for experiments at constant CO_2 during the dark, respectively. Flow rates were verified using a water-volume displacement method and a digital soap-bubble flow meter (model 650; Humonics Inc, Rancho Cordova CA, USA).

In an experiment designed to test the dilution of oscillation signals in the airstream between the plant chamber and the IRGA we compared maximum and minimum CO_2 concentrations emanating from the complete gas-exchange system with the signals emanating from the system when the post-chamber pre-IRGA cold-trap had been removed and the IRGA was directly connected to the outlet of the gas-exchange chamber. The dilution of the extremes of the oscillations averaged 7 μl l^{-1}.

Measurements of net CO_2 exchange

Intact seedlings of ca. 6 cm height, growing in 150-ml pots containing half-strength Johnson's solution (Winter 1973) were inserted into a gas-exchange cuvette located in the temperature-controlled growth chamber in which the seedlings had been maintained. For *T. grandis*, the entire shoot was sealed in the cuvette (total leaf area of 44–77 cm^2), whereas for *P. septenatum* one leaf was enclosed (area of 29–33 cm^2). Plant material in the cuvette was kept under a regime of 12 h light, 29 °C/12 h dark, 25 °C. The dew-point of the air entering the gas-exchange cuvette was 18 °C. The light intensity at the uppermost leaf inside the cuvette was 280 μmol m^{-2} s^{-1} for the experiments with *T. grandis* and 410 μmol m^{-2} s^{-1} for *P. septenatum*.

Plant material was incubated at 370 μl CO_2 l^{-1} in the gas-exchange cuvette overnight. Experiments were initiated about 2 h following the onset of the light period. After determining net CO_2 exchange rate at a constant 370 μl CO_2 l^{-1}, the CO_2 concentration was increased to 600 μl CO_2 l^{-1} and net CO_2 exchange was recorded following attainment of steady-state photosynthesis. For the experiments with oscillating CO_2 concentrations, gas exchange was recorded for 10 min in the presence of the plant tissue and then for 10 min in the absence of the plant tissue. Estimations of net CO_2 exchange did not alter when the sequence of collecting sample and control data was reversed. The output from the gas analyser was sampled electronically at 1-s intervals. CO_2 uptake by the tissue was calculated from the difference in the integrated CO_2 concentrations and expressed as a mean rate per second on a leaf-area basis.

To obtain CO_2–response curves of net CO_2 exchange in the light, CO_2 concentrations were increased in three steps from 370 to 850 μl CO_2 l^{-1}, decreased in six steps to 30 μl CO_2 l^{-1} and then increased in five steps to 600 μl CO_2 l^{-1}. Each CO_2 concentration was maintained until a steady-state rate of photosynthesis was attained.

Dark respiration rates were determined during the normal dark period. Measurements were taken at 370 μl CO_2 l^{-1}, at 600 μl CO_2 l^{-1} and subsequently at 370 μl CO_2 l^{-1}.

Results

The rate of net CO_2 uptake in the light by *T. grandis* was CO_2-dependent (Fig. 1). When exposed to a constant concentration of 600 μl CO_2 l^{-1}, the rate of net CO_2 uptake was 28 ± 3% (mean ± SE) greater than at a constant 370 μl l^{-1} ($P \leq 0.01$, paired *t*-test; columns 3 and 4 in Table 1). This CO_2-dependent increase at

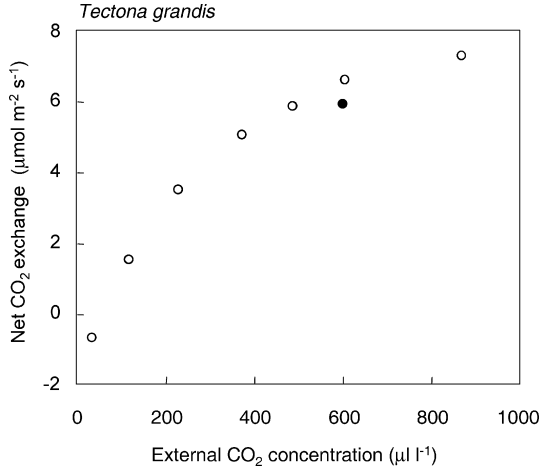

Fig. 1 CO_2–response curve of a whole shoot of a *Tectona grandis* seedling exposed to constant concentrations of CO_2 (*open circles*) or to oscillations in CO_2 concentration with half-cycles of 20 s (*closed circle*). Representative of four experiments on three plants

