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Photosynthetic characteristics of dwarf and fringe *Rhizophora mangle* L. in a Belizean mangrove

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ABSTRACT

Twin Cays (Belize) is a highly oligotrophic mangrove archipelago dominated by *Rhizophora mangle* L. Ocean-fringing trees are 3–7 m tall with a leaf area index (LAI) of 2.3, whereas in the interior, dwarf zone, trees are 1.5 m or less, and the LAI is 0.7. P-fertilization of dwarf trees dramatically increases growth. As a partial explanation of these characteristics, it was hypothesized that differences in stature and growth rates would reflect differences in leaf photosynthetic capacity, as determined by the photochemical and biochemical characteristics at the chloroplast level. Gas exchange and chlorophyll fluorescence were used to compare photosynthesis of dwarf, fringe and fertilized trees. Regardless of zonation or treatment, net CO_2 exchange (A) and photosynthetic electron transport were light saturated at less than 500 μmol photons m^{-2} s^{-1}, and low-light quantum efficiencies were typical for healthy C_3 plants. On the other hand, light-saturated A was linearly related to stomatal conductance (g_s), with seasonal, zonal and treatment differences in photosynthesis corresponding linearly to differences in the mean g_s. Overall, photosynthetic capacity appeared to be co-regulated with stomatal conductance, minimizing the variability of C_i at ambient CO_2 (and hence, C_i/C_a). Based on the results of *in vitro* assays, regulation of photosynthesis in *R. mangle* appeared to be accomplished, at least in part, by regulation of Rubisco activity.

Key-words: oligotrophy; phosphorus fertilization; Rubisco; stomatal conductance.

INTRODUCTION

The forest structure of neotropical, *Rhizophora mangle*-dominated mangroves has long been a topic of interest to ecologists (Lugo & Snedaker 1974). Perhaps their most obvious characteristic is that the mangles show distinct zonation, largely dependent on proximity to the ocean or estuary in which they are found (Lugo & Snedaker 1974; Lin & Sternberg 1992a, b; Feller 1995; Feller *et al*. 1999; Feller *et al*. 2003). Ocean fringing and inland canopies are distinguished, foremost, by their relative heights. For example, at the Twin Cays, Belize site used for this study, fringe trees are typically 4–6 m tall with relatively dense canopies, whereas the trees of the interior, dwarf zone rarely reach 2 m, have few branches, and only three to four pairs of active leaves on any branch at a given time (Feller 1995; Feller *et al*. 1999; Feller *et al*. 2003). These differences have been sustained over many years, that is, they do not reflect a transient or successional stage of the forest. This is clear both from more than 20 years of observations, but also from examination of current stems. In the fringe zone, internode lengths are typically on the order of 10 cm with elongation rates of up to 5 cm per month, whereas in the dwarf zone, internodes are indistinguishable between leaf scars, and shoot elongation occurs only as an anchor to new leaves (Feller *et al*. 2003). Regardless of their location, mangrove growth is nutrient limited and the limiting factor varies with zone (Feller *et al*. 2003). At Twin Cays, in the fringe zone, nitrogen limits growth; twice yearly fertilization with urea, over a period of 6 years, resulted in a statistically significant increase in shoot elongation and leaf and new shoot production. The dwarf zone, by comparison, is severely P-limited. Over the same period, superphosphate fertilization of dwarf trees had dramatically visible effects, including a more than 25-fold increase in shoot elongation, a nearly four-fold increase in leaf production, and a more than 10-fold increase in new shoot production (McKee *et al*. 2002). These effects are visible within 6 months of the first fertilizer application (see Fig. 1). In the absence of fertilization, the effects of nutrient limitation are exacerbated by additional abiotic stresses, including high irradiance, substrate salinity and anoxia, and high air, leaf and water temperatures. For example, the dwarf zone frequently experiences standing water 10–30 cm deep, and water surface temperatures may reach 50° (unpublished results). Except near the equinoxes, the dwarf zone is generally flushed by tides only infrequently, and may become hypersaline due to evaporation.

Inasmuch as *R. mangle* is the dominant species in this ecosystem, and both the fringe and dwarf zones are essentially monocultures, it is clearly adapted to these conditions. Nevertheless, it is reasonable to expect that the conditions impact the metabolism of the trees, in particular photosynthesis, and that growth and zonation reflect this.

Photosynthesis in *R. mangle* has been studied previously in Florida (Lin & Sternberg 1992a, b, c, 1993) using a combination of field and greenhouse studies. Nevertheless,

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Figure 1. *Rhizopora mangle* branches from the dwarf zone of Twin Cays (Belize). (a) Shoot apex of control trees, showing lack of internodal elongation and consequent leaf 'rosette' formation. (b) Branch elongation and leaf proliferation in response to P-fertilization. Fertilizer was applied 6 months prior to the photograph.

these studies left a number of questions open, and the soil-based Florida habitat is sufficiently different from the peat-based systems on Caribbean Cays that we considered further examination warranted. For example, in Florida, the dwarf, or scrub, zone was somewhat elevated with respect to the fringe, such that fluctuations in salinity with season were pronounced; seasonal rainfall patterns led to *lower* salinities in the dwarf than fringe zones for some periods of the year. On Caribbean Cays, such as that at Twin Cays, Belize, the dwarf zone is behind the fringe, but at a lower elevation, and remains flooded except under rare conditions. Here, salinity fluctuations tend to be between seawater and hypersaline levels.

In this study, we have examined photosynthesis in *R. mangle* in the Belizean system, comparing dwarf- and fringe-zone leaves and with particular attention to the effects of P-fertilization of dwarf trees. We hypothesized that differences in stature and growth rates would reflect differences in leaf photosynthetic capacity, as determined by photochemical and biochemical characteristics at the chloroplast level. Our results indicate that seasonal differences in photosynthetic capacity do occur, particularly in the fringe zone. Regardless of season, zonation or fertilization treatment, however, short-term control of photosynthetic capacity is strongly determined by irradiance and stomatal conductance.

MATERIALS AND METHODS

Field sites

Twin Cays is a small archipelago with two major islands located 12 km off the coast and approximately 3 km inside the barrier reef in Belize (Rützler & Feller 1996; Middleton & McKee 2001). It is a highly oligotrophic, peat-based island group, and even in years of record on-shore flooding (e.g. 1999), receives no terrigenous nutrients (Feller *et al.* 1999; Middleton & McKee 2001). The islands have distinct fringe/dwarf zonation, with the red mangrove, *Rhizophora mangle* L. (Rhizophoraceae) dominating in all zones. A transition zone, characterized by a height gradient and the presence of the unrelated mangroves, *Avicennia germinans* (L.) Stearns (Avicenniaceae), and *Laguncularia racemosa* (L.) Gaertn. f. (Combretaceae), separates the fringe and dwarf zones. In the fringe zone, the leaf area index (LAI) is approximately 2.3, and in the dwarf zone, it is 0.7 (Cheeseman, unpublished results). As a result, all dwarf tree leaves were fully exposed to the sky.

