

RESEARCH PAPER

Canopy CO₂ exchange of two neotropical tree species exhibiting constitutive and facultative CAM photosynthesis, *Clusia rosea* and *Clusia cylindrica*

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Abstract

Photon flux density (PFD) and water availability, the daily and seasonal factors that vary most in tropical environments, were examined to see how they influenced expression of crassulacean acid metabolism (CAM) in 3-year-old *Clusia* shrubs native to Panama. Instead of the commonly used single-leaf approach, diel CO₂ exchange was measured for whole individual canopies of plants in large soil containers inside a naturally illuminated 8.8 m³ chamber. In well-watered *C. rosea*, a mainly constitutive CAM species, nocturnally fixed CO₂ contributed about 50% to 24 h carbon gain on sunny days but the contribution decreased to zero following overcast days. Nonetheless, CO₂ fixation in the light responded in such a way that 24 h carbon gain was largely conserved across the range of daily PFDs. The response of *C. rosea* to drought was similarly buffered. A facultative component of CAM expression led to reversible increases in nocturnal carbon gain that offset drought-induced reductions of CO₂ fixation in the light. *Clusia cylindrica* was a C₃ plant when well-watered but exhibited CAM when subjected to water stress. The induction of CAM was fully reversible upon rewatering. *C. cylindrica* joins *C. pratensis* as the most unambiguous facultative CAM species reported in the genus *Clusia*.

Key words: *Clusia cylindrica*, *Clusia rosea*, CO₂ exchange, constitutive CAM, crassulacean acid metabolism, facultative CAM, photosynthetic pathway, tree canopy, whole-plant physiology.

Introduction

In contrast to the large number of studies on leaf-level photosynthesis and respiration, relatively few studies have addressed net CO₂ exchange of whole individual plants, particularly trees (Lloyd *et al.*, 1995; Medhurst *et al.*, 2006). Studies of whole plants growing in the field are better suited for predicting how growth responds to environment because they take into account the contributions to carbon gain of leaves of different developmental stages, canopy architecture, and heterotrophic tissues. Outdoor measurements of whole-plant net CO₂ exchange are scarce for species that exhibit CAM photosynthesis (crassulacean acid metabolism) (Kluge, 1977; Neales *et al.*, 1980; Sale and Neales, 1980; Nobel and Bobich, 2002; Rascher *et al.*, 2006), particularly for large species such as CAM trees within the Clusiaceae (Winter *et al.*, 2008).

CAM is a water-conserving mode of photosynthesis (Neales *et al.*, 1968; Winter *et al.*, 2005), the key features of which include the uptake of atmospheric CO₂ and the storage of the fixed carbon as malic acid at night, followed by the decarboxylation of malate and refixation of internally generated CO₂ by C₃ photosynthesis during the day (Holtum *et al.*, 2005). CAM is expressed to varying degrees in species from over 300 genera from 34 families that are mostly found in tropical and subtropical habitats subject to periodic water limitation (Smith and Winter, 1996; Holtum and Winter, 1999; Crayn *et al.*, 2004; Silvera *et al.*, 2005; Holtum *et al.*, 2007; Griffiths *et al.*, 2008).

A fascinating attribute of CAM plants is that the expression of the CAM cycle relative to C₃ photosynthetic fixation of atmospheric CO₂ in the light, may be strongly

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modulated by developmental and environmental factors (Winter and von Willert, 1972; Dodd *et al.*, 2002; Winter and Holtum, 2005, 2007; Cushman *et al.*, 2008; Winter *et al.*, 2008). The metabolic and ecological diversity expressed by CAM plants is epitomized within the approximately 300 shrub and tree species that constitute the neotropical genus *Clusia* (Clusiaceae; Lüttge, 2007). The genus, with its C₃ species, species with weak or pronounced CAM, and species that can switch between the C₃ and CAM options depending upon plant water status, has provided substantial insights into the evolutionary origins and functional significance of the CAM pathway (Zotz and Winter, 1993; Borland *et al.*, 1998; Gehrig *et al.*, 2003; Taybi *et al.*, 2004; Lüttge, 2006).

Essentially all studies of the expression of photosynthetic pathways in *Clusia* have focused on the responses of individual leaves and only recently has focus extended to whole-plant responses in the field (Winter *et al.*, 2008). The scaling-up from leaves to whole canopies is difficult to model for CAM plants because the light conditions and leaf ages that differ within a canopy determine not only the absolute rates of CO₂ fixation by the canopy as a whole but also the balance between light and dark fixation. Building upon recent experiments with *C. pratensis* (Winter *et al.*, 2008) and using a relatively simple flow-through outdoor glass chamber which is periodically sealed for brief monitoring using an open-path CO₂ analyser, whole-plant CO₂ exchange by *C. rosea* and *C. cylindrica* in their native tropical environments are analysed and compared for the first time.

C. rosea, probably the best known *Clusia* species and well-studied at the leaf level, is a largely constitutive CAM species with an extensive distribution from northern South America to Mexico, Florida, and the Caribbean. Highly successful outside its natural range, it is considered an invasive weed in lowland forests of Hawaii (Wagner *et al.*, 1999).

In an effort to understand better the functioning of CAM at the organismal level, the effects of daily PFD, temperature, and soil water status on whole canopy CO₂ exchange were separated by studying naturally illuminated *C. rosea* in the late wet season and during the dry season. A component of the study included the question of the possible significance of stress-induced up-regulation of CAM (i.e. a facultative component of CAM) in mature plants, a feature detected during an earlier laboratory study of seedlings (Winter *et al.*, 2008). Taken together, the results demonstrate that canopy dark and light fixation, despite their different sensitivities to daily PFD and soil water content, respond in a concerted manner that preserves carbon gain.

C. cylindrica is a species for which the limited photosynthetic pathway information available to date suggested that, despite a small capacity for CAM, C₃ photosynthesis is the major pathway of carbon acquisition (Holtum *et al.*, 2004; Winter *et al.*, 2005). Our exploratory study of whole canopy photosynthesis of *C. cylindrica* reveals that this species exhibits fully drought-inducible and reversible CAM.

Materials and methods

Plant material

C. rosea Jacq. and *C. cylindrica* Hammel were established from seeds collected from plants in their natural habitats in Panama. Plants were grown in topsoil from a local orchard in 400 l plastic containers wrapped with reflective insulation (see Fig. 4 in Winter *et al.*, 2008). The containers were placed upon polyethylene pallets (72×107×14 cm; McMaster-Carr, IL, USA) to facilitate their movement using a pallet-truck.

