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The analysis of photosynthetic performance in leaves under field conditions: A case study using *Bruguiera* mangroves

J.M. Cheeseman[1], B.F. Clough[2], D.R. Carter[1], C.E. Lovelock[2], Ong Jin Eong[3] & R.G. Sim[2]
[1]*Department of Plant Biology, University of Illinois, 505 S. Goodwin Ave., Urbana, IL 61801, USA*; [2]*Australian Institute of Marine Science, PMB 3, Townsville, Qld. 4810, Australia*; [3]*School of Biological Science, Universiti Sains Malaysia, 11800 Penang, Malaysia*

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Abstract

In this report, we analyze the photosynthetic capacity and performance of leaves under field conditions with a case study based on the mangroves *Bruguiera parviflora* and *B. gymnorrhiza*. Using a tower through a closed canopy at a field sight in North Queensland and portable infra-red gas analyzers, a large data set was collected over a period of 11 days early in the growing season. The set was used to analyze the relationship between net photosynthesis (P_{net}) and light, leaf temperature, stomatal conductance and intracellular CO_2 (C_i).

There are three objectives of this report: (1) to determine photosynthetic potential as indicated by the in situ responses of P_{net} to light and stomatal conductance, (2) to determine the extent to which photosynthetic performance may be reduced from that potential, and (3) to explore the basis for and physiological significance of the reduction.

The results indicate that even under harsh tropical conditions, the mangrove photosynthetic machinery is capable of operating efficiently at low light and with maximal rates of more than 15 μmol CO_2 m^{-2} s^{-1}. Though stomata were more often limiting than light, in any single measurement the average reduction of P_{net} from the maximum value predicted by light or conductance responses was 35%. Analysis of single leaf light and CO_2 responses indicated that photosynthetic performance was under direct photosynthetic, or non-stomatal, control at all light and conductance levels. Capacity was adjustable rapidly from a maximum value to essentially nil such that C_i varied inversely with P_{net} from ca. 150 μL L^{-1} at the highest rates of CO_2 exchange to ambient at the lowest.

Introduction

Despite an increasingly sophisticated understanding of photosynthetic processes at the chloroplast, biochemical and molecular levels, and careful characterizations of gas exchange in leaves under controlled conditions, our understanding of photosynthetic capacity and performance under natural conditions is, in general, rudimentary. Clearly, a major factor contributing to the problem is the extreme variability which characterizes measurements under field conditions. It is difficult to decide whether that variability is simply noise, whether it reflects simple differences between otherwise well-behaved leaves, or whether it reflects a more dynamic variability or control of the integrated photosynthetic processes.

In this paper, we will explore those differences with a case study based on two species of mangroves. Mangroves have long been interesting to physiologists and ecologists for the 'characteris-

tics which allow them to function in, and almost exclusively in, a harsh intertidal tropical environment (Tomlinson 1986). Broadly, mangrove growth conditions are characterized by high but variable salinities (Bunt et al. 1982), anaerobic rhizosphere substrates poor in nitrogen and phosphorus (Boto 1982), and prolonged periods of high irradiance at times of supraoptimal leaf temperatures (Björkman et al. 1988). Still, mangrove survival and dominance are apparently not compromised, and in the central, tropical portions of their range, canopies reach heights of more than 40 m (Tomlinson 1986).

In line with the attention given to photosynthesis under environmentally limiting conditions in general, the photosynthetic characteristics of mangroves and their relationship to salinity have also been considered extensively in recent years (e.g., Ball and Farquhar 1984a,b, Ball et al. 1987, Ball 1988, Ball et al. 1988, Björkman et al. 1988). As yet, however, no consistent picture has emerged which characterizes their photosynthesis. This is undoubtedly due in part to the fact that many of the studies have been performed under growth chamber conditions using seedlings collected from the extremes of their natural range. As it reflects the problem of data set variability, however, it also provides a more general opportunity to explore the characterization of field-level photosynthesis.