Fertilization protocols

For the N fertilization treatment of dwarf trees, we used one of three fertilization sites established in January, 1995, extending from the fringe into the dwarf zone (Feller *et al.* 2003). Trees were fertilized at 6-month intervals by coring below individual trees, inserting the fertilizer, and plugging the hole with peat. The N treatment was 300 g urea (45 : 0 : 0). A control treatment included coring and plugging, but no fertilization. The P-fertilization experiment used in this study was initiated in February, 2002. In this experiment, 150 g of P_2O_5 (0 : 20 : 0) were applied 1 m radially outward from the point at which an aerial root entered the substrate (treatment P2), or as close as possible to an aerial root anchoring point (treatment P3). Gas exchange measurements using these trees were performed approximately 7 months after fertilization. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) assays were performed 11 months after fertilization.

Greenhouse studies

Rhizophora mangle propagules were collected at Twin Cays in June, 2002 and planted individually in 15-cm-diameter pots with SB500 high porosity mix (Sun Gro Horticulture,

Inc., Bellevue, WA, USA). Greenhouse temperatures were 30 °C day and 27 °C night, with supplemental illumination provided by white metal halide lamps to assure a minimum photoperiod of 17 h and a minimum irradiance of 200 μmol m^{-2} s^{-1} at plant height. Supplemental humidification was used to maintain a relative humidity of approximately 50%. One 15 mL spoonful of Instant Ocean (Aquarium Systems Inc., Mentor, OH, USA) sea salts was added to the soil surfaces twice weekly, and plants were watered once or twice daily, as needed, with sufficient water to assure flow through the pots. Reverse osmosis water was used. The trees were fertilized four times weekly with 15 : 5 : 15 (N : P : K) fertilizer (150 p.p.m. N) (Sierra Horticultural Products, Marysville, OH, USA). Once weekly, the plants were fertilized with $MgSO_4$ (1.2 g L^{-1}; Magma Grow, PQ Corporation, Valley Forge, PA, USA) and iron (0.9 g L^{-1} Sequestrene 330Fe; Becker Underwood, Inc., Ames, IA, USA).

Logistics

The measurements reported here were performed during four visits to the field site, in February 2001, in June and September/October 2002, and in February 2003. The initial survey of photosynthetic characteristics under ambient conditions used dwarf and fringe trees along the main channel through the archipelago. Thereafter, our studies – including the P-fertilization and Rubisco activity measurements – were concentrated at one site designated as the Lair Channel (88.10139°W, 16.82944°N). Additional measurements of unfertilized, dwarf trees were made at a site designated as the Dock (88.10453°W, 16.82570°W).

Gas exchange

Net CO_2 assimilation was measured by infrared gas analysis using a LiCor 6400 photosynthesis system (Li-Cor Corp., Lincoln, NE, USA) in one of three configurations. For the initial field survey (Fig. 2), ambient air and normal incident illumination were used. Thereafter, a LiCor model 6400-02B red/blue photodiode array provided illumination and reference cell CO_2 was controlled at 370 μmol mol^{-1} (unless otherwise specified) using the system CO_2 injector. For greenhouse studies (Fig. 3), CO_2 was controlled by injection, and illumination was provided using a red/blue LED array integral to LiCor model 6400-40 leaf chamber fluorometer, with blue light accounting for 10% of the total photon flux. For all individual leaf, spot measurements, photosynthesis and conductance were monitored graphically (the LiCor 'view' mode) to assure that data were taken only after the parameters stabilized and before stomates began to close.

Fluorescence

Fluorescence measurements, for field studies, were made using a Walz PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany) with a model 2030-B leaf-clip holder. Fluorescence and gas exchange measurements were made on the same leaves, sequentially, with gas exchange first. Data are those reported by the PAM-2000 system software, namely the electron transport rate (ETR) was calculated assuming equal partitioning of excitation between the photosystems and a leaf absorptance of 0.85. For greenhouse studies, the LiCor model 6400-40 leaf chamber with integral fluorometer was used without modification to the software or sampling protocols other than adjustment of the flash intensity and duration to achieve maximal fluorescence with the minimal exposure of the leaves to the saturating flashes. Again, ETR was estimated from ϕ_{II} [the instantaneous photochemical efficiency of photosystem II (PSII)] and incident irradiance assuming equal partitioning of excitation between the photosystems and a leaf absorptance of 0.85.

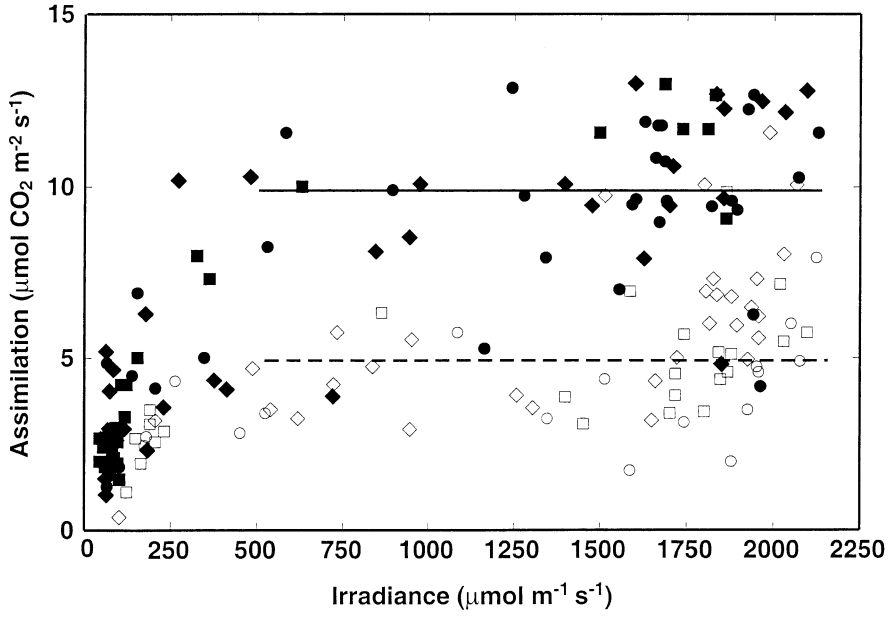

Figure 2. The relationship between net CO_2 assimilation and incident irradiance in leaves of *R. mangle* in the dwarf and fringe zones at Twin Cays. Data were collected in field surveys using natural light, February 2001. Horizontal lines show the mean assimilation rates at ≥500 μmol quanta m^{-2} s^{-1}. Symbols: ●, control fringe; ■, N-fertilized fringe; ◆, P-fertilized fringe; corresponding open symbols are dwarf zone data.

