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Are plants growing close to the floors of tropical forests exposed to markedly elevated concentrations of carbon dioxide?

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Abstract. The study tested the frequently expressed perception that the concentration of CO₂ in the vicinity of establishing seedlings growing close to tropical forest floors is generally high. CO₂ concentration was monitored 10 cm from the forest floor over several days during wet and dry seasons at three Panamanian lowland and montane tropical forest sites. Air was sampled at a low flow rate with a peristaltic pump to minimise contamination by air from other strata. The average CO₂ concentrations observed during the dry and wet seasons were 387 and 423 µL CO₂ L⁻¹ air, respectively, a relatively small enrichment compared with the above-canopy CO₂ concentrations. The highest CO₂ concentration recorded at 10 cm was 494 µL L⁻¹. The generally modest levels of enrichment—far below concentrations required to saturate photosynthesis—were nonetheless sufficient to significantly increase the rates of CO₂ uptake relative to above-canopy CO₂ concentrations by shade-grown seedlings of *Piper cordulatum* C. DC., an understory shrub and *Virola surinamensis* (Rol.) Warb., a late successional tree species.

Introduction

The photosynthetic organs of establishing tropical forest seedlings are generally located within 20 cm of the forest floor, an environment dominated by low photosynthetic photon flux densities (PPFD) that may frequently approach or fall below the PPFD required to provide sufficient energy for net CO₂ assimilation (the light compensation point). At low PPFD the energy requirement of photosynthetic carbon metabolism is sensitive to CO₂ concentration such that the light intensity required for net positive CO₂ assimilation reduces with increasing CO₂ concentration. The photosynthetic performance and thus survival of seedlings in the understory may therefore be favoured at elevated CO₂ levels.

It is a common assumption that the potentially detrimental biological effects of low light levels near tropical forest floors are ameliorated because the forest floor atmosphere is enriched in CO₂. Elevated CO₂ levels at night, principally generated by processes that include the diffusion of respiratory CO₂ from the soil, decomposition of leaf litter and leaf respiration, are maintained by the low vertical and horizontal displacement of air masses that is characteristic of closed, dense vegetation stands. After dawn, the floor to canopy stratification of CO₂ levels tends to decrease. The morning reductions in CO₂ concentrations are mainly the

product of photosynthetic CO₂ assimilation and increased turbulent mixing, the result of increases in wind speed associated with temperature differences between the atmospheres near the canopy and the forest floor. Enhanced concentrations of CO₂ near the forest floor will only be of benefit to photosynthesis by seedlings, whether actively growing or ecdormant, if they are maintained for an appreciable time into the light period because the light intensity close to floors of tropical forests may remain low for substantial periods after sunrise.

The results of Medina *et al.* (1986), who used Matheson-Kitagawa CO₂ detector tubes to measure CO₂ concentration profiles at 0, 1 and 20 m and reported 500 µL L⁻¹ CO₂ at 20 m from the forest floor in tropical forests of the upper Rio Negro Basin, Venezuela, have been widely cited as providing support for the postulate that CO₂ concentration considerably greater than above-canopy level is maintained well into the light period for up to 20 m above the forest floor (cf. Lüttge 1997). Similar concentrations have been reported by Buchman *et al.* (1997), by using infrared gas-analysis techniques. However, there is also a body of results in the literature for a range of neotropical and Australasian tropical forest sites that indicates that daytime CO₂ concentration between 0.5 m and the top of canopies rarely deviate by more than 50 µL L⁻¹ from concentrations measured in the above-canopy atmosphere (Lemon *et al.* 1970; Björkman *et al.*

1972; Allen and Lemon 1976; Kira and Yoda 1989; Würth *et al.* 1998). The few reports of CO₂ concentration in the 5–20-cm height range, the range within which many seedlings establish (i.e. become independent of their seed or cotyledonary reserves) or ecodormant seedlings are found, vary considerably from ambient to above 600 $\mu\text{L L}^{-1}$. It is not clear to what extent the variation reported in CO₂ concentrations is a real biological phenomenon and to what extent it is a phenomenon generated by the variety of measurement techniques used. In contrast, there is a general agreement that the CO₂ concentrations within 1 cm of the forest floor and in the leaf litter are considerably greater than those found above the canopy (cf. Medina *et al.* 1986).

Some of the variation observed in CO₂ concentration near the floor of tropical forest is undoubtedly a product of seasonal, daily and site-to-site variations of factors that affect the diffusion of CO₂ from the soil, the exchange of CO₂ between the canopy and the atmosphere, microbial activity and net photosynthesis. Such factors include leaf-litter cover, soil texture, soil and air temperatures, soil moisture, rainfall, soil nutrient contents, wind speed and light intensity.

A component of the variation in forest floor CO₂ concentration may also result from sampling artefacts. When CO₂ is monitored using infrared gas analysis, air is typically sampled at rates of 500–1000 mL min⁻¹. At such sampling rates, air may be channelled from different heights to the sampling port and the CO₂ concentration measured may not represent the concentration present at the sampling height. Air channelled from above may result in underestimations of the CO₂ concentration whereas air channelled from the forest floor might be expected to result in overestimation.

High air humidity characteristic of lower forest atmosphere may also affect CO₂ measurements if infrared analysis is used. If humid air samples are not dehumidified, incorrect estimates of CO₂ concentration may occur if water condenses in the lines leading to the CO₂ analyser. Condensation is particularly a problem when gas lines containing moist air are cooled at night or are in contact with cool surfaces such as soil. This is a particular problem in tropical environments. Not only does liquid water absorb CO₂ and act as a CO₂ buffer, but the high level of water vapour broadens the infrared absorption lines associated with the 4.26 μm CO₂ absorption band such that CO₂ concentration may be overestimated. In addition, many types of plastic tubing absorb water and hysteretic water vapour effects may occur as the tubing degasses.