Plants were grown outdoors under well-watered conditions at the Smithsonian Tropical Research Institute Santa Cruz Experimental Research Facility at Gamboa, Republic of Panama (9° 07' N, 79° 42' W). When used in experiments, the plants were between 33 and 40 months old.

Measurements of whole-plant CO₂ exchange

A plant plus its container were placed inside a naturally illuminated chamber constructed of glass panels and an aluminium framework (8.8 m³, approximately 1.9×1.9×2.4 m; Winter *et al.*, 2008). The chamber was aspirated with external air (10.5 m³ min⁻¹; blower model 4C054, Grainger Industry Supply, OH, USA) which was mixed by four fans in the chamber. Temperature was regulated by a split air-conditioning system (model VI124C2H, Innovair, FL, USA) controlled by a data-logger (CR-5000, Campbell Scientific, UT, USA) and a shaded copper-constantan thermocouple located inside the chamber. Unless stated otherwise, the air-conditioner was programmed to turn on when the air temperature within the chamber was above 32 °C and to turn off when the air temperature was below 29 °C.

Whole-plant gas-exchange was quantified at 30 min intervals from the rate at which the CO₂ concentration inside the chamber changed when air-flow into the chamber was blocked for 2 min, an action that converted the chamber from an open to a closed system. The air-conditioner within the chamber was turned off during the measurement interval to reduce interference on CO₂ estimations of temperature fluctuations that occurred when the air-conditioner switched-on and switched-off during its normal operation. During the measurement process the CO₂ concentration inside the chamber was monitored at 5 s intervals by a LI-7500 open-path CO₂ analyser (Li-Cor, NE, USA).

For experiments with *C. rosea*, the soil and roots within the pots were isolated from the chamber by black plastic film. Between CO₂ measurements, when the chamber was in the open flow mode, the head-space between the soil and the plastic seal was continually flushed with air from inside the chamber using an air-pump (20 l min⁻¹). During measurements, when the chamber was operating in the closed mode, no air was pumped through the head-space of the pot. Measurements of plants from which the shoots had been removed demonstrated that when no air was pumped through the head-space above the soil, leakage of soil- and

root-derived respiratory CO₂ from the head-space through a narrow outlet tube into the chamber was not detectable during the 2 min interval when shoot CO₂ flux was measured. For *C. cylindrica*, all measurements of net CO₂ exchange included root and soil respiration.

Measurements of PFD were taken outside the chamber using a quantum sensor (LI-190SA, Li-Cor, NE, USA). PFD inside the chamber was approximately 15% below that outdoors.

The example in Fig. 1 demonstrates, for a *C. cylindrica* in the C₃ mode, that the change in the concentration of CO₂, shown as raw output from the open-path gas analyser, was linear throughout the measurement period (between 10 s and 130 s). Temperature increased by 2.3 °C during the measurement interval in the middle of a sunny day when there was a high constant radiation load of over 2000 μmol photons m⁻² s⁻¹. At night, in the absence of external

radiation, the increase in temperature was small (0.2 °C), probably the result of heat emitted by the equipment inside the chamber.

CO₂ flux in the closed chamber was calculated as

$$F_c = \frac{v\bar{P}(1000 - \bar{W})}{8.314(\bar{T} + 273)} \cdot \frac{dC'}{dt}$$

where, during the measurement interval, F_c is the flux of CO₂ (μmol s⁻¹), v is the volume of the chamber (m³) corrected for the volumes of the pot, plant, and equipment inside the chamber, C' is the dilution-corrected CO₂ mole fraction (μmol mol⁻¹), \bar{P} is the average pressure (kPa), \bar{T} is the average temperature (°C), \bar{W} is the mean water mole fraction (mmol mol⁻¹), and 8.314 is the Universal Gas Constant (Pa m³ mol⁻¹ °K⁻¹).

C' is

$$C' = \frac{C}{1 - \frac{W}{1000}}$$

where W , the water mole fraction (mmol mol⁻¹), and C , the CO₂ mole fraction (μmol mol⁻¹), were calculated from LI-7500 measurements of the mole densities (mmol m⁻³) for CO₂ (D_c) and H₂O (D_w) by

$$C = \frac{D_c 8.314(T + 273)}{P}$$

$$W = \frac{D_w 8.314(T + 273)}{1000P}$$

$\frac{dC'}{dt}$ was calculated from the difference in D_c and D_w , measured 10 s and 130 s after the chamber was sealed.

During normal chamber operation with the air-conditioner switched on (at a nominal operational set-point of 32 °C), chamber air temperature tracked the changes in ambient temperature but was slightly higher than ambient, i.e. the air-conditioner provided coarse rather than fine control (Fig. 2C). On average, the air temperature within the chamber was about 2–3 °C above outside ambient in the light depending upon radiation load. During the dark, when external temperatures were always below the set-point of the air-conditioner, the chamber averaged 2.7 °C above ambient.

Responses to temperature were studied by eliminating temperature control (air-conditioner switched off) or by reducing the set-point of the air-conditioning unit to 22 °C for 2 d (Fig. 2D–I).

Experimental procedures

Five individuals of *C. rosea* and two individuals of *C. cylindrica* were studied between September 2007 and June 2008. During this period, which covers the end of the wet season and the entire dry season in Panama, the natural variation in daily PFD was used to construct light–response relationships of daytime and night-time CO₂ fixation. Canopy CO₂ exchange of individual plants was studied for between 11–46 day–night cycles. During the entire study