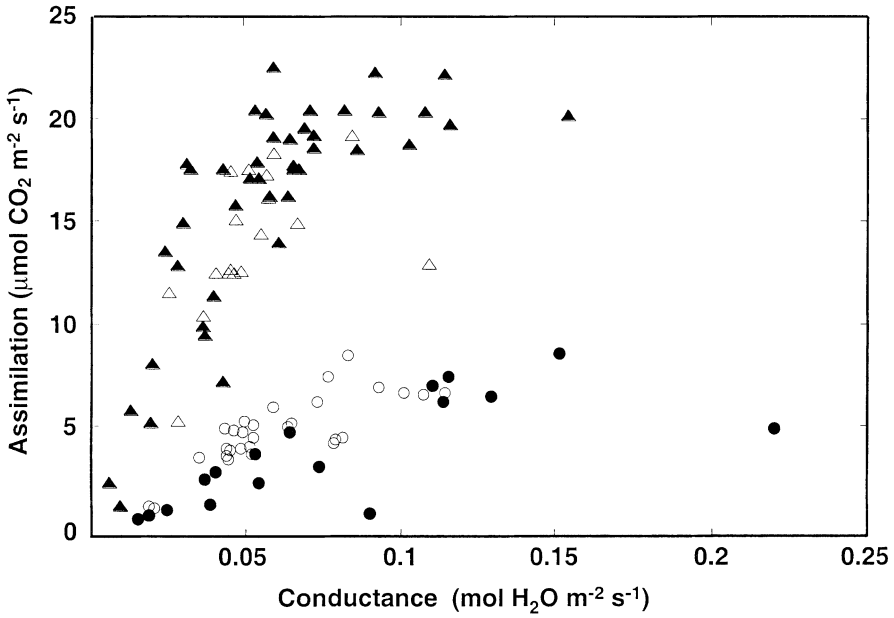

Figure 3. The relationship between stomatal conductance and assimilation in control (unfertilized) *R. mangle* in fringe (closed symbols) and dwarf (open symbols) zones at ambient (370 μmol mol^{-1}, ●, ○) and elevated CO_2 (1400 μmol mol^{-1}, ▲, △). Data were collected in October. CO_2 was elevated around each leaf only during the period in which it was in the IRGA chamber.

Rubisco

For Rubisco analyses, 10 cm^2 (approximately 0.5 g) leaf samples were collected in the field with a cork borer (Cheeseman *et al*. 1997). Leaves were selected as being fully expanded and fully exposed to the sky, such that they would receive direct radiation at some period during the day. The actual irradiance at time of harvest was a function of leaf angle and position with respect to other trees, and solar position (no canopy-internal, shade leaves were sampled). Leaves were used only if their surface was uniformly illuminated (as judged visually) at the time of harvest. Samples were immediately frozen in liquid nitrogen (LN_2) in HistoPrep Omniset tissue cassette boxes (Fisher Scientific, Pittsburgh, PA, USA) and stored in LN_2 until extraction. Prior to sampling, stomatal conductance, transpiration, leaf temperature and incident irradiance were measured with a LiCor 1600 null balance porometer. For analysis, samples were quickly weighed, ground to a fine powder in a mortar under LN_2, and extracted in a buffer containing 100 mM HEPES, 20 mM $MgCl_2$, 5 mM Na_2EDTA, 50 mM KCl, 10% glycerol (v/v), 1% Triton X-100 (v/v), 2% polyvinyl pyrrolidone (PVP)-40 (w/v), and freshly added 2-mercaptoethanol (0.11% v/v) and ascorbate (1 mM), pH 7.6. The inclusion of 1% detergent greatly improved extraction of enzymes; at lower concentrations (0.2%), palisades cells remained intact and clumped, the supernatant was nearly colourless, and activities were low. Six millilitres of buffer were used to extract each sample. When the sample was just thawed and well-mixed, a 200 µL portion was filtered through a G8 glass fibre filter (Fisher Scientific) in a 10 mL disposable syringe. This was diluted 1 : 2 with distilled water and assayed for initial Rubisco activity immediately. 'Fully activated' Rubisco was prepared by addition of $MgCl_2$ and $KHCO_3$ to 10 mM, followed by incubation on ice for at least 10 min. All reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Rubisco activities were determined using the enzyme-linked assay (Sharkey, Savitch & Butz 1991), following NADH oxidation at 340 nm. The assay was modified to increase sensitivity as follows (Archie Portis, personal communication): instead of using the phosphocreatine/phosphocreatine kinase system to regenerate ATP, 4 mM phospho*enol*pyruvate (PEP) and pyruvate kinase (EC 2.7.1.40; 200 U mL^{-1}) were used. The glyceraldehyde-3-phosphate generated by the action of phosphoglycerate kinase (EC 2.7.2.3; 400 U mL^{-1}) and glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12; 200 U mL^{-1}) was coupled to a second NADH oxidation using triose-phosphate isomerase (EC 5.3.1.1; 2300 U mL^{-1}) and glycerophosphate dehydrogenase (EC 1.1.1.8; 200 U mL^{-1}). Thus, four molecules of NADH were oxidized per CO_2 fixed rather than the two in the original method.

Stable isotope analysis

Carbon isotopes in leaf samples were determined at the Geophysical Laboratory, Carnegie Institute of Washington. Approximately 800 μg of dried leaf material was weighed into a tin capsule (Costech Analytical Technologies, Inc., Valencia, CA, USA), and sealed. Samples were introduced via the EA carousel (Wooller, Collins & Fogel 2001) into the autosampler (A2100) of a CE Instruments, NA 2500 series, elemental analyser (ThermoFinnigan Italia S.p.A., Milan, Italy). Isotope ratios of the combustion gases were analysed using continuous-flow, stable isotope ratio mass spectrometry (DeltaplusXL; ThermoFinnigan MAT GmbH, Bremen, Germany). Carbon isotope ratios ($\delta^{13}C$) are expressed relative to Pee Dee Belemnite ($\delta^{13}C$ defined as 0.0‰). Acetanalide (C_8H_9NO) was analysed as a check on the accuracy and precision of isotopic ratios and elemental compositions by the elemental analyser. Precision for $\delta^{13}C$ was ± 0.14 SD (C% ± 2.89 SD).

Statistics

All statistical analyses were performed using StatView 4.51 statistical software (SAS Institute, Cary, NC, USA).

RESULTS

Light and conductance responses of net CO_2 assimilation

We began our studies of photosynthesis in *R. mangle* by surveying net CO_2 exchange at ambient irradiance and atmospheric CO_2 in February, 2001 (Fig. 2). In the fringe zone, the mean A_{max} was 9.9 μmol m^{-2} s^{-1}, whereas in the dwarf zone, it was about 47% less, 5.3 μmol m^{-2} s^{-1}. The mean stomatal conductances for the surveyed leaf populations were also significantly different ($P < 0.0001$): in the fringe zone, g_s was 0.098 ± 0.005 mol m^{-2} s^{-1} compared with 0.068 ± 0.004 mol m^{-2} s^{-1} in the dwarf zone.

Photosynthesis appeared to be light saturated at or below 500 μmol photons m^{-2} s^{-1} in both zones (Fig. 2), despite the fact that these leaves were unshaded. This relatively low saturation irradiance was confirmed, at the leaf population level, by comparison of CO_2 assimilation rates at fixed irradiances of 350, 500, 750 and 1000 μmol m^{-2} s^{-1} using a red/blue LED source rather than ambient light. In individual cases, it was also confirmed by switching between light intensities (e.g. 350–500, or 750–1000 μmol m^{-2} s^{-1}) once a stable chamber CO_2 concentration was reached. No significant differences were found between the rates as a function of irradiance in either growth zone. With individual leaves, there were no changes in assimilation rate in any of the 'switching' tests.