$\delta^{13}\text{C}$ values of the understorey vegetation, a potentially important diagnostic tool for the estimation of water-use efficiency and modelling of forest CO₂ fluxes, are influenced by forest understorey CO₂ concentrations. It has been postulated that the vertical stratification of stable carbon isotope ratios frequently observed in plant tissue beneath rainforest canopies is correlated with the proportions of isotopically light respiratory CO₂ and isotopically heavy

tropospheric CO₂ fixed by the vegetation, i.e. with the vertical stratification of the CO₂ column (Medina and Minchin 1980; Medina *et al.* 1986). However, plant carbon isotope contents may also vary in response to the ratio of internal to external CO₂ concentrations ($C_i:C_a$) during photosynthesis (Farquhar *et al.* 1982), a ratio influenced by both plant metabolism and external CO₂ concentration. As light intensity decreases, intracellular CO₂ concentration tends to increase because photosynthetic rate of CO₂ uptake is reduced and diffusion of CO₂ through stomata becomes less limiting. At higher $C_i:C_a$ ratios, the equilibrium carbon isotope effect is expressed and an increased discrimination against ¹³C would be expected (O'Leary 1981; Holtum *et al.* 1983). Medina *et al.* (1986) argued that since many understorey plants exhibit low stomatal conductances and most of the CO₂ assimilation by understorey plants occurs during illumination by light flecks when $C_i:C_a$ is reduced (i.e. when stomatal limitation is increased), the carbon isotope content of the vegetation will reflect the proportion of respiratory to atmospheric CO₂ assimilated. However, it is probable that the relative contributions to plant $\delta^{13}\text{C}$ values of source air and $C_i:C_a$ effects will vary temporally in response to environmental and developmental factors. For example, in *Alocasia macrorrhiza*, an Australian rainforest understorey species, the extent to which stomata limit assimilation during photosynthetic induction in response to sun flecks depends on both light intensity and the value of stomatal conductance prior to exposure (Björkman *et al.* 1972; Kirschbaum and Pearcy 1988; Pearcy 1990). Studies at Barro Colorado Island, Panama and in French Guiana indicate that at these sites between as little as 20% and as much as 70% of the differences in $\delta^{13}\text{C}$ values between the canopy and the understorey vegetation could be accounted for by source air effects (Sternberg *et al.* 1989; Buchmann *et al.* 1997). Despite these uncertainties, Sternberg *et al.* (1989) expressed the opinion that leaf $\delta^{13}\text{C}$ values could be used as indicators of plant water-use efficiency without analysing the isotopic composition of the air near the plants under study because carbon isotope ratios of forest air can be estimated from $\delta^{13}\text{C}$ values of decomposing litter and CO₂ concentrations.

The aims of the experiments described here were to quantify the CO₂ concentration in the region where seedling establishment occurs, 10 cm from the forest floor, in a variety of neotropical rainforest types and to establish the relative rates of photosynthesis in low-light-adapted understorey seedlings exposed to light levels and CO₂ concentrations measured 10 cm from the forest floor. Particular care was taken to reduce the chances of artefactual measurements during the quantification of CO₂ concentration. Precautions included using a peristaltic pump to provide a slow air sampling speed of 15 mL min⁻¹, provision of multiple air sampling orifices to minimise channelling, dehumidification of the air flow prior to the air entering air lines and the

placement of instrumentation in warmed containers at least 5 m from the sampling sites.

Materials and methods

Experimental sites

CO₂ concentrations were measured at the following three forest floor sites and one above-canopy site in the Republic of Panama:

(i) Parque Natural Metropolitano (9°59'N, 79°33'W, 30 m above sea level)—The forest floor sampling site was located 10 m from the Smithsonian Tropical Research Institution Pacific canopy crane in the understorey of a lowland tropical dry forest (*sensu* Holdridge *et al.* 1971) with a mean annual rainfall of *c.* 1700 mm and a pronounced dry season from mid-December to April. Above-canopy atmospheric CO₂ concentrations were monitored continuously for a year at the Tupper Building, Smithsonian Tropical Research Institute, which is located 2 km south of the Parque Natural Metropolitano experimental site.

(ii) Barro Colorado Island (9°10'N, 79°51'W, 80 m above sea level)—The sampling site was located 10 m from the Smithsonian Tropical Research Institution 40-m research tower in the understorey of lowland tropical moist forest with a mean annual rainfall of *c.* 2600 mm and a pronounced dry season from mid-December to April (Leigh *et al.* 1982; Windsor 1990).

(iii) Fortuna Natural Reserve (8°43'N, 82°14'W, *c.* 1300 m above sea level)—The sampling site was located approximately 150 m from the Fortuna Natural Reserve Jorge L. Araúz Centre for Scientific Investigations in the understorey of a lower montane tropical forest with a mean annual rainfall of *c.* 3900 mm (Cavelier 1992).