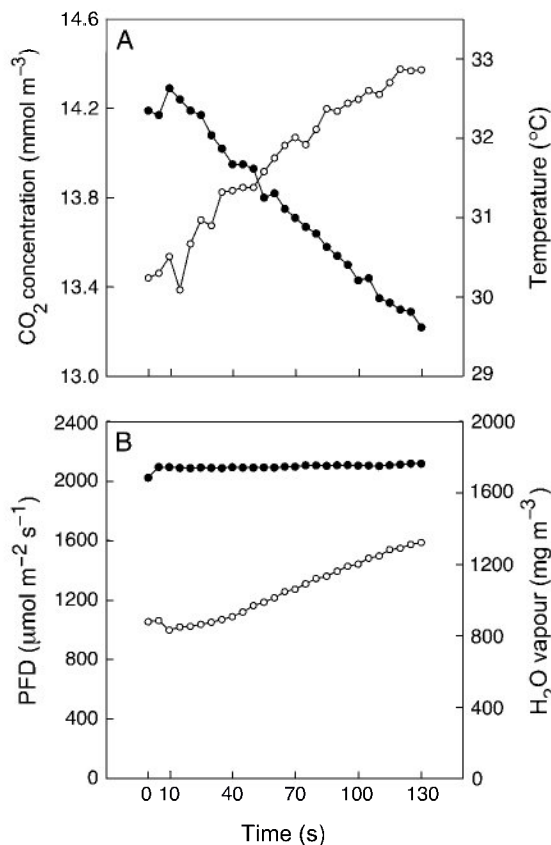


Fig. 1. An example of conditions during a measurement in the light (18 March 2008, beginning at 12.30 h). (A) CO₂ concentration (closed circles) and air temperature (open circles), (B) concentration of water vapour (open circles) and PFD (closed circles). At time 0, the chamber was converted from an open to a closed system and the air-conditioner was switched off. Net CO₂ exchange was estimated from the change in CO₂ concentration between measurements at 10 s and 130 s. The plant in the chamber was a 37-month-old *Clusia cylindrica* (leaf area 15.64 m²; dry masses of leaves, stems plus aerial roots, and below-ground roots were 1.76, 3.47, and 1.92 kg, respectively). Measurements include plant and soil net CO₂ exchange.

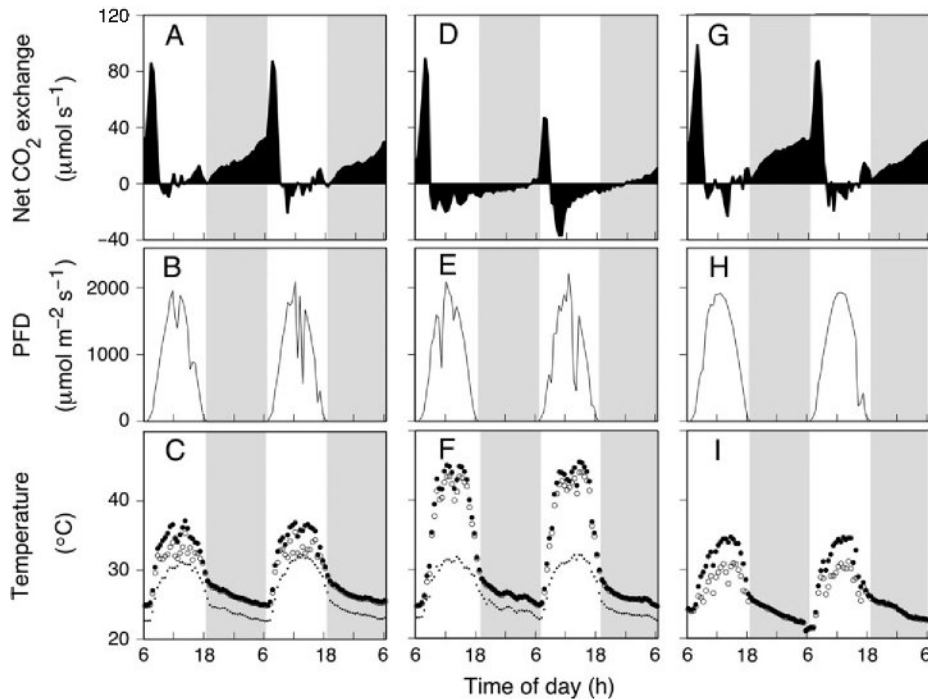


Fig. 2. Net CO₂ exchange (A, D, G), PFD (B, E, H), and temperatures of the ambient air (dots) and air inside the measuring chamber at the beginning (open circles) and end (closed circles) of 2 min measurement intervals (C, F, I) for a *Clusia rosea* (34-months-old, leaf area 11.29 m²; dry masses of leaves, stems plus aerial roots, and below-ground roots were 3.13, 2.69 and 1.72 kg, respectively) in a chamber within which the air-conditioner was at a set-point of 32 °C (A, B, C; 5–7 January, 2008), turned off (D, E, F; 15–17 January, 2008), or at a set-point of 22 °C (G, H, I; 11–13 January 2008). Measurements are for net CO₂ exchange of the shoot only.

over 200 day–night cycles were monitored. Details of the dates, plant sizes, the specific environmental conditions and treatments for each experiment are provided in the figures and figure legends. At the end of experiments, leaves, stems, aerial roots, and subterranean roots were harvested and dried at 70 °C. Before drying, leaf area was determined with a leaf area meter (model 3100, Li-Cor, NE, USA).

Results

Clusia rosea: temperature response

Under the standard chamber conditions described in Fig. 2A–C, a canopy of a well-watered *C. rosea* exhibited features typical of CAM that included dark CO₂ fixation (Phase I of CAM), a pronounced peak of early morning CO₂ uptake (Phase II of CAM), reduced CO₂ exchange in the middle of the day (Phase III of CAM), and afternoon CO₂ uptake (Phase IV of CAM).

In order to test the sensitivity of CO₂ exchange by *C. rosea* to large above-ambient air temperatures, the chamber air-conditioner was disconnected (Fig. 2D–F). Without air-conditioning, midday temperatures were up to 12 °C greater than ambient. Under these extreme daytime temperatures CO₂ efflux during the middle of the day and early afternoon increased, and both afternoon and nocturnal CO₂ uptake were essentially abolished. The morning peak of CO₂ uptake was reduced considerably during the second day of the treatment.

When the air-conditioner was at a set-point of 22 °C, which was 10 °C below the standard set-point, the average daytime chamber temperatures were similar to the outside temperatures, but the rise in temperature in the chamber during the 2 min measuring intervals was greater than when the air-conditioner was at a set-point of 32 °C (Fig. 2C, I). The day–night patterns of net CO₂ exchange were outwardly similar and carbon gain in the light was essentially identical for a plant in the chamber when the air-conditioning set-points were 22 °C or 32 °C, but nocturnal carbon gain was 27% higher when the mean night-time temperature decreased by 2.7 °C from 26.6 °C to 23.9 °C (Fig. 2A, C, G, I).