Constant, saturating irradiance measurements were used in subsequent surveys, comparing fringe and dwarf zone photosynthetic capacity. Table 1 summarizes these results. In all cases, measurements were made on a leaf in the second pair from the apex; generally, these are the most active on the trees (data not shown). In both June and October, assimilation rates were lower in the fringe zone than in February; there was no such distinct pattern in the dwarf zone. Only in February were there significant differences between zones for either A_{max} or mean stomatal conductance at light saturation.

Overall, as shown in Table 1, stomatal conductance in *R. mangle* was low. More than half of the values were less than 0.1 mol m^{-2} s^{-1}. At light saturation, CO_2 assimilation was a linear function of stomatal conductance over most of the naturally occurring range of conductances (Fig. 3). Although the highest conductances were found in the fringe zone, overall there were no statistical differences in the means between the zones. The $\delta^{13}C$ values were, however, consistently less negative in the dwarf-zone than fringe-zone leaves, indicating that, averaged over the entire period of leaf development and activity, the conductances of dwarf-zone leaf stomata were somewhat less than those of fringe-zone leaves (Farquhar, O'Leary & Berry 1982).

Both in February and October, higher rates of light-saturated photosynthesis corresponded with lower conductance and C_i, indicating real differences in photosynthetic capacity; if capacities had been the same, we would expect lower conductance to result in lower C_i. Interestingly, the greater capacity was in the fringe zone in February, but in the dwarf zone in October. This difference disappeared under elevated CO_2, although low stomatal conductance still limited photosynthesis in more than half of the samples (Fig. 3). Assimilation was nearly three-fold greater at elevated CO_2 than at ambient, with a maximal rate of >20 μmol m^{-2} s^{-1}. The rate at moderate CO_2 (C_i = 480 μmol mol^{-1}) was equal to that at high CO_2 (Table 2), indicating a limitation by RuBP regeneration.

The quantum efficiency of CO_2 fixation and V_{cmax}

Two important parameters for characterization of photosynthetic capacity are low light quantum efficiency of CO_2 assimilation (ϕ_{CO2}), and V_{cmax}. The latter is usually estimated from the slope of A–C_i curves at low CO_2, with the assumption that Rubisco activity has remained constant during the generation of the curve (Long & Bernacchi 2003). Both of these measurements are problematic in rhizophoracean mangroves, including *R. mangle*, particularly under field conditions, because stomates begin to close within a few

Table 1. Summary of maximal net CO_2 assimilation rates (A_{max}), stomatal conductance, intercellular CO_2 (C_i) and $\delta^{13}C$ in dwarf- and fringe-zone *R. mangle* leaves in February, June and October

		February	June	October
A_{max}	Fringe	9.9 ± 0.3 (50)	6.6 ± 0.4 (64)	3.9 ± 0.6 (17)
	Dwarf	5.3 ± 0.4 (58)***	7.3 ± 0.4 (47)	4.9 ± 0.6 (29)
Conductance	Fringe	0.110 ± 0.006	0.072 ± 0.004	0.078 ± 0.013
	Dwarf	0.068 ± 0.006***	0.080 ± 0.005	0.060 ± 0.004
C_i	Fringe	164 ± 8	194 ± 8	261 ± 8
	Dwarf	185 ± 8	188 ± 10	200 ± 5***
$\delta^{13}C$	Fringe	–26.5 ± 0.4 (6)	–25.6 ± 0.2 (66)	–28.1 ± 0.4 (27)
	Dwarf	–24.7 ± 0.1 (3)	–24.3 ± 0.1 (28)***	–27.1 ± 0.2 (65)**

*** indicates values which were statistically different at $P < 0.001$; ** indicates $P < 0.01$. Values are means ± SEM (n); sample sizes for A_{max}, conductance and C_i were equal. Data are restricted to control (unfertilized) trees in each zone. A_{max} data were restricted to irradiances >500 μmol m^{-2} s^{-1}. Units: A_{max} = μmol CO_2 m^{-2} s^{-1}; conductance = mol H_2O m^{-2} s^{-1}; C_i = μmol mol^{-1}; $\delta^{13}C$ = ‰.

Table 2. Conductance and assimilation as functions of external CO_2 in dwarf- and fringe-zone leaves of *R. mangle*. Data are means ± SEM for control (unfertilized) trees only, in October

	Zone	Ambient CO_2	Moderate CO_2	High CO_2
Conductance (mol m^{-2} s^{-1})	Fringe	0.071 ± 0.009	0.084 ± 0.019	0.056 ± 0.005
	Dwarf	0.060 ± 0.004	0.071 ± 0.004	0.050 ± 0.005
Assimilation (μmol m^{-2} s^{-1})	Fringe	3.9 ± 0.6	13.0 ± 2.7	16.0 ± 0.8
	Dwarf	4.9 ± 0.3	15.5 ± 0.6	14.2 ± 0.8
Intercellular CO_2 (μmol mol^{-1})	Fringe	261 ± 8***	517 ± 15	772 ± 31
	Dwarf	200 ± 5***	440 ± 23	805 ± 31

With the exception of the fringe zone at moderate CO_2 ($n = 5$), the minimal sample size was 18 for any condition. All leaves were exposed to 750 μmol photons m^{-2} s^{-1} which was saturating, even at the highest CO_2. Leaf temperatures were between 34 and 39 °C. Intercellular CO_2 concentrations were significantly different (***$P < 0.0001$) between zones in the ambient CO_2 treatment; otherwise there were no significant differences between dwarf and fringe means within any of the CO_2 treatments. Atmospheric CO_2 was controlled at 360, 830 and 1400 μmol mol^{-1} for the three treatments.

minutes of enclosure in an IRGA chamber (Cheeseman *et al*. 1991, 1997; Cheeseman 1994). The CO_2 assimilation drops concomitantly, becoming unresponsive to irradiance or variations in chamber CO_2 levels, and intercellular CO_2 approaches ambient, giving a 'horse-shoe' appearance to plots of assimilation versus intercellular CO_2. To date, we have been unable to circumvent this problem by manipulating vapour pressure deficit (VPD), CO_2, air flow rates through the leaf chamber, or leaf temperature.