Table 1 Net CO_2 uptake by shoots of *Tectona grandis* seedlings at constant and oscillating CO_2 concentrations. Percentage reduction in CO_2-stimulated CO_2 uptake was calculated as [(D−E)/(D−C)×100], where the capital letters indicate values in the columns from left to right. The means of rates for 20-s and 40-s oscillations did not differ from each other (paired*t*-test), but differed from rates at constant 370 and 600 µl CO_2 l^{-1} ($P \le 0.01$, paired*t*-test). The rates at constant 370 and 600 µl CO_2 l^{-1} differed ($P \le 0.01$, paired *t*-test)

Plant No.	Expt. No.	Net CO_2 uptake (µmol m^{-2} s^{-1})				Reduction in CO_2-stimulated rate under oscillating CO_2(%)	
		Constant	Constant	Oscillating			
		370 µl CO_2 l^{-1}	600 µl CO_2 l^{-1}	600 µl CO_2 l^{-1}			
				20 s	40 s	20 s	40 s
1	1	4.98	6.90	6.13	–	40.1	–
	2	–	6.46	5.77	–	–	–
	3	–	6.26	5.81	–	–	–
	4	5.01	7.35	6.75	–	25.6	–
2	1	6.56	7.96	7.37	–	42.1	–
	2	7.26	7.77	7.64	–	25.5	–
	3	7.19	8.68	7.59	–	73.2	–
	4	7.61	9.32	8.37	8.36	55.6	56.1
	5	7.35	9.07	8.64	8.66	25.0	23.8
3	1	4.99	6.58	6.20	6.08	23.9	31.4
	2	4.74	6.67	6.58	5.96	5.2	36.8
	3	6.05	7.71	7.04	7.44	40.4	16.3
	4	5.90	7.78	7.13	7.08	34.6	37.2

600 µl CO_2 l^{-1} was reduced to 19 ± 3% (SE; $P \le 0.01$, paired *t*-test) when the tissue was exposed to symmetric oscillations with a mean of 600 µl CO_2 l^{-1}, a half-cycle of 20 s and an amplitude of ca. 170 µl CO_2 l^{-1} (Fig. 2, Table 1). Similarly, in the subset of plants exposed to 40-s oscillations, the 30 ± 3% (SE; $P \le 0.01$, paired *t*-test) increase of net CO_2 uptake was reduced to 20 ± 2% (SE; $P \le 0.01$, paired *t*-test). That is, in every experiment performed with *T. grandis* under oscillating CO_2 conditions of less than 1 min, net CO_2 uptake diminished. The reduction of the stimulation of photosynthetic CO_2 uptake associated with the increase from 370 to 600 µl CO_2 l^{-1} was 36 ± 5% (SE) in the presence of oscillations with a 20-s half-cycle and 34 ± 6% (SE) in the presence of oscillations with a 40-s half-cycle (Table 1).