Clusia rosea: the relationship between carbon gain and PFD in well-watered plants

The extent and pattern of net CO₂ exchange by well-watered *C. rosea* was modulated by PFD. For example, extensive changes in day–night patterns of net CO₂ exchange were observed during and following overcast days. Figure 3 illustrates that for an extremely overcast day (day 3), (i) net CO₂ uptake during the following dark period was abolished, (ii) the maximum rate of CO₂ uptake during the morning peak was less but the duration of the peak was 1.7-times longer than that observed during previous days when PFD was higher, and (iii) CO₂ loss during the middle of the day was reduced. The effect of the overcast day persisted into the following day during which nocturnal CO₂ uptake remained dampened, particularly early in the

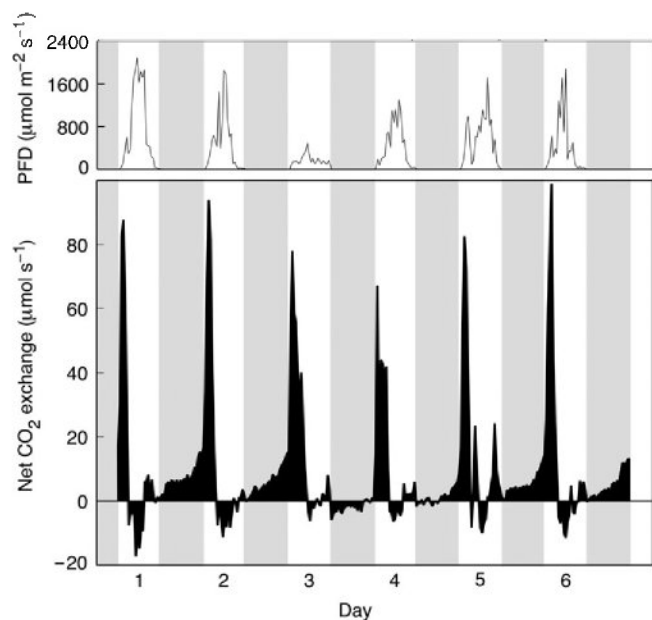


Fig. 3. The effect of an extremely overcast day (day 3) on the day–night pattern of net CO₂ exchange by a well-watered *C. rosea* (see legend of Fig. 2 for details of plant size) in a naturally illuminated chamber between 14 and 20 November, 2007. Shaded sections indicate darkness. Measurements are for net CO₂ exchange of the shoot only.

night. Light and dark CO₂ exchange patterns had reverted to standard by day 5.

The contributions of light and dark CO₂ uptake to net carbon gain were differentially correlated with daily PFD (Fig. 4). At low daily PFD, CO₂ uptake in the light dominated the 24 h carbon gain. The apparent light compensation point for net dark CO₂ uptake, i.e. the daily PFD below which net CO₂ uptake during the following night is not observed, was between 10 mol m⁻² d⁻¹ and 15 mol m⁻² d⁻¹. In 2007, the daily PFD at the study site was below 16 mol m⁻² d⁻¹ for only 37 d. CO₂ uptake in the dark increased linearly with daily PFD such that dark and light uptake contributed equally to the 24 h carbon gain when PFD reached about 30 mol m⁻² d⁻¹. In 2007, the daily PFD at the study site was ≥30 mol m⁻² d⁻¹ for 185 d.

The principal contributor to CO₂ uptake in the light by well-watered *C. rosea* was the morning peak of uptake that generally attained a maximum between 1–1.5 h post-sunrise but lasted 3–4 h (Fig. 3). The carbon gain during the peak was not correlated with net CO₂ uptake during the preceding night and was only weakly correlated with PFD during the peak (Fig. 5). Approximately 75% of the negative relationship observed between carbon gain during the light and daily PFD (Fig. 4) was attributable to an increasing loss of CO₂ during the middle of the day (Phase III) and reduced afternoon CO₂ fixation (Phase IV) (Fig. 6).

Clusia rosea: the effects of water-stress

C. rosea from which water was withheld for up to 25 d still exhibited all four phases of CAM (Fig. 7). In comparison to

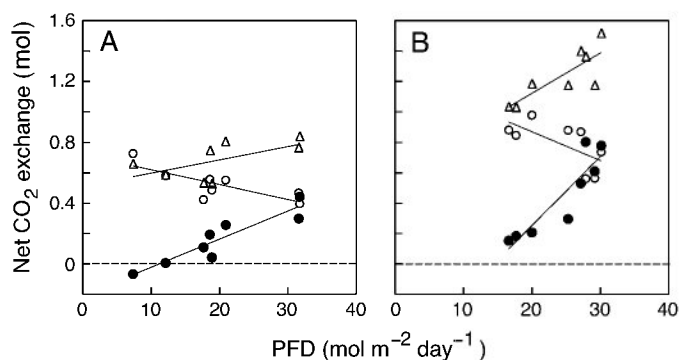


Fig. 4. Relationships between daily PFD and 24 h carbon gain (open triangles), carbon gain in the dark (closed circles) and carbon gain during the light (open circles) for two well-watered *C. rosea* monitored in a naturally illuminated chamber. For the plant in (A), the values are for 10–17 November 2007 (see legend of Fig. 2 for details of plant size). For the plant in (B), the values are for 23–25 April and 7–11 May 2008. The plant in (B) had a leaf area of 14.21 m² and dry masses of leaves, stems plus aerial roots, and below-ground roots of 4.01, 3.95, and 2.82 kg, respectively. Fitted lines are least square linear regressions. Measurements are for net CO₂ exchange of the shoot only.

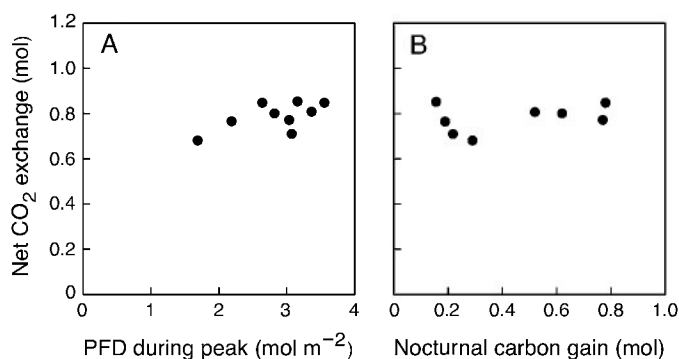


Fig. 5. Relationship between carbon gain during the morning peak of CO₂ uptake and PFD during the peak (A) or nocturnal carbon gain during the preceding dark period (B). The plant in the chamber was a well-watered *C. rosea* (see legend of Fig. 4B for details of plant size; values are for 23–25 April 2008, and 7–12 May 2008). Measurements are for net CO₂ exchange of the shoot only.

plants with an adequate water supply, stressed plants exhibited higher carbon gain during the night and reduced CO₂ uptake during the day (Fig. 8). Upon rewatering, following a decrease in soil water content to about 50% of field capacity, the contribution to 24 h carbon gain of dark fixation fell and the contribution from light fixation rose (Table 1). The 24 h carbon gains of the stressed and rewatered plants were similar.