Therefore, we adopted an indirect approach to these measurements. For low-light quantum efficiency, our approach was based on observations that in well-watered plants at low-to-moderate salinity under greenhouse conditions, conductance sometimes remained constant for prolonged periods in the chamber (Cheeseman 1994). Thus, we combined (1) gas exchange and fluorescence measurements in the greenhouse, with (2) the highly linear and tight relationship between electron transport rates (ETR, calculated from fluorescence) and irradiance, which we have repeatedly found at low light under both greenhouse and field conditions (Cheeseman *et al*. 1997). The responses of CO_2 assimilation and ETR under greenhouse conditions are shown in Fig. 4. Both relationships were linear at low irradiance, saturated at about 500 μmol m^{-2} s^{-1}, and were somewhat reduced at intensities above 75% of full sunlight. The ratio of the slopes of the ETR and CO_2 assimilation responses at irradiances below 100 μmol m^{-2} s^{-1} was 5.6, indicating the absorbance of 11.1 photons per CO_2 fixed. A very similar estimate, 5.5 ± 0.45 electrons per CO_2 was obtained using the point-wise ratio of ETR to assimilation corrected for net CO_2 rate in the dark after illumination ($A - A_{dark}$) over the same range of irradiances. For purposes of this analysis, it is the linearity of the low-light responses and their ratio which are the critical features.

Under field conditions, in leaf populations (Fig. 5) and with individual leaves (data not shown), ETR was a linear function of irradiance at low light, and scatter around the line was low (cf. Cheeseman *et al*. 1997, Lüttge *et al*. 2003).

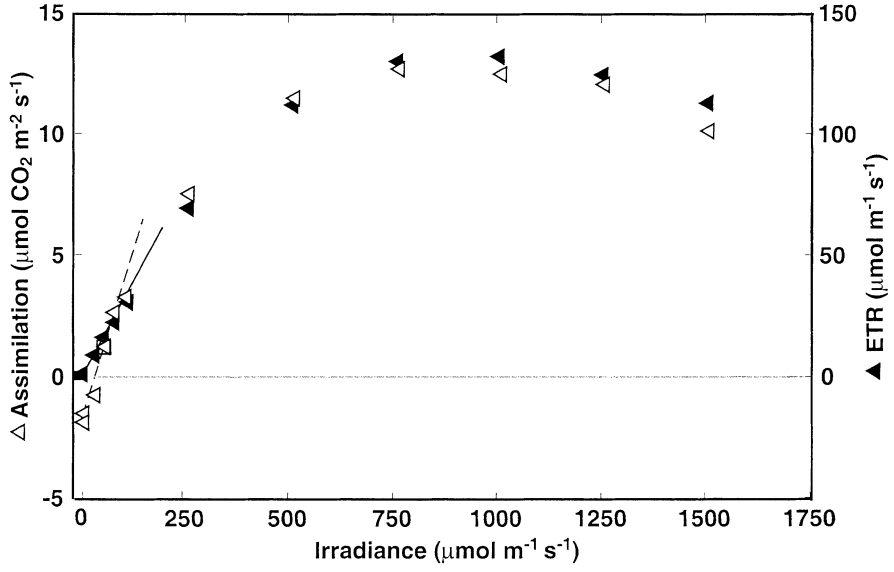

Figure 4. Light responses of net CO_2 assimilation and electron transport rates in a single leaf of a *R. mangle* seedling under greenhouse conditions at ambient O_2 and with CO_2 regulated at 370 μmol mol^{-1}. The study was run from high to low irradiance in a plant which was illuminated at the start. The response study was followed by a 25-min period of dark adaptation during which $F_v : F_m$ reached 0.822. F_s was substantially constant throughout the study, indicating that there was no change in the proportion of the PSII reaction centres which were active. Open symbols, gas exchange; closed symbols, ETR (note differences in *y*-axes). Throughout the study, mean conductance was 0.1 mol m^{-2} s^{-1}, declining only when irradiance went to zero. Lines show regressions fit to data for irradiance <100 μmol m^{-2} s^{-1}. For net assimilation, $\phi_{CO2} = 0.055 \pm 0.005$ (SE of regression coefficient); for ETR, $\phi_{ETR} = 0.304 \pm 0.003$.

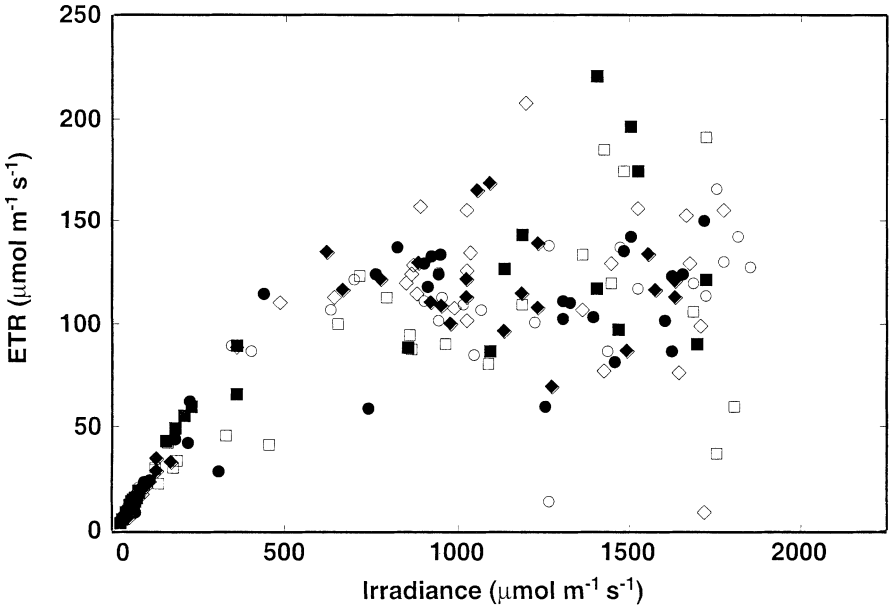

Figure 5. Irradiance response of electron transport rate (ETR) through PSII in *R. mangle* under field conditions. Data are from surveys of dwarf and fringe zone control trees and were collected immediately following the measurements in Fig. 2. The slope of the linear response at irradiances less than 100 μmol photons m^{-2} s^{-1} was 0.268. The mean ETR at light saturation (> 500 μmol m^{-2} s^{-1}) was 122 ± 4 μmol m^{-2} s^{-1}, with no differences between zones or fertilization treatments. Symbols: ●, control fringe; ■, N-fertilized fringe, ◆, P-fertilized fringe; corresponding open symbols are dwarf zone data.

Fringe and dwarf leaves behaved similarly. The slope at irradiances below 100 μmol m^{-2} s^{-1} in the field (Fig. 5) was 88% of that in Fig. 4 (in the greenhouse). If we assume that greenhouse and field leaves had the same absorptance, and the same ratio of ϕ_{ETR} to ϕ_{CO2}, the low light quantum efficiency of CO_2 fixation for field trees at atmospheric CO_2 and O_2 can be estimated as 0.048 on an incident light basis, or approximately 0.057 on an absorbed light basis. Regardless of arguments that these assumptions may engender, this approach gives an estimate of the efficiency of mangrove leaves under field conditions that is not otherwise available, and indicates that they have operating efficiencies under normal atmospheric conditions that are comparable with other C_3 plants from less extreme environments (Singsaas, Ort & Delucia 2001).

An alternative approach to the use of A–C_i curves for estimation of photosynthetic capacity was also needed. As noted above, A–C_i relationships in rhizophoracean mangroves appear horseshoe-shaped, with C_i approaching ambient as conductance and assimilation decrease (Cheeseman 1994; Cheeseman *et al*. 1991), regardless of C_a manipulations. Therefore, we approached the problem by comparing Rubisco activities in leaf extracts directly.