Atmospheric sampling and measurement of CO₂ concentration

CO₂ concentration was measured at 10 cm above the forest floor. At each site, air was continuously sampled at a rate of 15 mL min⁻¹ with a peristaltic pump (EP-1 Econopump, Biorad, CA, USA) through two circular ports (2 mm internal diameter) located 3 cm apart. The air was dehumidified by passage through a column (9.5 × 3 cm) of self-indicating Drierite (97% CaSO₄, 3% CoCl₂, 10–20 mesh; W. A. Hammond Drierite Co, Ohio, USA) attached to the inlet ports. Following filtration through a 0.45-µm Acro-50 PTFE filter (Gelman Sciences, Michigan, USA) the air flowed through 3.2-mm internal-diameter tubing into a Licor 6252 infrared gas analyser (Li-Cor, Nebraska, USA) operating in the absolute mode. The analyser was calibrated by standard gases (Scott, Pennsylvania, USA) and gas-mixing pumps (Wösthoff, Bochum, Germany).

In order to minimise disturbance of the leaf litter cover and the surrounding understorey vegetation the peristaltic pump, CO₂ analyser and chart recorder were located in a sealed shelter at least 5 m from the CO₂ sampling sites. The response time for detection of changes in the CO₂ concentration at the sampling site was approximately 5 min.

Measurements of wind velocity and light intensity

Wind velocity, recorded as m s⁻¹, was determined at 10 cm (all sites) and 2 m (Barro Colorado Island only) with a thermal air velocity sensor (model 8355, TSI Inc., Minnesota, USA). Means were calculated from 90 measurements taken at 10-s intervals over 15-min periods. Photosynthetic photon flux density was measured with a Li-Cor 190S quantum sensor (Li-Cor, Nebraska, USA).

Leaf gas-exchange

Shade-adapted seedlings of two locally growing species, *Piper cordulatum* C. DC., an understorey shrub, and *Virola surinamensis* (Rol.) Warb., a late successional tree, were cultivated under low light conditions (at an average PPFD of *c.* 10 µmol photons m⁻² s⁻¹) for 6 months to 1 year in pots. *Piper* seedlings were grown under natural understorey conditions at Barro Colorado Island (for PPFDs, see Würth

et al. 1998) whereas *Virola* seedlings were grown in a naturally ventilated shade house at the Smithsonian Tropical Research Institution, Panama City.

Net CO₂ exchange was measured for whole intact leaves in a through-flow gas-exchange system (Walz, Effeltrich, Germany). Dewpoint and leaf temperatures of 20 and 27°C, respectively, were maintained during the light and the dark.

Following incubation of whole intact leaves at 360 µL CO₂ L⁻¹ in the gas-exchange cuvette overnight, steady-state leaf photosynthesis was measured at 4.6 µmol photons m⁻² s⁻¹ and 360, 400 or 450 µL CO₂ L⁻¹ during the following light period. Dark respiration rates were determined at 360 µL CO₂ L⁻¹ following completion of the CO₂ responses. Estimations of light compensation points assumed similar rates of respiration at 360 and 450 µL CO₂ L⁻¹. Gas-exchange experiments were repeated on leaves from three separate plants of each species.

Results

Barro Colorado Island

The mean 24-h CO₂ concentration over the 4 days of measurement during the dry season at Barro Colorado Island was 391 ± 4 (s.d.) µL L⁻¹ (maximum 443 µL L⁻¹, minimum 358 µL L⁻¹) whereas the mean 24-h CO₂ concentration over the 4 days of measurement during the early wet season was 429 ± 4 (s.d.) µL L⁻¹ (maximum 485 µL L⁻¹, minimum 389 µL L⁻¹), a difference of 38 µL L⁻¹ (9.7%) (Fig. 1).

During both the dry and the wet season, the CO₂ concentration exhibited day–night fluctuations of broadly similar 24-h periodicity (Fig. 1a, c). CO₂ concentrations tended to increase during the dark from a daily low observed at about 1600–1800 hours. Maximum CO₂ concentration was generally reached by mid-morning, after which it tended to fall until the late afternoon.

Average wind velocities at 10 cm were generally low, rarely exceeding 0.1 m s⁻¹ during the middle of the day when velocities were at their daily maximum (Fig. 1b, d). Maximum wind gusts, of about 0.2–0.3 m s⁻¹ during the wet season and 0.4–0.5 m s⁻¹ during the dry season, were also observed between midday and mid-afternoon. Average wind velocity at 2 m was approximately 3.7 times that at 10 cm.

On 11 July 1999 (during the wet season), the PPFD at 10 cm from the forest floor attained approximately 0.5, 2, 5 and 6.5 µmol photons m⁻² s⁻¹ at about 0800, 0900, 1000 and 1300 hours, respectively. At about 0800, 0930, 1030 and 1300 hours, the PFD above the canopy was 98, 527, 803 and 1678 µmol photons m⁻² s⁻¹, respectively. Maximum background PPFD observed (i.e. excluding sunflecks) during wet or dry season visits to the research site was between 10 and 20 µmol photons m⁻² s⁻¹.