Clusia cylindrica

Well-watered *C. cylindrica* exhibited characteristics of a C₃ plant (Fig. 9). Net CO₂ exchange was positive during the light and negative during the dark. Following the

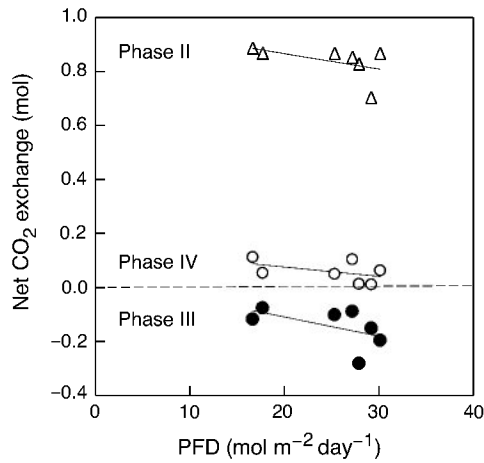


Fig. 6. Relationship between net CO₂ exchange and daily PFD for a well-watered *C. rosea* (see legend of Fig. 4B for details of plant size; values are for 23–25 April, 2008, and 7–10 May 2008) during the morning CO₂ uptake peak (CAM phase II) (open triangles), midday stomatal closure (CAM phase III) (closed circles) and afternoon CO₂ fixation (CAM phase IV) (open circles). Fitted lines are least square linear regressions. Measurements are for net CO₂ exchange of the shoot only.

imposition of water-stress by withholding watering from day 6, daytime CO₂ uptake was progressively reduced until it was confined to a distinct peak of CO₂ uptake in the early morning. By day 11 the diurnal carbon balance of the plant and soil system was negative because of extensive CO₂ loss for most of the day. Day 11 also witnessed the first appearance of net CO₂ uptake during the dark as CAM was induced. By day 15, the nocturnal carbon balance became positive. Following rewatering on day 19, when the soil water content had decreased to 54% of field capacity, the 24 h net CO₂ exchange reverted to the C₃ pattern observed at the onset of the experiment.

The measurements of net CO₂ exchange by *C. cylindrica* included both shoot and soil–root responses because, unlike *C. rosea* which has a defined central stem at the base of the plant, the multiple stems and aerial roots at the base of *C. cylindrica* made it extremely difficult to isolate the shoot from the soil. In order to estimate respiratory CO₂ fluxes from the soil surface, the shoot was removed on day 28 (Fig. 9). Net CO₂ exchange during the subsequent 3 d indicated a net loss of 0.178 mol CO₂ during the light and 0.116 mol CO₂ during the dark. If this loss of respiratory CO₂ from soil and roots is taken into account then, for the plant plus soil shown in Fig. 9, the diurnal carbon gain of the shoot of the rewatered plant 4 d before decapitation would have been 16% greater and the nocturnal CO₂ loss would have been 30% less.

The drought-stress induced shift from C₃ to CAM was confirmed in a second experiment with *C. cylindrica* (leaf area 11.88 m²; dry masses of leaves, stems plus aerial roots, and below-ground roots were 1.16, 2.13, and 1.38 kg, respectively). In this experiment (no figure shown), which was run for 8 d under well-watered conditions and 25 d in the absence of watering, the shoot was removed when water

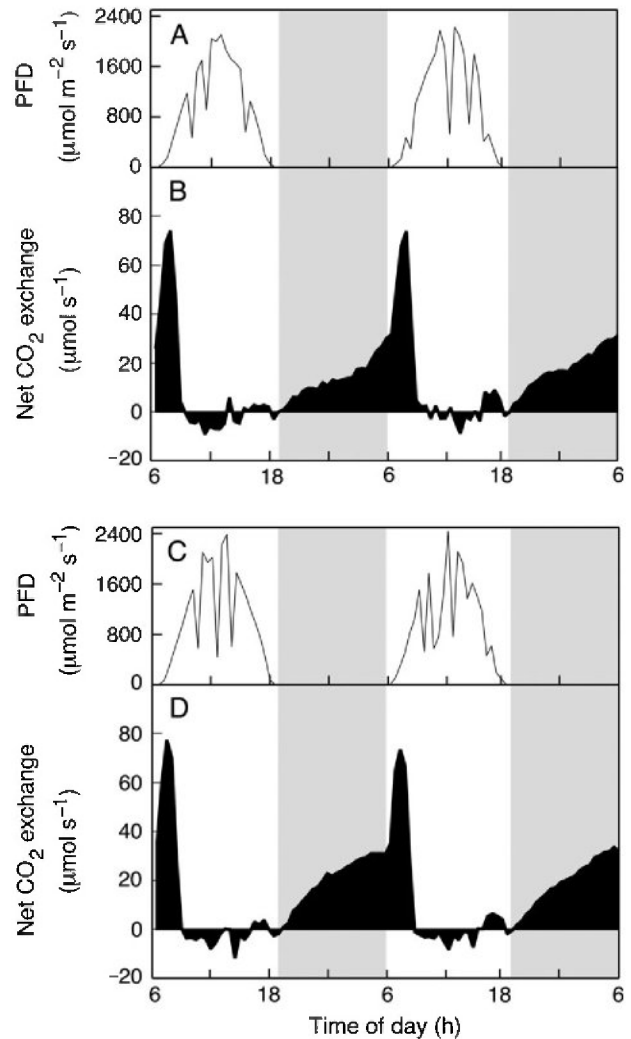


Fig. 7. Effect of reduced water availability on the pattern of day–night CO₂ exchange for a 36-month-old *C. rosea* (leaf area 10.10 m²; leaf dry mass 2.73 kg) 2 d after water was withheld (A, B; 10–12 February 2008) and 18–20 d after water had been withheld (C, D; 26–28 February 2008). Shaded sections indicate darkness. Measurements are for net CO₂ exchange of the shoot only.

stress was most severe and the plant was performing pronounced CAM. Under these conditions, respiratory CO₂ loss from the soil surface, averaged over 3 d, was 0.135 mol CO₂ during the light and 0.128 mol CO₂ during the dark. If the loss of respiratory CO₂ from roots plus soil is taken into account the calculated nocturnal carbon gain of the shoot performing pronounced CAM is 0.182 mol, i.e. 3.4 times higher than the nocturnal carbon balance of the whole plant plus soil (0.054 mol).