In this report, therefore, we will consider the photosynthetic characteristics of *Bruguiera* mangroves in a closed forest canopy in North Queensland. Our objectives will be

1) to determine photosynthetic potential as indicated by the in situ responses of net CO_2 fixation (P_{net}) to light and stomatal conductance,
2) to determine the extent to which photosynthetic performance may be reduced from that potential, and
3) to explore the underlying basis for and possible physiological significance of the reduction.

Throughout the report, the interactions of these environmental and physiological controls with biochemical controls on P_{net} will be considered through the relationships between P_{net} and intercellular CO_2 (C_i) under ambient conditions.

Materials and methods

Two mangrove species, *Bruguiera parviflora* (Roxb.) Wright and Arnold and *B. gymnorrhiza* (L.) Lamk., were studied at a field site ca. 1 km from the mouth of the Daintree River (16°16'S, 145°25'E) in North Queensland during a two week period in October 1988. River salinity varied from fresh water at low tide to full strength sea water (27 p.p.t.) at high tide. Though the site was not flooded daily, there was little drainage of the soil between tides. Soil salinity from the surface to 40 cm was constant at about 23 p.p.t.

Access to the canopy was made possible by the on-site construction of a 17 m, multi-platform tower approximately 40 m inland from the river bank. A light profile of the canopy was obtained using a series of photosynthetically active radiation (PAR) sensors which were extended from the tower, and at the conclusion of the study, all leaves which fell within the profile region were harvested and their surface areas measured using a LI-COR leaf area meter. The leaf area index (LAI) in the portion of the canopy most easily accessed from the tower and sampled predominantly in this study was 7.1. Within the top meter (15.5–14.5 m), the LAI reached 4.3, and the mean irradiance (PAR) declined to 13% of the level incident at the top of the canopy. With the exception of widely spaced seedlings of *B. gymnorrhiza*, there were essentially no leaves below 6 m above the ground. At the tower, *B. gynmorrhiza* was present only within the canopy.

Though *Bruguiera* mangroves are not deciduous, they have a period of rapid leaf replacement at the beginning of the spring growing season. Consequently, the leaves measured in this study were all recently but fully expanded, with no appearance of cuticular weathering. Neither species has salt glands.

In situ measurements of gas exchange were made with two LI-COR 6200 portable photosynthesis systems. Two different leaf chambers were used, the 1 L chamber supplied by LI-COR, and a chamber designed and built at AIMS which allowed one-hand operation. There were no discernable measurement differences associated either with the IRGA units or the chambers. In general, the sampling protocol was based on time (20 s samples) rather than on

changes in chamber CO_2 concentration, though the latter was sometimes used when P_{net} was very high.

IRGA data were downloaded at the end of each day; the combined data set was manipulated and summarized using the Statistix 3.0 statistical software package (Analytical Software, St. Paul MN 55113, USA). Both light and conductance response curves were analyzed using the complex hyperbolic response model (e.g., Leverenz 1987):

$$P_{net} = \frac{P_{max} + (\phi i) - \{(P_{max} + (\phi i))^2 - 4\Theta(\phi i\, P_{max})\}^{0.5} - R_d}{2\Theta} \quad (1)$$

For light response studies, i is the light intensity, ϕ is the quantum yield at low light, or the initial slope of the response curve; P_{max} is the maximum rate of net photosynthesis at high light, and R_d is the dark respiration rate. Θ is a measure of the convexity of the curve – a value of 1.0 is a strictly 'Blackman-type' response (Leverenz 1987), linear to P_{max} then breaking and constant; a Θ value of 0 indicates a rectangular hyperbolic response. When used to model conductance responses, $g_{w.v.}$ (the value reported by the LI-COR systems) was substituted for i in Eq. (1), and R_d was replaced by a simple y-intercept.

Fitting of curves to Eq. (1) was performed using the Marquardt algorithm and a BASIC program modified from Schreiner et al. (1985). Manipulation of curves to boundaries of data sets (Fig. 4) was done with a program by the senior author from whom copies are available on request.