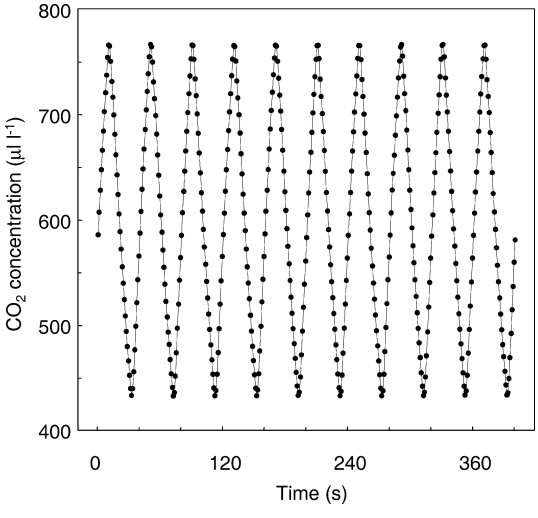

Fig. 2 CO_2 concentrations experienced by the shoot of the *T. grandis* seedling illustrated in Fig. 1 during 10 complete oscillations each with a half-cycle of 20 s. CO_2 concentration was sampled every 1 s. The mean CO_2 concentration during the experiment depicted was 598.9 ± 0.2 µl CO_2 l^{-1}, the mean of the maxima was 766.0 ± 0.2 µl CO_2 l^{-1} and the mean of the minima was 433.5 ± 0.1 µl CO_2 l^{-1} (values ± SE). Similar regular kinetics were observed during experiments with oscillations of 40 s half-cycle

Similar observations were made for photosynthetic CO_2 uptake by *P. septenatum* (Table 2, Fig. 3). The stimulation in net CO_2 exchange in response to an increase in the CO_2 concentration from a constant 370 µl CO_2 l^{-1} to a constant 600 µl CO_2 l^{-1} was 52 ± 2% (SE; $P \le 0.01$, paired *t*-test). This increase was reduced to 36 ± 2% (SE; $P \le 0.01$, paired *t*-test) when the tissue was exposed to 20-s oscillations, i.e. reduction of the stimulation of CO_2 uptake was 31 ± 3% (SE) in the presence of 20-s oscillations (Table 2).

For shoots of *T. grandis* the rates of respiratory net CO_2 loss during the dark were examined at 25 °C at a constant 370 and a constant 600 µl CO_2 l^{-1} (Table 3). The rate of net CO_2 production in the presence of 600 µl CO_2 l^{-1} averaged 4.8 ± 1.3% (SE) less than the average of the rates at 370 µl CO_2 l^{-1}. The differences were significant at a level of $P \le 0.01$ (paired *t*-test).

Discussion

The potential for short-term fluctuations in CO_2 concentration, typical of FACE systems, to alter photosynthetic carbon gain from that observed under constant CO_2 concentrations has been commented upon a number of times (Evans and Hendrey 1992; Cardon et al. 1994, 1995; Nagy et al. 1994; Hendrey et al. 1997; McLeod and Long 1999; Pepin and Körner 2002). Rates of net CO_2 exchange under rapidly fluctuating CO_2

Table 2 Net CO_2 uptake by leaves of *Pseudobombax septenatum* seedlings at constant and oscillating concentrations of CO_2. Percentage reduction in CO_2-stimulated CO_2 uptake was calculated as [(D−E)/(D−C)×100], where the capital letters indicate values in table columns from left to right. The means of the rates at constant 370, constant 600 μl CO_2 l^{-1} and for 20-s oscillations differed from each other ($P \leq 0.01$, paired *t*-test)

Plant No.	Expt. No.	Net CO_2 uptake (μmol m^{-2} s^{-1})			Reduction in CO_2-stimulated rate under oscillating CO_2(%)
		Constant 370 μl CO_2 l^{-1}	Constant 600 μl CO_2 l^{-1}	Oscillating 600 μl CO_2 l^{-1}	20 s
1	1	8.44	12.76	10.89	43.3
	2	7.21	10.70	9.77	26.6
2	1	6.11	9.53	8.47	31.0
3	1	7.00	10.86	9.99	22.5
4	1	5.66	8.36	7.35	37.4
	2	7.03	10.93	10.02	23.3

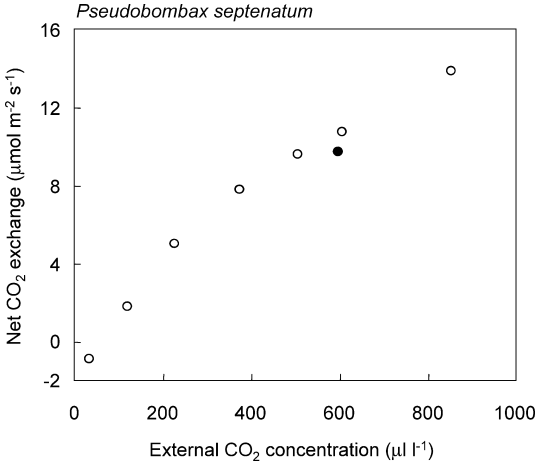

Fig. 3 CO_2–response curve of a leaf of a *Pseudobombax septenatum* seedling exposed to constant concentrations of CO_2 (*open circles*) or to oscillations in CO_2 concentration with half-cycles of 20 s (*closed circle*). The mean CO_2 concentration during the oscillation experiment depicted was 599 ± 2 μl CO_2 l^{-1}, the mean of the maxima was 775 ± 2 μl CO_2 l^{-1} and the mean of the minima was 440 ± 3 μl CO_2 l^{-1} (values ± SE)