Discussion

Clusia rosea

Measurements of entire shoots of *C. rosea* under close to natural illumination in a tropical environment clearly demonstrate that *C. rosea* has a strong constitutive CAM

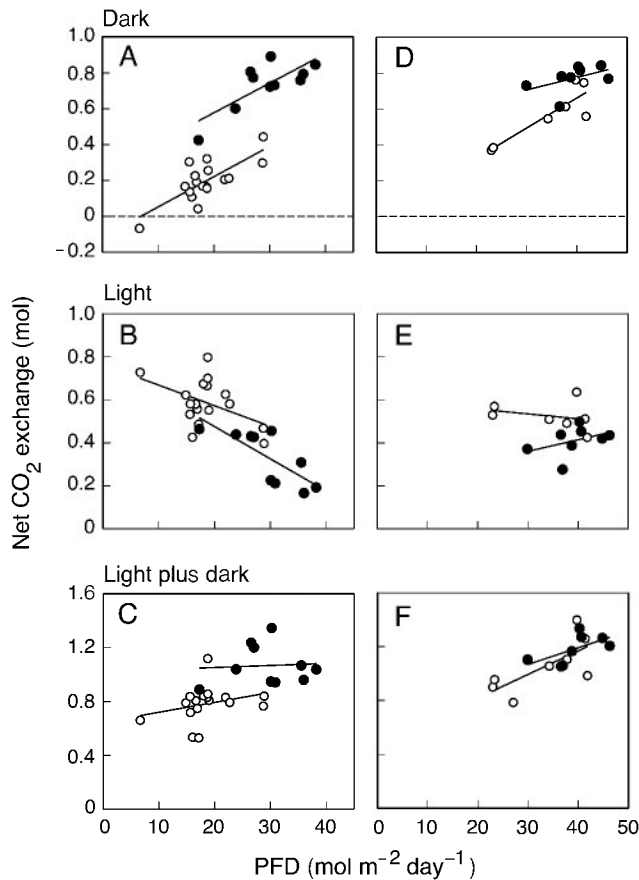


Fig. 8. Effect of reduced water availability on the relationship between PFD and carbon gain in the dark (A, D), carbon gain in the light (B, E), and 24 h carbon gain (C, F) for two *C. rosea* in a naturally illuminated chamber. For the plant in (A), (B), and (C) see legend of Fig. 2 for details of plant size. Water was withheld for 25 d. Open symbols are for the plant when it was well-watered for 7 d and then water was withheld for 10 d (11–27 November 2007), and closed symbols are for the same plant when water had been withheld for between 16 d and 25 d (3–12 December 2007). For the second plant (D, E, and F, see legend of Fig. 7 for details of plant size) water was withheld for 23 d. Open symbols are for the first 8 d after water was withheld (9–16 February 2008), and closed symbols are for the last 8 d after it had been withheld (24 February to 2 March 2008). Fitted lines are least square linear regressions. Measurements are for net CO₂ exchange of the shoot only.

component. In well-watered plants on sunny days approximately 50% of the 24 h carbon gain was obtained during the dark.

The most striking feature of canopy gas-exchange by *C. rosea* was that the highest rates of CO₂ uptake occurred during an early morning CO₂ fixation phase that often lasted several hours. This peak, which immediately followed sunrise, was present essentially under all conditions encountered. The prominence of the peak in terms of its contribution to total carbon gain has not been fully appreciated in single-leaf, spot gas-exchange measurements with this species in the field (Ball *et al.*, 1991; Franco *et al.*, 1994; Haag-Kerwer *et al.*, 1996).

Table 1. Net CO₂ balance during the day and night for a *Clusia rosea* shoot (see legend of Fig. 2 for details of plant size) in a naturally illuminated chamber

Water was withheld for 25 d (18 November 2007 until 12 December 2007) after which the plant was rewatered. Values referring to the water-stress treatment are the mean \pm standard error for the last 6 d. Values after rewatering are the mean \pm standard error for 6 d beginning on day 3 after rewatering. Daily PFD was 33.0 ± 6.5 mol m⁻² (mean \pm SD; $n=12$) during the measurement intervals. The asterisk indicates significant difference at $P \leq 0.05$; n.s. not significantly different (*t* test, two-tailed, equal variance).

Period	Net CO ₂ balance (mol CO ₂ shoot ⁻¹)		
	Water-stressed	Rewatered	
Night	0.791 \pm 0.027	0.581 \pm 0.057	*
Day	0.261 \pm 0.044	0.471 \pm 0.045	*
Day plus night	1.051 \pm 0.063	1.052 \pm 0.076	n.s.

Morning CO₂ fixation peaks (Phase II in the terminology of Osmond, 1978) were originally described for CAM plants exposed to abrupt dark–light changes in the laboratory. This phase of CO₂ uptake is characterized by a transition from CO₂ fixation by PEPC to fixation by Rubisco (Borland and Griffiths, 1996). In *C. rosea* and other *Clusia* species, the contribution of PEPC to CO₂ fixation may be prolonged as indicated by the insensitivity of the peak to PFD (Fig. 6), low levels of instantaneous ¹³C discrimination (Roberts *et al.*, 1996, 1997), and by acidification that continues into the early morning (Franco *et al.*, 1994; Zott and Winter, 1994; Haag-Kerwer *et al.*, 1996; W. Wanek, K. Winter, unpublished data). A convenient way to quantify the relative contributions of PEPC and Rubisco during the peak would be to test the O₂-sensitivity of CO₂ uptake (Osmond and Björkman, 1975; Winter and Tenhunen, 1982), a simple technique which, to our knowledge, has never been applied to CAM tissues in the field, let alone entire shoots. If PEPC activity is significantly prolonged into the light in *C. rosea*, and perhaps in other *Clusia* species (Borland and Griffiths, 1997), then the relationship between CAM plant carbon isotope ratio and contribution of dark and light CO₂ fixation to total carbon gain established by Winter and Holtum (2002) may have to be expanded to consider this *Clusia*–CAM peculiarity. Construction of the original relationship did not include *Clusia* plants performing pronounced CAM and, moreover, used plants grown in the laboratory under 12/12 h light/dark cycles with rectangular rather than sinusoidal transitions in light intensity. It is noteworthy, however, that the carbon isotope ratio of mature outer-canopy leaves of the *C. rosea* plants used in this study was 19.6 ± 0.6 ‰ (SD, $n=6$; Cernusak *et al.*, 2008), a value from which the Winter–Holtum relationship would predict that 40% of the plant carbon was obtained via CO₂ fixation in the dark. This number is close to the 50% observed during sunny days in this study. The deviation from expected is consistent with a proportion of the carbon being fixed during cloudy days when the contribution of dark CO₂ fixation to 24 h carbon gain is reduced (Fig. 4).