Analysis of samples collected in the dark indicated that *R. mangle* lacks 2-carboxy-arabinitol 1-phosphate (CA1P) as a night-time inhibitor of Rubisco. Both the carbamylation state and total activity of night-time samples were high (Table 3). Total activity during the day was, on average, about 30% higher with a similar carbamylation state.

When sampling for Rubisco activity, each day-time harvest was preceded with measurement of irradiance and conductance. The relationship between irradiance at the time of harvest and Rubisco activity is shown in Fig. 6, based on a subset of the total data, restricted to intensities below 500 μmol m^{-2} s^{-1}, that is below light saturation of net assimilation. For both initial and fully activated assays, the relationships were linear and the regressions were highly significant. There was no correlation between Rubisco activity and irradiance at saturating light intensities. The relationship between Rubisco activity and stomatal con-

Table 3. Comparison of Rubisco activities (in μmol CO_2 m^{-2} s^{-1}) in leaves harvested in the dark and light from the dwarf zone, February 2003

	All samples	Control	N-fertilized	P-fertilized
Dark				
Initial activity	17.0 ± 0.9	16.3 ± 0.9	19.8 ± 1.0	14.9 ± 1.7
Total activity	21.0 ± 1.6	22.5 ± 0.4	24.4 ± 2.0	16.1 ± 2.7
Initial/total	0.825 ± 0.042	0.722 ± 0.016	0.823 ± 0.084	0.931 ± 0.058
Light				
Initial activity	22.1 ± 1.4	25.1 ± 2.9	23.4 ± 2.0	18.8 ± 2.1
Total activity	28.5 ± 1.6	32.8 ± 3.7	29.3 ± 1.8	24.6 ± 2.1
Initial/total	0.768 ± 0.024	0.773 ± 0.032	0.797 ± 0.043	0.743 ± 0.044

Dark samples were collected more than 90 min before sunrise. Light samples were collected throughout the day, from 0830 to 1700 h; Data are means ± SEM for three samples in each treatment in the dark, and 12–16 samples per treatment in the light. All samples were included in the analysis, regardless of irradiance or conductance at the time of harvest.

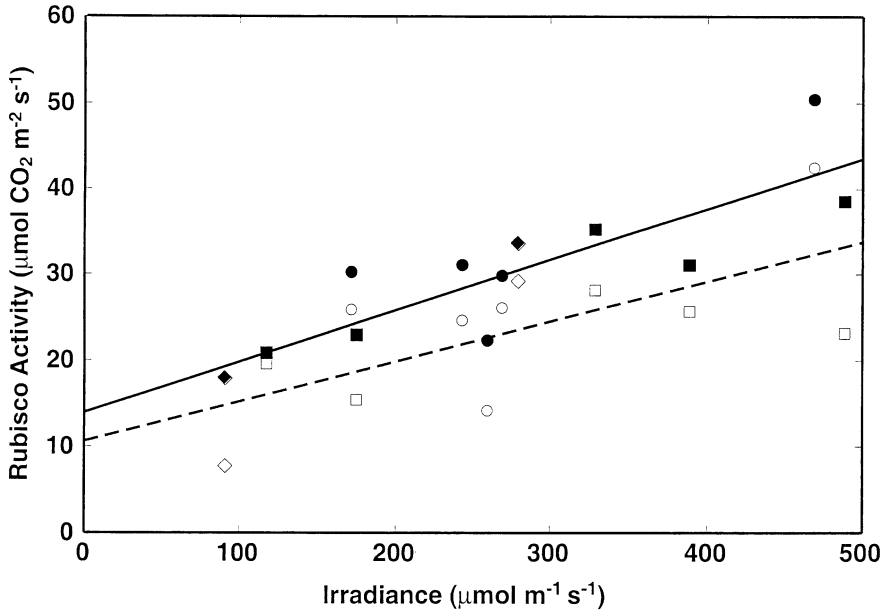

Figure 6. The relationship between Rubisco activity, assayed *in vitro*, and irradiance at time of harvest in *R. mangle* leaves from the Lair Channel site dwarf zone, February 2003. The data set was restricted to samples with irradiance less than 500 μmol m^{-2} s^{-1} at the time of harvest. Symbols: ●, ○, control; ■, □, N-fertilized; ◆, ◇, P-fertilized; open symbols are initial activities; closed are fully activated. Lines were fitted to the combined data, without regards to fertilization treatment. Based on the ratio of the slopes, the mean activation state was 0.78. Regression coefficients are significant at $P < 0.001$. For initial activity, $r^2 = 0.45$; for fully active, $r^2 = 0.73$.

ductance at light saturation (again using a restricted subset of the total data set) is shown in Fig. 7. Again, there were linear and significant relationships for both initial and total activity. (Note that in both of these analyses, samples from all fertilization treatments were combined as there were no differences in activity associated with those treatments – see next section).

Fertilization effects

As noted in the Introduction, the trees at Twin Cays are severely nutrient limited. The effect of fertilization on growth is most dramatic with P addition to trees in the dwarf zone (McKee *et al*. 2002; and Fig. 1). As is characteristic for plants under severe nutrient limitation, this growth stimulation was not accompanied by an increase in tissue P levels; that is, the initial growth stimulation is accompanied by nutrient dilution. For this study, our analysis of fertilization effects was limited to the dwarf zone.

Both N and P fertilization increased the mean, light-saturated assimilation rates and stomatal conductance of the leaves, with P having the greatest effects (Fig. 8). Overall, however, the data comprise a single, linear cluster, indicating a uniform relationship between assimilation and conductance regardless of treatment. With respect to Rubisco activities, although the differences between treatments were statistically marginal ($P > 0.05$ in all cases), N-fertilized and control trees appeared to have higher activities than P-fertilized trees both in the dark and in the light (Table 3). The P-fertilized trees showed more com-