Parque Natural Metropolitano

The mean 24-h CO₂ concentration over the 4 days of measurement during the dry season at Parque Natural Metropolitano was 383 ± 4 (s.d.) µL L⁻¹, whereas the mean 24-h CO₂ concentration over the 2.5 days of measurement during the early wet season was 419 ± 4 (s.d.) µL L⁻¹, a

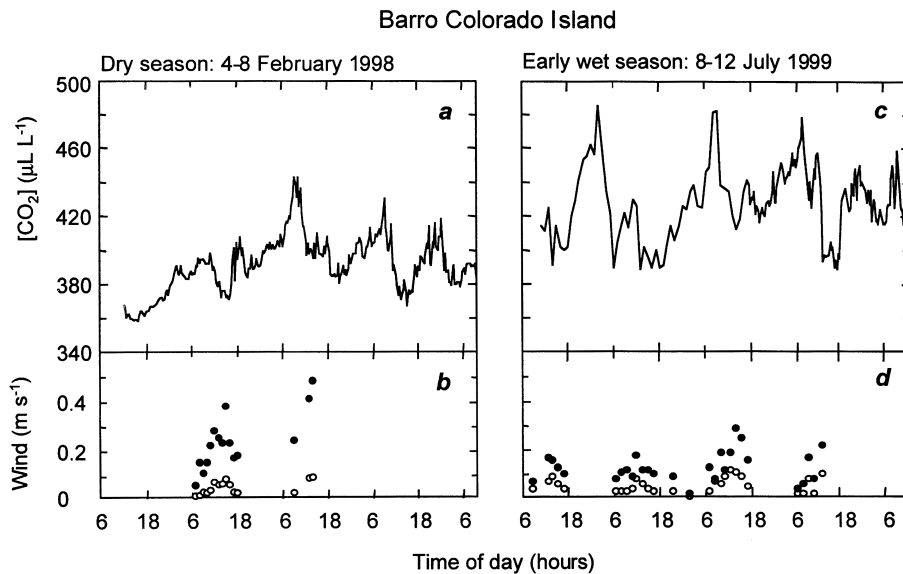


Fig. 1. CO_2 concentration and wind velocity near the forest floor of a lowland tropical moist rainforest at Barro Colorado Island, Republic of Panama, for 4 days during the dry season in 1998 (*a* and *b*) and for 4 days during the wet season in 1999 (*c* and *d*). CO_2 concentration was measured continuously at 10 cm from the forest floor. The values illustrated are for 20-min intervals, with the exception that those during the first 39 h in (*c*) are for 60-min intervals. Wind velocities measured at 10 cm from the forest floor are the means (○) or maxima (●) of 90 measurements undertaken over 15-min intervals. The mean 24-h (0600–1800 hours and 1800–0600 hours) CO_2 concentrations measured during the wet season were significantly higher than the comparable dry season values (Student's *t*-test, unpaired, two-tailed, $P = 0.05$).

difference of $36 \mu\text{L L}^{-1}$ (9.3%) (Fig. 2). For comparison, 20-day mean maximum and minimum above-canopy CO_2 concentrations were 388 ± 5 and 360.8 ± 2 (s.d.) $\mu\text{L L}^{-1}$, respectively, during the dry season and 398 ± 12 and 364 ± 6 (s.d.) $\mu\text{L L}^{-1}$, respectively, during the wet season.

In a manner similar to that observed at Barro Colorado Island, during both the dry and the wet season the CO_2 concentration at Parque Natural Metropolitan exhibited day–night fluctuations of broadly similar periodicity (Fig. 2*a, b*). CO_2 concentrations tended to increase during the dark from a daily low observed at about 1600–1800 hours. Maximum CO_2 concentration was generally reached by mid-morning, after which it tended to fall until the late afternoon. On 10 of 11 days of continuous measurement during the dry season, the daily minimum CO_2 concentration, which averaged 378 ± 4 (s.d.) $\mu\text{L L}^{-1}$, occurred in the afternoon between 1330 and 1700 hours (Table 1). In contrast, the daily maximum CO_2 concentration, which averaged 405 ± 4 (s.d.) $\mu\text{L L}^{-1}$, occurred in the morning between 0700 and 1000 hours on 10 of 11 days.

During a January dry season day, the PPFD at 10 cm from the forest floor ranged between 0.1 and $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ as the PPFD above the canopy ranged between 4 and $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Average wind velocity at 10 cm during the wet season was generally low, rarely exceeding 0.06 m s^{-1} (Fig. 2*c*).

Table 1. Maximum and minimum CO_2 concentration and the time of observation for 11 consecutive days during the 1998 dry season at Parque Natural Metropolitan, Panama City

Date	Max. [CO_2] (ppm)	Time (hours)	Min. [CO_2] (ppm)	Time (hours)
23 January 1998	400	0810	380	0040
24 January 1998	406	0720	360	1558
25 January 1998	396	2340	358	1448
26 January 1998	404	0750	361	1402
27 January 1998	404	0716	371	1512
28 January 1998	416	0808	368	1642
29 January 1998	404	0640	364	1600
30 January 1998	405	0355	381	1337
31 January 1998	420	0948	377	1544
1 February 1998	400	0728	363	1413
2 February 1998	405	0739	362	1608
Mean	405		368	

Fortuna

The mean 24-h CO_2 concentration over the 2.5 days of measurement during the wet season in the Fortuna montane rainforest was 421 ± 21 (mean \pm s.d.) $\mu\text{L L}^{-1}$ (minimum of $396 \mu\text{L L}^{-1}$, maximum of $494 \mu\text{L L}^{-1}$). The day–night periodicity of CO_2 concentration was less marked at Fortuna than at the other sites studied (Fig. 3). Continual rain and cooler temperatures (minimum of 14°C to a maximum of 26°C) were experienced during the measurement period.