Table 3 Net CO_2 production by shoots of five seedlings of *Tectona grandis* during the dark. Plants were sequentially exposed to 370, 600 and 370 μl CO_2 l^{-1}. The rate of net CO_2 production in the presence of 600 μl CO_2 l^{-1} averaged $4.8 \pm 1.3\%$ (SE) less than the average of the rates at 370 μl CO_2 l^{-1}. The differences were significant at a level of $P \leq 0.01$ (paired *t*-test)

Plant No.	Net CO_2 production (μmol m^{-2} s^{-1})		
	370 μl CO_2 l^{-1}	600 μl CO_2 l^{-1}	370 μl CO_2 l^{-1}
1	0.695	0.685	0.720
2	0.805	0.785	0.850
3	0.740	0.715	0.740
4	0.740	0.690	0.730
5	0.825	0.780	0.840

concentrations have not been measured in real time because of the technical difficulty of accurately estimating the differences between the rapidly changing CO_2 concentrations in the reference and sample airstreams. We circumvented this problem by separating in time the measurements of the reference and sample airstreams and integrated the rapidly changing CO_2 concentrations in both airstreams over a number of oscillations (10 min). Mean rates of net CO_2 exchange could thus be calculated, and treatments at oscillating and constant CO_2 concentrations could be compared.

In both *T. grandis* and *P. septenatum*, rapid oscillations of CO_2 at frequencies and amplitudes commonly experienced by vegetation inside FACE systems consistently reduced by about a third the increase in net carbon gain associated with an increase in CO_2 concentration from 370 to 600 μl CO_2 l^{-1} (Tables 1, 2). Oscillations in atmospheric CO_2 should only influence photosynthetic rate in C_3 plants if the concentration of dissolved CO_2 at the site of the Rubisco is altered and if the activity of the carboxylase is limited by CO_2 at some point during the oscillation. Change in the concentration of CO_2 at the site of Rubisco should reflect the amplitude and frequency of oscillation, and the rate at which carbon diffuses from the atmosphere to the chloroplast. The oscillations will be dampened as CO_2 traverses the boundary layer, passes through the stomate into the sub-stomatal cavity, dissolves in the cell milieu and diffuses to the chloroplast.

Clearly, the photosynthetic CO_2-assimilating apparatus in *T. grandis* and *P. septenatum* can respond to extremely rapid changes in external CO_2 concentration. Analogous rapid responses have been reported in wheat for measurements of fluorescence under non-photorespiratory conditions (Hendrey et al. 1997). Chlorophyll fluorescence yield (F_t) in wheat leaves responded to half-cycles as short as 2 s when exposed to oscillations of amplitude 225 μl CO_2 l^{-1} around a mean of 575 μl CO_2 l^{-1}, and electron transport through photosystem II (J) was reduced by about 10% when exposed to 30-s half-cycles and 20% when exposed to half-cycles of 60 s or greater oscillating around a mean of 650 μl CO_2 l^{-1} with an amplitude of 215 μl CO_2 l^{-1}.

A model has been proposed to explain the decrease in photosynthetic net carbon gain in the presence of oscillating CO_2 concentrations (see Fig. 1 in Hendrey et al. 1997). The model assumes that the concentrations of CO_2 within the oscillating range fall within the partially saturated portion of the photosynthetic CO_2–response curve (see Fig. 1), and that during oscillations

the leaf tissue is exposed to the maximum and minimum oscillatory concentrations of CO_2 for a duration sufficient to permit steady-state photosynthesis to occur, i.e. the oscillations are rectangular in shape. Under such conditions the mean of the two extreme steady-state rates of photosynthesis will lie below the curve. Our observations with *T. grandis* and *P. septenatum* are consistent with the model in that net carbon gain under short-term oscillations fell below the curve (Figs. 1, 3). However, the situation is more complex because in our experiments, which were designed to emulate FACE conditions, the external CO_2 concentration changed continuously, never reaching a steady state.