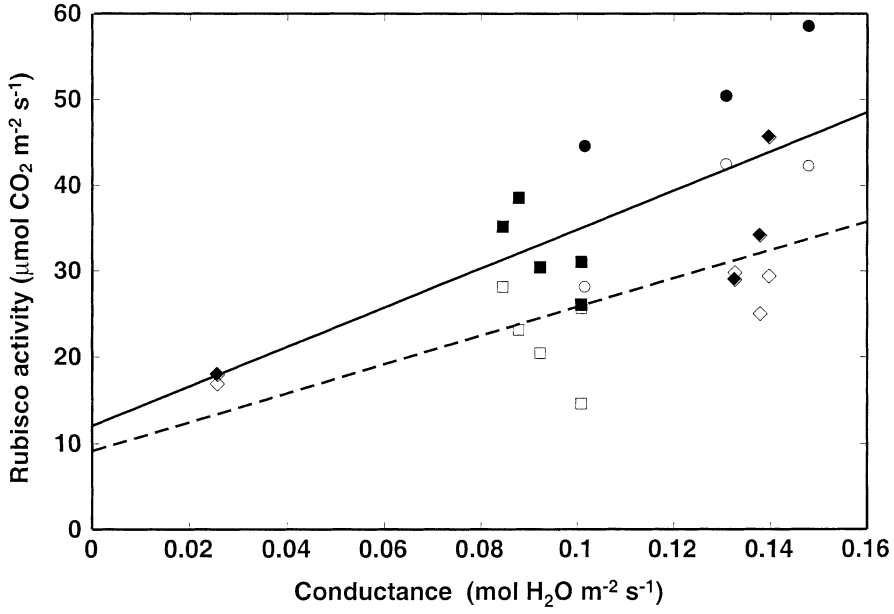

Figure 7. The relationship between Rubisco activity, assayed *in vitro*, and stomatal conductance at time of harvest in *R. mangle* leaves from the Lair Channel site dwarf zone, February 2003. The data set was restricted to samples with irradiance greater than 500 μmol m^{-2} s^{-1} at the time of harvest. Symbols: ●, ○, control; ■, □, N-fertilized; ◆, ◇, P-fertilized; open symbols are initial activities; closed are fully activated. Lines were fit to the combined data, without regards to fertilization treatment. Regression coefficients are significant at $P < 0.001$. For initial activity, $r^2 = 0.44$; for fully active, $r^2 = 0.47$. Based on the ratio of the slopes, the mean activation state was 0.73.

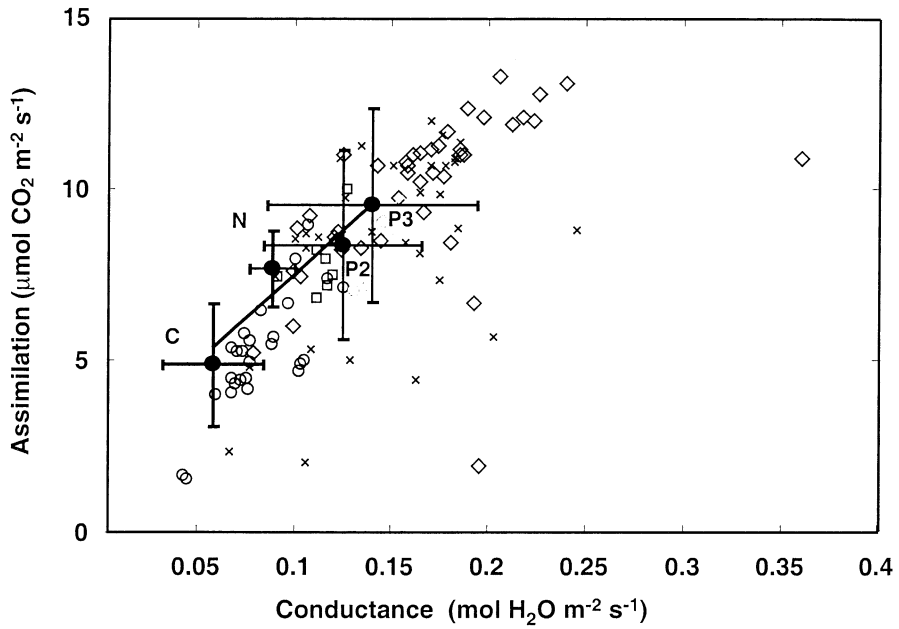

Figure 8. The relationship between net CO_2 assimilation and stomatal conductance in dwarf *R. mangle* at the Lair Channel site. Data were collected at the end of September 2002 at saturating irradiance and ambient CO_2 (approximately 365 μmol mol^{-1}). P2 indicates plants which received P-fertilizer at a single point, 1 m from the anchoring point of an aerial root, P3 indicates plants fertilized immediately adjacent to a single aerial root. Control and N-fertilized trees were from the experimental transects set up in 1995. Large symbols with error bars indicate means ± SD for all samples in each treatment. The regression line was fit to the means, $r^2 = 0.92$. Symbols: ○, control; □, N-fertilized; ×, P-fertilized (P2); ◇, P-fertilized (P3).

plete activation of Rubisco in the dark than N-fertilized or control leaves, with a reverse trend during the day, although, again, the differences were only marginally statistically significant.

DISCUSSION

This research was stimulated by two observations of *R. mangle* growth under the oligotrophic conditions of the Belizean cays. These were, first, that fringe trees are much larger than the interior, dwarf trees, and, second, that P-fertilization dramatically increased the growth rates of dwarf trees. In both cases, these observations should reflect long-term, integrated differences in carbon inputs through photosynthesis. Hence, we hypothesized that the growth rates reflect differences in photosynthetic capacity; for example, that fringe and P-fertilized dwarfs would have higher photosynthesis rates and efficiencies than control dwarfs. Overall, the light responses of photosynthesis and ETR indicated that fringe and dwarf leaves operate with similar photochemical efficiencies and saturation characteristics. Seasonal and zonal differences in A_{max}, C_i, the A–g_s relationship and *in vitro* Rubisco activities, however, imply that photosynthetic capacity is variable, even over short periods, and that P-fertilization of the dwarf trees enhanced it significantly.

Regardless of their location or treatment, photosynthesis by red mangrove leaves at Twin Cays saturated at a low irradiance, between 350 and 500 μmol m^{-2} s^{-1}. Although this seems like a low irradiance for saturation in a tropical tree in a simple, unshaded canopy, these characteristics are similar to those we have previously reported for *R. stylosa*, and for *B. parviflora* (Cheeseman *et al.* 1991, 1997). They are also similar to the characteristics reported for *Bruguiera gymnorrhiza* grown under low light conditions (Takemura *et al.* 2000). Slightly higher light saturation levels have been reported for *Avicennia marina* (Ball & Critchley 1982; Naidoo, Tuffers & von Willert 2002). As in our previous studies with *B. parviflora* (Cheeseman *et al.* 1991), the light saturation level in the *Avicennia* studies did not depend on the irradiance of the growth environment under field conditions, or on the salinity of the field site.

Comparisons of our results with other studies of *R. mangle*, however, reveal a number of differences, particularly with regards to seasonality and zonation. For example, the light-saturated rate of photosynthesis at ambient CO_2, in this study, was only greater in the fringe zone during February (Table 1), a pattern seemingly opposite to that reported by Lin & Sternberg (1992a) for Florida trees. In similarity to their results, however, when photosynthesis rates differed, fringe zone conductances were higher than those in the dwarf zone. These differences most likely reflect differences and seasonal variations in the water sources, air temperatures, elevations and salinity gradients in the two habitats. On the other hand, in their comparison of *Avicennia marina* and *Bruguiera gymnorrhiza*, Naidoo *et al.* (2002) found that CO_2 exchange was largely unaffected by salinity in *B. gymnorrhiza* while being enhanced at higher salinity in *A. marina*. Again, it must be kept in mind that the term 'mangrove' is one of convenience, identifying quite unrelated species found in inter-tidal tropical forests. The species differ markedly, however, in many physiological and structural characteristics, and consequently, comparisons between families must always be made cautiously.