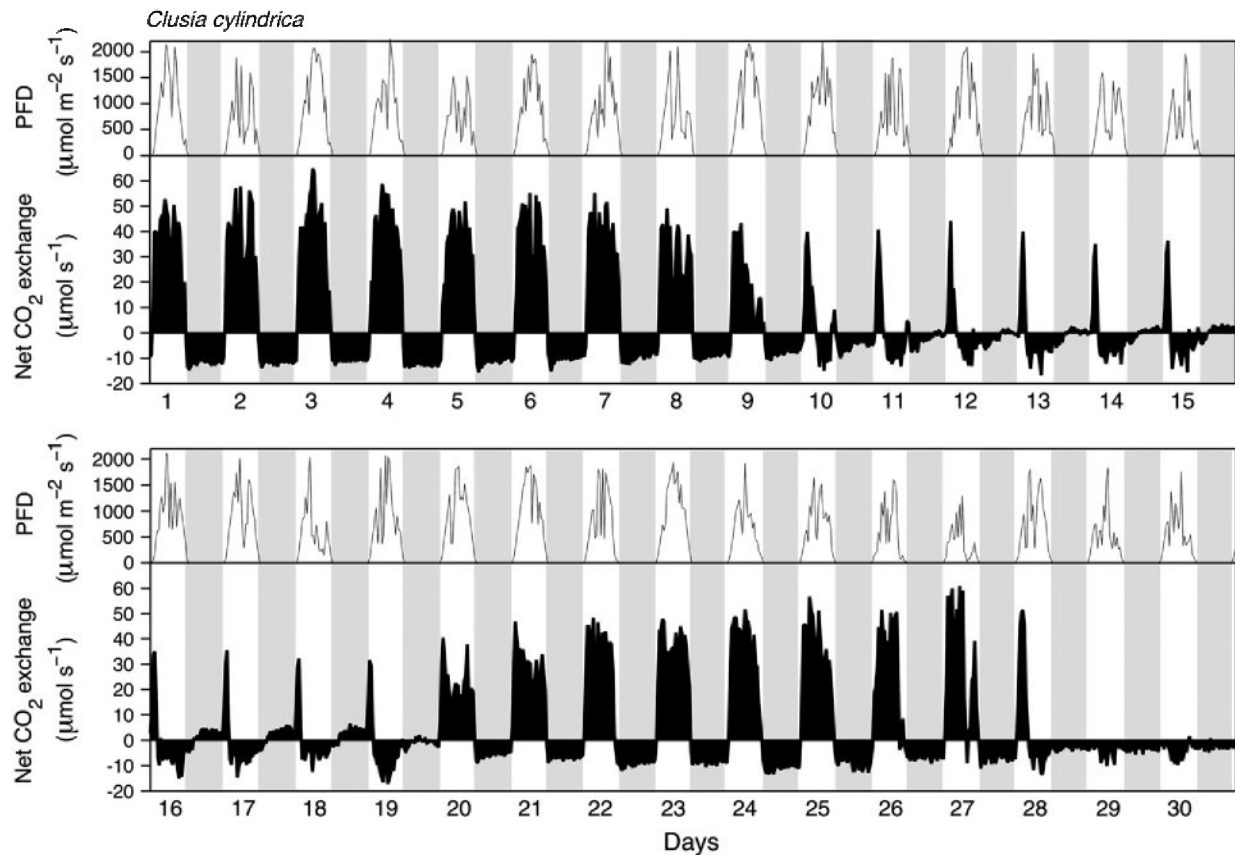


Fig. 9. Net CO₂ exchange for *C. cylindrica* (plant plus the soil in which it grew; see legend of Fig. 1 for details of plant size) in response to drought and rewatering. The plant was monitored for 28 d in a naturally illuminated chamber (day 1 was 15 March 2008). The plant was well-watered except for between days 6 and 18, inclusively, when water was withheld. On day 28 the shoot was removed from the chamber and net CO₂ exchange from the soil and below-ground biomass was monitored. Shaded sections indicate darkness.

Of the environmental parameters that influence photosynthesis, light is the factor that varies most on a day-to-day basis. A major finding of this study is that although CO₂ fixation in the dark was strongly responsive to the day-to-day variations in PFD, the 24 h carbon gain was not. That is, 24 h carbon assimilation remained high across the range of daily PFDs experienced by the plants in the field.

Nocturnal carbon gain had a threshold of daily PFD below which it was abolished. This threshold was crossed following extremely overcast or rainy days. By contrast, on none of the measuring days was daily PFD sufficiently low to curtail severely CO₂ uptake in the light by the canopies of well-watered plants. In fact, the reduction in dark CO₂ fixation was generally accompanied by an increase in light CO₂ fixation.

CAM mainly contributed to total C gain on bright days when the driving force for water loss was high and thus the water-use efficient attributes of CAM became paramount. The water conserving features of CAM are less relevant when daily PFDs are low. Similar responses of light and dark CO₂ fixation to low light during rainy days were observed in the C₃-CAM species, *C. uvitana*, for which it was demonstrated that the principal contributor to these responses was low light, not reduced temperature (Zotz and Winter, 1993). It is noteworthy that net dark CO₂ fixation is

suppressed in these sun-acclimated plants at low light intensities which still support significant CAM in shade-adapted species such as *Aechmea magdalenae* (Skillman and Winter, 1997; Skillman et al., 2005; Skillman, 2008).