We have not examined the effects of rapid oscillations on stomatal aperture, a response that can indirectly affect photosynthetic carbon gain. Cardon et al. (1994, 1995) demonstrated in *Zea mays* and *Phaseolus vulgaris* that the average stomatal conductance during 3- to 20-min oscillations with medians of 333–340 μl CO_2 l^{-1} and amplitudes of 100–160 μl CO_2 l^{-1} could be driven far from the steady-state condition observed at the median CO_2 concentration. Both the extent and the direction of the departure from the steady state was dependent upon species-specific asymmetries in stomatal opening and closing kinetics as well as the frequency and amplitude of oscillations in CO_2.

A small but consistent reduction of 4.8% in respiratory carbon loss was observed at constant 600 μl CO_2 l^{-1} in comparison to that observed at a constant 370 μl CO_2 l^{-1}. It is unlikely that the reduction in carbon loss is the result of leakage of CO_2 from tissues reported by Jahnke and Krewitt (2002) and Pons and Welschen (2002) as the entire shoot of each *T. grandis* plant was enclosed in the gas-exchange chamber and the stem was tightly sealed with the non-porous synthetic rubber sealant Terostat VII (Henkel-Teroson, Heidelberg, Germany), rather than a semi-porous gasket. Similarly, one cannot ascribe the small differences in respiratory loss to changes in the water vapour content of the airstream, which was dehumidified in an electronically controlled water vapour trap at 2 °C prior to IRGA analysis.

The decrease in the rate of respiratory carbon loss from the shoots from *T. grandis*, which was measured during the normal dark period of the plants at 25 °C, was about 2-fold that reported for 12 C_3 and C_4 grassland species (Tjoelker et al. 2001), 35–70% of that observed for sweetgum, *Liquidambar styraciflua* (Hamilton et al. 2001), and about one-third of that reported by Amthor (1997) who analysed the data for 36 species in 45 studies. The decrease in respiratory CO_2 loss in *T. grandis* is small enough to be accounted for by direct effects of CO_2 on mitochondrial enzymes (Drake et al. 1999). Respiratory CO_2 loss at night may be reduced by phosphoenolpyruvate carboxylase (PEPC) as is the case for weak crassulacean acid metabolism (CAM) plants (Holtum and Winter 1999) but in non-CAM plants, doubling the ambient CO_2 concentration is unlikely to affect the rates of net CO_2 loss in the dark via PEPC (Melzer and O'Leary 1987; Amthor 1997).

The calculated increase in 24-h carbon gain associated with the change from 370 to 600 μl CO_2 l^{-1} was 29.8% when 600 μl CO_2 l^{-1} was only provided during the daylight hours, and 30.4% when 600 μl CO_2 l^{-1} was also provided at night. In the context of FACE experiments, this reduction in dark respiration observed in seedlings of the C_3 plant *T. grandis* represents a trifling increase in net carbon gain. However, bearing in mind the variety of values published for the effects of increasing CO_2 concentration on respiratory dark loss (see Amthor 1997; Drake et al. 1997; Curtis and Wang 1998) there is clearly a need for further studies on whole intact plants rather than leaf segments or detached leaves.

In conclusion, we have demonstrated that short-term oscillations in CO_2 concentration matter. However, it is unclear whether the responses are species-specific, whether plant CO_2 exchange acclimates to oscillating CO_2 in the long-term, whether the reduction in net carbon gain persists and, if so, whether the reduction translates into reduced growth. Moreover, in our experiments the oscillations were regular in periodicity and uniform in amplitude and shape, although this is not the case in FACE systems. Even so, our observations raise the possibility that FACE systems may underestimate the potential fertilising effects of above-ambient CO_2 concentrations on plants.

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