Electron transport rates, as reflected in PSII fluorescence, showed a similar saturation at low irradiance (Figs 4 & 5), as well as high, low irradiance efficiency. ETR was also independent of zonation (Fig. 5), and of fertilization treatment, even when growth was greatly stimulated. The basic light responses were, again, similar to those we have reported for other members of the Rhizophoraceae

(Cheeseman *et al*. 1991, 1997), and to results reported for other mangroves (Naidoo *et al*. 2002), and other tropical trees (Lüttge *et al*. 2003).

Overall, conductance was the most immediately obvious determinant of photosynthesis at light saturation, even at elevated levels of ambient CO_2 (Fig. 3). Fringe, dwarf and fertilized dwarf data fell on the same A–g_s curves (Figs 3 & 8), although their locations on the curves differed. For example, P-fertilized dwarf trees lay further out on the assimilation versus conductance curve than the controls (Fig. 8), and except in the June measurements (Table 1), the mean conductance in the fringe zone was higher than that in the dwarf. Greater overall conductance of fringe zone leaves was also indicated by differences in $\delta^{13}C$ (Table 1). Although the absolute $\delta^{13}C$ numbers and seasonal variations differ between studies, a similar conclusion has been reached in other studies, including those performed with *R. mangle* (Lin & Sternberg 1992b; McKee *et al*. 2002). Interestingly, Medina & Francisco (1997) found that $\delta^{13}C$ was much less variable in *R. mangle*, in the rivers and fringes of Venezuela than it was in *A. germinans* or *Laguncularia racemosa*. The reduced discrimination against the heavier C isotope by dwarf zone leaves indicates a more restricted CO_2 supply, and thus, a lower, average conductance over the lifespan of the leaves.

These results are consistent with results from other mangrove studies. Andrews *et al*. (Andrews, Clough & Muller 1984; Andrews & Muller 1985), for example, found a strictly linear A–g_s relationship in *R. stylosa* at Hinchinbrook Island, Queensland, as did Cheeseman *et al*. (1997) with that species under the more extreme environmental conditions of Western Australia. Lin and Sternberg found a slightly curvilinear relationship for *R. mangle*, but under greenhouse conditions, when salinity was cycled from lower to higher levels (Lin & Sternberg 1993). Unfortunately, their field study did not present the comparable figures (Lin & Sternberg 1992a). In *A. marina*, the same linear relationship between A and g_s was found for both sun- and shade-grown plants (Ball & Critchley 1982). Finally, the most extensive documentation of the tight correlation between photosynthesis and conductance was the study by Clough of 19 species of mangroves (including several in the Rhizophoraceae) in nine estuaries in N. Queensland. Most striking from that study was the fact that in each estuary, all the species' responses could be fitted to a single line, even when species from several different families were included (Clough & Sim 1989).

Based on the equations used to calculate C_i (Farquhar & von Caemmerer 1982), V_{cmax} (photosynthetic capacity) can be estimated from the linear, CO_2-limited portion of A–C_i curves. The amenability of different species of mangroves to this manipulation, however, varies considerably. Ball and Farquhar, for example, used A–C_i analyses to compare responses of *Avicennia marina* and *Aegiceras corniculatum* to long- and short-term variations in salinity (Ball & Farquhar 1984a, b). Both conductance and carboxylation efficiency effects were evidenced in the A–C_i curves, especially in *A. corniculatum*. During transient increases in salinity, the lower portion of the A–C_i curves (i.e. V_{cmax}) was insensitive to salinity, whereas conductance adjusted to maintain the photosynthetic 'operating point' at the break between the CO_2-limited and regeneration-limited conditions.

In the present study, the closure of stomates after relatively short periods in the IRGA chamber precluded the use of A–C_i analysis. On the other hand, the associated decreases in photosynthesis and increases in C_i suggested that V_{cmax} is pronouncedly dynamic in this species, a feature which would, in any case, complicate A–C_i analysis (Long & Bernacchi 2003). Such dynamics were supported by results from the Rubisco studies (Figs 6 and 7). Over the range of subsaturating irradiances, Rubisco activity doubled (Fig. 6), indicating adjustment of photosynthetic capacity to the radiation environment beyond initial light activation. At saturating light, Rubisco activity was linearly related to stomatal conductance (Fig. 7). In practice, this may reflect the down-regulation of Rubisco during stomatal closure, perhaps associated with development of water stress or higher leaf temperatures, rather than up-regulation when stomates open. In any case, the mechanism of regulation is, as yet, unclear. It is possible that our results reflect dynamic control by a 'daytime inhibitor' (Kane *et al*. 1998; Parry *et al*. 1997).

Finally, we return to the original observations and the question of why fringe and P-fertilized dwarf plants grow more rapidly than the unfertilized dwarfs. On the one hand, although our results show similarity of photosynthetic capacity in dwarfs and fringe trees in the absence of fertilization for much of the year, they also show that for part of the year, light-saturated rates and photosynthetic capacities are substantially higher in the fringe zone (Fig. 2). Coupled with the higher LAI there, this would result, over time, in greater integrated carbon gain for the fringe trees.

The relationship between photosynthetic capacity and stomatal conductance may also partially explain the enhanced performance associated with P-fertilization (Fig. 8). Though there have been numerous empirical models derived to include photosynthesis and conductance interactions (Cheeseman & Lexa 1996), that developed by Farquhar & Wong (1984) is most useful here. They proposed that stomatal conductance could be described as proportional to a parameter, T, 'loosely related to the ATP content of the mesophyll chloroplasts.' Conductance and assimilation would be linked, as there would be only one value of conductance at which ATP production and consumption would be balanced. This is particularly attractive here because of the observation that reducing the severe limitation of growth by P was associated with increases in both g_s and A. Thus, we hypothesize that one limitation of photosynthesis before fertilization was the availability of P_i itself for photophosphorylation and the export of products to the cytosol.

A complementary hypothesis, linked to the overall low conductances measured and the increase with fertilization, is that stomatal opening may itself be limited by the ability of the plants to supply leaves with water. In that case, it follows that, in addition to being able to support greater

rates of photosynthetic P utilization, P-fertilized dwarf trees would have to show an increase in root system and stem hydraulic conductivity. Indeed, this seems to be the case; Lovelock, Ball and Feller (unpublished results) have measured stem hydraulic conductivity, finding that P-fertilized trees had twice the conductivity of controls.

In conclusion, the results of these studies have shown remarkable similarities between dwarf- and fringe-zone leaves, and dwarf-zone leaves which have received growth-stimulating fertilization treatments: all plants showed light saturation of photosynthesis and ETR at the same, relatively low irradiance, similar low light quantum efficiencies, and similar light-saturated assimilation rates when stomatal conductances were relatively high. High conductance was not, however, the norm, and for the most part, seasonal, zonal and treatment differences in photosynthesis corresponded to differences in the mean stomatal conductances. Overall, photosynthetic capacity appeared to be co-regulated with stomatal conductance, minimizing the variability of C_i at ambient CO_2. This regulation appeared to be accomplished, at least in part, by regulation of Rubisco activity.

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