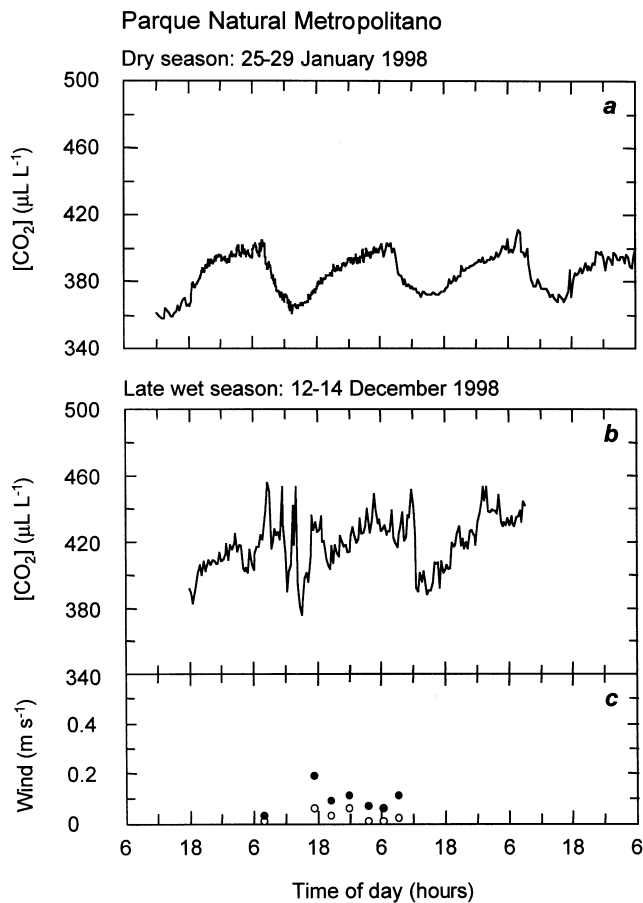


Fig. 2. CO₂ concentration and wind velocity near the forest floor of a lowland tropical dry forest at Parque Natural Metropolitano, Republic of Panama, for 4 days during the dry season in 1998 (a) and for 2.5 days during the wet season in 1998 (b and c). CO₂ concentrations were measured continuously at 10 cm from the forest floor. The values illustrated are for 20-min intervals, with the exception that the first 6 h are for 4-min intervals. Wind velocities measured at 10 cm from the forest floor are the means (○) or maxima (●) of 90 measurements undertaken over 15-min intervals. The mean 24-h (0600–1800 hours and 1800–0600 hours) CO₂ concentrations measured during the wet season were significantly higher than the comparable dry season values (Student's *t*-test, unpaired, two-tailed, *P* = 0.05).

During the 3 days preceding measurements, approximately 83 mm of rain fell, whereas during the 3 days of measurement rainfall was 64.5, 12.8 and 40 mm, respectively. During the second light period of the study (5th December 1999) light intensities at 10 cm in the understorey remained low throughout the day averaging 5.1, 5.6 and 3.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 1000, 1220 and 1500 hours, respectively. Comparable light intensities in a gap 200 m from the site were 505, 420 and 312 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Gas-exchange measurements

Piper cordulatum and *V. surinamensis* exhibited similar net rates of CO₂ uptake (mean \pm s.e.) of 0.252 ± 0.011 and 0.219 ± 0.004 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, when exposed to 360 μL

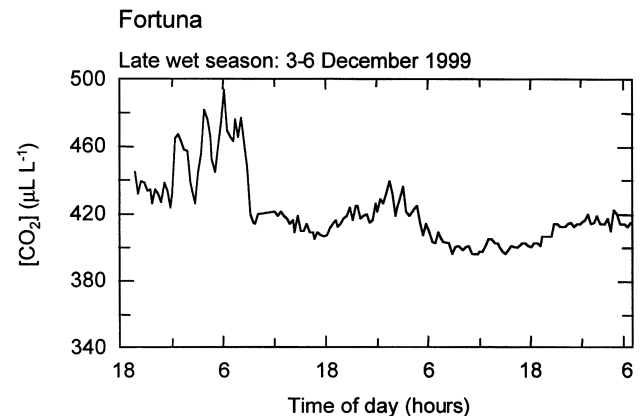


Fig. 3. CO₂ concentration and wind velocity near the forest floor of a lower montane rainforest at Fortuna Natural Reserve, Republic of Panama, for three days during the wet season in 1999. CO₂ concentration was measured continuously at 10 cm from the forest floor. The values illustrated are for 20-min intervals.

CO₂ L⁻¹ at 4.6 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Table 2). In all leaves tested, the rates of CO₂ assimilation increased proportionally when the CO₂ concentration was increased from 360 to 400 or 450 $\mu\text{L L}^{-1}$. The average stimulation observed when the CO₂ concentration was increased from 360 to 450 $\mu\text{L L}^{-1}$ was $18.1 \pm 1.6\%$ for *P. cordulatum* and $19.2 \pm 2.2\%$ for *V. surinamensis*.

Dark respiration rates at 360 $\mu\text{L L}^{-1}$ were -0.063 ± 0.003 (mean \pm s.e.) and -0.100 ± 0.006 (mean \pm s.e.) $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *P. cordulatum* and *V. surinamensis*, respectively.

Light compensation points (mean \pm s.e.) calculated for the fully expanded leaves of *P. cordulatum* and *V. surinamensis* exposed to 360 $\mu\text{L L}^{-1}$ were 0.9 ± 0.06 and 1.4 ± 0.04 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, low values indicating that the plants tested were indeed shade-acclimated. When the leaves were exposed to 450 $\mu\text{L L}^{-1}$ the light compensation points decreased by $12.6 \pm 1.0\%$ and $11.6 \pm 1.3\%$, respectively, from the levels observed at 360 $\mu\text{L L}^{-1}$.