Resilience of 24 h CO₂ uptake by *C. rosea* canopies was also exhibited under conditions of drought stress. *C. rosea* from which water was withheld for up to 25 d exhibited higher nocturnal carbon gain in the latter part of the drying cycle than at the onset of the drying cycle which compensated for the drought-related decrease in CO₂ fixation in the light. A stress-induced stimulation of CAM was also indicated by a decrease in nocturnal carbon gain following rewatering (Table 1). The effects of water stress on canopy gas-exchange could be clearly separated from the effects of seasonal and day-to-day changes in PFD (Fig. 8A–C). Although *C. rosea* is now generally accepted as a constitutive CAM species, these observations demonstrate that it has a facultative CAM component, even in older field-grown plants, a conclusion that extends previous laboratory studies with seedlings (Winter et al., 2008). The least negative carbon isotope ratios reported for field-collected *C. rosea* from Panama of -16.7% (a hemi-epiphyte) and -17.0% (a terrestrial plant) (Holtum et al., 2004) are 2.5–3.0 ‰ less negative than those reported for well-watered plants used in the present study (Cernusak et al., 2008), which

would indicate that drought-related up-regulation of CAM does occur in *C. rosea* under natural conditions.

On the basis of the differences in carbon gain at average dark temperatures of 23.9 °C and 26.6 °C (Fig. 2), it is estimated that under the standard measuring conditions nocturnal carbon gain was underestimated by about 27%, although the overall patterns of CO₂ exchange in the light and dark were similar under both temperature regimes. Carbon gain during the light was little affected. Daytime temperatures of up to 12 °C above ambient which led to leaf temperatures of exposed leaves above 50 °C that are unlikely to be experienced *in situ*, resulted in a marked reduction in net carbon gain in the light and in the dark. Interestingly, the *C. rosea* canopy studied here did not exhibit, either at ambient temperatures or immediately following exposure to abnormally high temperatures, the massive midday CO₂ effluxes that have been occasionally reported in leaf studies of field-grown *C. rosea* at ambient temperatures (Haag-Kerwer *et al.*, 1996; Cernusak *et al.*, 2008). An improved temperature tracking and control system, which is under development, should assist in the attempts to deconvolute the effects of day and night temperatures on canopy CO₂ exchange in the light and in the dark.

Clusia cylindrica

This study demonstrates that *C. cylindrica* is a facultative CAM plant, exhibiting drought-induced C₃ to CAM shifts that are totally reversible upon rewatering (Fig. 9). The induction and loss of CAM occurred rapidly in the whole plant, with net CO₂ uptake in the dark present 4 d after water was withheld and absent 2 d after watering was reinitiated. The extent of the C₃ to CAM shifts, their reversibility and the rapidity of the responses to changed soil water status parallel those of *C. pratensis*, the most clear-cut example of facultative CAM hitherto reported in the genus *Clusia* (Winter *et al.*, 2008). Morphology and ITS sequence-based cladograms of Central American *Clusia* indicate that *C. pratensis* and *C. cylindrica* are not particularly closely related (Hammel, 1986; Gehrig *et al.*, 2003). Of the clades into which the species of *Clusia* have been assigned, *C. cylindrica* is in the *C. flava* group where it is a sister-species to *C. rosea*, whereas *C. pratensis* is in the *C. minor* group in which it is a sister-species to *C. minor*, a widely studied species with a strong facultative CAM component (Lüttge, 2008). Both groups contain species with pronounced CAM, species with weak CAM and species in which CAM has not been detected (Holtum *et al.*, 2004).

The ability to switch rapidly between C₃ and water-conserving CAM photosynthesis presumably assists *C. cylindrica* to occupy a diversity of habitats and exhibit a range of life-forms. In Panama, it has been collected from sites from sea-level to 800 m and may grow as a tree, a shrub, a hemi-epiphyte or an epiphyte. Indeed, differences in the expression of CAM related to habitat and season have been reported for *C. cylindrica* in Panama (Holtum *et al.*, 2004). A *C. cylindrica* growing as a tree in an exposed site close to sea-level in Bocas del Toro had a C₃-like δ¹³C

value of −28.3‰ whereas a nearby hemi-epiphytic individual exhibited a less C₃-like −24.9‰, and two epiphytic plants growing at 700 m and 780 m in montane forests had C₃-like δ¹³C values of −30.3‰ and −32.9‰, respectively. A plant growing in a 200 l container at the Smithsonian Tropical Research Institute Santa Cruz Experimental Research Facility at Gamboa did not exhibit day–night fluctuations in titratable acidity when measured in July, during the wet season, but exhibited fluctuations when measured in January, during the early part of the dry season (Holtum *et al.*, 2004).

The combination of the capacity for CAM, inferred from nocturnal acid fluctuations, and C₃-type isotope ratios as observed in a number of Panamanian species of *Clusia* (Holtum *et al.*, 2004) can be explained in principle by the operation of (i) weak CAM all of the time and/or (ii) C₃ photosynthesis for most of the time and strong CAM for short periods. The gas-exchange characteristics of whole canopies of both *C. cylindrica* and *C. pratensis* suggest that the latter phenotype is the preferred option. This hypothesis needs to be confirmed for other species of *Clusia*.

Conclusions

Continuous monitoring over many consecutive days of whole canopy CO₂ exchange of *Clusia* plants grown in large soil containers provided a coherent portrayal of plant–environment interactions that cannot be readily extrapolated from spot measurements of single leaves.

Daily carbon gain in entire shoots of 3-year-old *C. rosea* was buffered across the range of daily PFDs experienced in the field and in response to reduced water availability because changes in CO₂ fixation in either the dark or the light tended to counterbalance each other. As a consequence, carbon gain during overcast days remained high despite a marked reduction in nocturnal CO₂ uptake. Similarly, up-regulation of CAM in response to drought stress assisted in compensating for the reduction in carbon gain in the light. In contrast to the moderate facultative CAM component in the largely CAM constitutive *C. rosea*, the expression of CAM in *C. cylindrica* appeared to be overwhelmingly controlled by drought stress, establishing *C. cylindrica* as a truly facultative CAM species. The expression of CAM, particularly the facultative components, should assist these two tropical *Clusia* species to maintain carbon gain during periods of reduced water availability which, in Panama, may involve an annual dry season of up to 4 months.

Year-round *in situ* studies of whole-plant gas-exchange of *Clusia* species that can successfully grow as terrestrial trees, epiphytes, and hemi-epiphytes, especially when shoot and root responses are measured separately, are expected to greatly advance our understanding of how carbon assimilation and productivity are controlled by daily and seasonal conditions and will aid in quantifying the relevance of CAM for the performance and survival of these plants in current and future climates.

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