Discussion

CO₂ concentrations 10 cm from the forest floor

Pronounced 24-h fluctuations and seasonal differences in CO₂ concentrations at 10 cm from the forest floor were consistently observed at Barro Colorado Island, a moist tropical forest site and at Parque Natural Metropolitano, a lowland tropical dry forest site, during both the dry and wet seasons. Wet season 24-h average CO₂ concentrations were 36–38 $\mu\text{L L}^{-1}$ greater than dry season averages.

The range in 24-h CO₂ concentration of between 389 and 485 $\mu\text{L L}^{-1}$, measured at 10 cm from the forest floor at Barro Colorado Island during the wet season in 1999 (July), was similar to the 24-h range of between 380 and 440 $\mu\text{L L}^{-1}$ at 50 cm for 2 days during the wet season in November 1991 and 1992 at Barro Colorado Island (Zotz and Winter 1994).

Table 2. Photosynthetic rate at $4.6 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ exhibited by leaves from three seedlings of *Piper cordulatum* and *Virola surinamensis* exposed to $360 \mu\text{L CO}_2 \text{ L}^{-1}$, and percentage change in photosynthetic rate when CO_2 concentration is raised from 360 to 400 and $450 \mu\text{L CO}_2 \text{ L}^{-1}$

Asterisks indicate values significantly different from the $360 \mu\text{L CO}_2 \text{ L}^{-1}$ mean at $P=0.05$ (Student's *t*-test, paired, single-tailed)

	CO ₂ fixation at [CO ₂] $360 \mu\text{L L}^{-1}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Percentage change in CO ₂ fixation from 360 to $400 \mu\text{L L}^{-1}$	Percentage change in CO ₂ fixation from 360 to $450 \mu\text{L L}^{-1}$
<i>Piper cordulatum</i>			
Plant 1	0.242	6.2	15.0
Plant 2	0.274	12.8	20.5
Plant 3	0.240	7.5	18.9
Mean \pm s.e.	0.252 ± 0.011	$8.8 \pm 2.0^*$	$18.1 \pm 1.6^*$
<i>Virola surinamensis</i>			
Plant 1	0.222	7.1	15.0
Plant 2	0.223	9.0	20.2
Plant 3	0.211	13.4	22.4
Mean \pm s.e.	0.219 ± 0.004	$9.8 \pm 1.9^*$	$19.2 \pm 2.2^*$

During the latter study, CO_2 concentrations ranging between 355 and $385 \mu\text{L L}^{-1}$ were measured at 35 m in the canopy.

The 24-h range of between $358 \mu\text{L L}^{-1}$ and $443 \mu\text{L L}^{-1}$ observed during the dry season is comparable to that reported by Würth *et al.* (1998), who measured a maximum of 430 , mean of 373 and median of $381 \mu\text{L L}^{-1}$ during daylight hours for 2 days early in the late dry season in 1995 (May).

The fluctuations and absolute levels of CO_2 observed at the Fortuna montane forest site during the last 48 h of the sampling period were dampened in comparison to those observed during the initial 12 h, and at Barro Colorado Island and Parque Natural Metropolitano (Figs 1–3), a situation that may have been influenced by the extremely wet conditions that prevailed during the 3-day study. Carbon dioxide levels may have been affected by the absorption of CO_2 by liquid water and temperature-related reductions in the CO_2 -producing processes of microbial and plant respiration and the CO_2 -consuming process of photosynthesis. Wind effects at the site were probably small as the velocities were low (averages of 0.018 , 0.022 and 0.041 m s^{-1} at 1000 , 1220 and 1600 hours, respectively, on 5 December 1999), although blustery above the canopy and at a research centre 150 m from the site.

The fluctuations observed at the three sites studied were relatively small compared with some reports (Medina and Minchin 1980; Buchmann *et al.* 1997) but are consistent with others from a range of tropical forests (e.g. Lemon *et al.* 1970; Björkman *et al.* 1972; Allen and Lemon 1976; Kira and Yoda 1989; Würth *et al.* 1998). While one cannot rule out short-term CO_2 fluctuations of less than about 5-min periodicity, such short-term temporal resolution was beyond the capacity of the sampling system due to the residence

times of gas within the dehumidification chamber and within the tubing leading to the gas analyser. It is unlikely that the omission of such rapid changes would have made a substantial difference to the patterns illustrated in Figs 1–3.

Although the measurements indicate that during both the wet and dry seasons the CO_2 concentrations at the three Panamanian forest sites averaged 20 – $60 \mu\text{L L}^{-1}$ greater than the above-canopy concentration, there may be a period of several days following the first rains of the dry-to-wet season transition when bursts of respiratory CO_2 , presumably soil-derived, increase the CO_2 concentrations above the values we have observed. During the seasonal transition period above-canopy CO_2 concentrations monitored from the Tupper building at the Smithsonian Tropical Research Institute (c. 16 m above ground level) reached $450 \mu\text{L L}^{-1}$, indicating that concentrations 10 cm from the ground were, at least for a short period during the year, far higher than those observed during the forest floor sampling periods.

Plant gas exchange under light and CO_2 conditions experienced 10 cm from the forest floor

When shade-adapted seedlings of *P. cordulatum* and *V. surinamensis* were exposed to light intensities and temperatures measured at the forest floors of Barro Colorado Island and Parque Natural Metropolitano, an increase in the CO_2 concentration from 360 to $450 \mu\text{L L}^{-1}$ stimulated CO_2 assimilation (mean \pm s.e.) 18.1 ± 1.6 and $19.2 \pm 2.2\%$, respectively (Table 2). Although the CO_2 concentrations near the forest floors at Barro Colorado Island and Parque Natural Metropolitano decreased during the day, during most mornings there was a period of several hours when concentrations remained above $360 \mu\text{L L}^{-1}$. At Barro Colorado Island, the region 10 cm from the floor was

Table 3. Percentage daylight hours (0600–1800 hours) during which the CO₂ concentration, 10 cm from the forest floor at Barro Colorado Island and Parque Natural Metropolitano, remained within certain CO₂ limits in the wet and dry seasons (see Methods for dates)

CO ₂ concentration (μL L ⁻¹)	Parque Natural Metropolitano		Barro Colorado Island	
	Dry season (%)	Wet season (%)	Dry season (%)	Wet season (%)
325–375	52.6	1.0	22.4	0
375–425	47.4	54.8	71.3	58.4
425–475	0	44.2	6.3	41.6

exposed to (i) CO₂ concentrations within 25 μL L⁻¹ of 350 μL L⁻¹ for 1% of the daylight hours during the wet season, and 44.2% of time to concentrations within 25 μL L⁻¹ of 450 μL L⁻¹, and (ii) to CO₂ concentrations within 25 μL L⁻¹ of 350 μL L⁻¹ for 52.6% of the daylight hours during the dry season but spent no time within 25 μL L⁻¹ of 450 μL L⁻¹ (Table 3). At Parque Natural Metropolitano, the region 10 cm from the floor was not exposed to (i) CO₂ concentrations within 25 μL L⁻¹ of 350 μL L⁻¹ during the wet season but 41.6% of daylight time was spent within 25 μL L⁻¹ of 450 μL L⁻¹, and (ii) during the dry season, the region was exposed to concentrations within 25 μL L⁻¹ of 350 μL L⁻¹ for 22.4% of the daylight hours, and only 6.3% was spent within 25 μL L⁻¹ of 450 μL L⁻¹ (Table 3).

Clearly, at both sites, establishing and ecodormant seedlings spend considerable time performing photosynthesis at elevated CO₂ concentrations, particularly during the wet season. Würth *et al.* (1998) quantified CO₂ fixation at an understorey site at Barro Colorado Island. They observed that 32% of the incident light was between 3 and 10 μmol m⁻² s⁻¹, accounting for about one-third of the estimated daily CO₂ uptake, 24% of the light was between 10 and 20 μmol m⁻² s⁻¹, accounting for about one-third of the estimated CO₂ uptake, and PPFD greater than 20 μmol m⁻² s⁻¹ constituted about 40% of the incident light, accounting for one third of the estimated CO₂ uptake. If the estimations of Würth *et al.* (1998) are applicable to the Barro Colorado Island site studied by us—a reasonable assumption since the two sites were only 200 m apart—and to the site at Parque Natural Metropolitano, then one can conclude that the elevated CO₂ concentrations at 10-cm height reported here were sufficient to stimulate CO₂ uptake over 95% of the daylight hours during the wet season and between 47 and 78% of the daylight hours during the dry season. Such an increase in carbon balance would favour seedling establishment, especially during the wet season. The estimate is a conservative one as Würth *et al.* (1998) based their calculations on a light compensation point of 3 μmol m⁻² s⁻¹.

Shade-adapted seedlings of *P. cordulatum* and *V. surinamensis* exposed to 360 μL L⁻¹ CO₂ exhibited light compensation points of between 0.9 and 1.4 μmol m⁻² s⁻¹, respectively. The light compensation points decreased by

between 12.6 and 11.6%, respectively, at 450 μL L⁻¹ CO₂, demonstrating that even at the low light intensities present at the forest floor both early in the morning and late in the afternoon, the seedlings had a capacity to maintain net carbon balance. Winter and Virgo (1998) have reported that elevated CO₂ enhances growth in *P. cordulatum* growing under extremely low light intensities.

The effects of enhanced CO₂ concentrations during the dark on the growth of tropical forest understorey seedlings are not known, although there is evidence from soybean of growth stimulation (Griffin *et al.* 1999).

The rates of CO₂ uptake exhibited by leaves growing near the forest floor at Fortuna, in CO₂ concentrations averaging 421 μL L⁻¹, should be greater than if exposed to 330–360 μL CO₂ L⁻¹, the CO₂ concentrations measured above the canopy at the same site (Zotz *et al.* 1997). Calculation of the level of stimulation at Fortuna is difficult because of the effects of altitude on CO₂ partial pressures. Although the average daily mole fractions of CO₂ (i.e. μmol CO₂ mol⁻¹ air = μL CO₂ L⁻¹ air) during the wet season were similar at the high-altitude Fortuna site and the two lowland sites (Figs 1–3), ambient CO₂ partial pressures were about 18.5% lower at Fortuna. Since ambient CO₂ partial pressure and stomatal conductance dictate the leaf internal CO₂ partial pressure, the source of CO₂ for Rubisco, it would be expected that the rate of photosynthesis in plants would decrease with altitude (Körner *et al.* 1988; Cordell *et al.* 1999; Crayn *et al.* 2001).

One might expect that the capacity to extract water-use efficiency information from variations in tissue δ¹³C signals of understorey plants would be enhanced when the vertical stratification of CO₂ concentration and the associated CO₂-related effects on leaf isotopic content are less pronounced. It remains to be determined whether such information could be successfully extracted solely from isotopic determinations of leaf tissues, leaf litter and local CO₂ concentrations, i.e. without measuring the ¹³C:¹²C ratio of the air as suggested by Sternberg *et al.* (1989).

In conclusion, measurement of CO₂ concentrations at 10 cm from the forest floor at three Panamanian tropical forest sites during wet and dry seasons show regular 24-h fluctuations of CO₂ concentrations, which may be 20% greater than the above-canopy concentration. The absolute

values observed, which were measured using a system designed to reduce the effects of measurement artefacts, were at the lower end of values reported in the literature and were insufficient to saturate photosynthesis with respect to CO₂. Nevertheless, the CO₂ concentrations reported were sufficient to (i) increase the rates of CO₂ uptake (relative to above-canopy CO₂ concentrations) by plants, such as establishing or ecodormant seedlings, subjected to understorey photon flux densities by up to 19% and (ii) reduce the light compensation points by about 12%.

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References

- Allen LH, Lemon E (1976) Carbon dioxide exchange and turbulence in a Costa Rican tropical rain forest. In 'Vegetation and the atmosphere. Vol. 2.' (Ed. JL Monteith) pp. 265–308. (Academic Press: London)
- Björkman O, Ludlow MM, Morrow PA (1972) Photosynthetic performance of two rainforest species in their native habitat and analysis of their gas exchange. *Carnegie Institute Year Book* **71**, 94–102.
- Buchmann N, Guehl J-M, Barigah TS, Ehleringer JR (1997) Interseasonal comparison of CO₂ concentrations, isotopic composition and carbon dynamics in an Amazonian rainforest (French Guiana). *Oecologia* **110**, 120–131.
- Cavelier J (1992) Fine root biomass and soil properties in a semideciduous and a lower montane rain forest in Panama. *Plant and Soil* **142**, 187–201.
- Cordell S, Goldstein G, Meinzer FC, Handley LL (1999) Allocation of nitrogen and carbon in leaves of *Metrosideros polymorpha* regulates carboxylation capacity and δ¹³C along an altitudinal gradient. *Functional Ecology* **13**, 811–818.
- Crayn DM, Smith JAC, Winter K (2001) Carbon-isotope ratios and photosynthetic pathways in Rapataceae. *Plant Biology*, in press.
- Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9**, 121–137.
- Griffin KL, Sims DA, Seeman JR (1999) Altered night-time CO₂ concentration affects growth, physiology and biochemistry of soybean. *Plant, Cell and Environment* **22**, 91–99.
- Holdrige LR, Grenke WC, Hatheway WH, Liang T, Tosi JA jr (1971) 'Forest environments in tropical life zones: a pilot study.' (Pergamon Press: Oxford)
- Holtum JAM, O'Leary MH, Osmond CB (1983) Effect of varying CO₂ partial pressure on photosynthesis and on carbon isotope composition of carbon-4 of malate from the Crassulacean acid metabolism plant *Kalanchoe daigremontiana* Hamet. et Perr. *Plant Physiology* **71**, 602–609.
- Kira T, Yoda K (1989) Vertical stratification in microclimate. In 'Tropical rain forest ecosystems'. (Eds H Leith, MJA Werger) pp. 59–71. (Elsevier: Amsterdam)
- Kirschbaum MUF, Pearcy RW (1988) Gas exchange analysis of the relative importance of stomatal and biochemical factors in photosynthetic induction in *Alocasia macrorrhiza*. *Plant Physiology* **86**, 782–785.
- Körner C, Farquhar GD, Rocksandic Z (1988) A global survey of carbon isotope discrimination in plants from high altitude. *Oecologia* **74**, 623–632.
- Leigh EG, Rand AS, Windsor DM (1982) 'Ecology of a tropical forest: seasonal rhythms and long-term changes.' (Smithsonian Institution Press: Washington)
- Lemon E, Allen LH, Müller I (1970) Carbon dioxide exchange of a tropical forest. Part II. *BioScience* **20**, 1054–1059.
- Lüttge U (1997) 'Physiological ecology of tropical plants.' (Springer Verlag: Berlin)
- Medina E, Minchin P (1980) Stratification of δ¹³C values in Amazonian rain forests. *Oecologia* **45**, 377–378.
- Medina E, Montes G, Cuevas E, Rokzandic Z (1986) Profiles of CO₂ concentration and δ¹³C values in tropical rain forests of the upper Rio Negro Basin, Venezuela. *Journal of Tropical Ecology* **2**, 207–217.
- O'Leary MH (1981) Carbon isotope fractionation in plants. *Phytochemistry* **20**, 553–567.
- Pearcy RW (1990) Sunflecks and photosynthesis in plant canopies. *Annual Review in Plant Physiology and Plant Molecular Biology* **41**, 421–453.
- Sternberg LSL, Mulkey SS, Wright SJ (1989) Ecological interpretation of leaf carbon isotope ratios: influence of respired carbon dioxide. *Ecology* **70**, 1317–1324.
- Windsor DM (1990) 'Climate and moisture variability in a tropical forest: longterm records from Barro Colorado Island, Panama.' (Smithsonian Institution Press: Washington)
- Winter K, Virgo A (1998) Elevated CO₂ enhances growth in the rain forest understorey plant, *Piper cordulatum*, at extremely low light intensities. *Flora* **193**, 323–326.
- Würth MKR, Winter K, Körner CH (1998) *In situ* responses to elevated CO₂ in tropical forest understorey plants. *Functional Ecology* **12**, 886–895.
- Zotz G, Winter K (1994) Photosynthesis and carbon gain of the lichen, *Leptogium azureum*, in a lowland tropical forest. *Flora* **189**, 179–186.
- Zotz G, Büdel B, Meyer A, Zellner H, Lange OL (1997) Water relations and CO₂ exchange of tropical bryophytes in a lower montane forest in Panama. *Botanica Acta* **110**, 9–17

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