In situ light response studies were accomplished using a portable integrating sphere/gas exchange chamber (Ireland et al. 1989) provided by Dr Neil Baker, University of Essex. The sphere was plumbed into the LI-COR system replacing the standard leaf chamber. Illumination was supplied by a 12 V projector lamp powered by a truck battery, and was limited to irradiances less than approximately 200 μmol $m^{-2}\,s^{-1}$. Irradiance was reduced from the maximum using a diaphragm, keeping the lamp voltage constant, and was measured using a PAR sensor fitted into the sphere to create the least non-uniformity (Ireland et al. 1989). At each irradiance, two 20 s samples were taken and the chamber CO_2 level was restored to ambient by opening the auxiliary valve for 40 to 60 s. In this way, C_a was maintained without disturbing the leaf.

Unfortunately, in this configuration and under conditions at the site, it was not possible to measure either leaf temperature or chamber humidity. Consequently, the formula for the calculation of photosynthetic rates was simplified:

$$P_{net} = \frac{PV_T}{8.314(T_a + 273)S}\,\frac{dC}{dt} \quad (2)$$

where P is atmospheric pressure, V_T is the total system volume, T_a is the IRGA temperature (the sphere and the IRGA were located close to each other and shaded), S is the leaf area in the chamber (ca. 6–10 cm^2), and dC/dt is the change in the CO_2 concentration per unit time measured by the IRGA. Though this simplification introduces errors in the estimates of P_{net}, they are likely to be systematic; the results presented from those studies are, therefore, qualitatively comparable if not quantitatively correct.

Absorbance coefficients of leaves were also determined with the integrating sphere. The mean absorbance was 0.75 ± 0.02 throughout the canopy. Because data were not taken on each leaf, the use of a mean value to adjust estimates of quantum yield (ϕ) to an absorbed light basis is simply a scalar adjustment which has not been applied to the reported values.

Measurements of O_2 evolution were performed with a Hansatech LD2 Leaf Disc Unit, and in all cases, discs were sealed in the chamber within 10 min of removal from the tree. The leaf disc chamber was connected to a circulating water bath maintained at 25°C. The O_2 electrode was zeroed by purging the chamber with N_2 and calibrated by injecting 1 ml of air into the sealed chamber using a gas-tight syringe (Delieu and Walker 1983). After measurement of dark respiration, light responses were determined by stepwise increases of light intensities, between which the chamber was given a two min flush with 5% CO_2 in water-saturated air (5 $cm^3\,min^{-1}$). At each light intensity, O_2 evolution was recorded for 5 min.

Results

The basic premise of the research and analysis to be presented here is that in order to characterize photosythetic performance under field conditions, it is necessary, first, to accept that it will exhibit substantial variability, and then to accumulate a data set which incorporates as much of that variability as possible. The complete data set for this study consisted of more than 650 samples collected over a period of 11 days. All times and canopy positions were well represented.

The acceptance of natural conditions also precludes the design and execution of experiments which require precise environmental history or control. Therefore, we developed an a posteriori analytical approach, collecting the data set under prevailing conditions and restricting it later to those elements which met a defined set of environmental criteria.

The results of such analyses were qualitatively similar, in this study, for both mangrove species and for exposed and interior leaves. Therefore, analytical details and figures will be presented for top-of-the-canopy *Bruguiera parviflora* only. The results for interior leaves of both species will be summarized in the tables.

Initial analysis

We began with an analysis of the largest possible, least restricted subset of the data, considering, first, simple correlations which might be expected to have physiological meaning. Thus, restricting the data to *B. parviflora* at the top of the canopy ($n = 359$), there were high correlations between light and leaf temperature (0.58), net photosynthesis (P_{net}) and conductance (0.57), and P_{net} and transpiration (0.68), the latter probably reflecting the relationship of both variables to conductance. A strong negative correlation between conductance and time of day (−0.57) was probably related to the concomitant increase in leaf temperature. Further examination of the relationship between P_{net} and T_{leaf} indicated a broad peak between 29 and 34°C (data not shown). Finally, there was a significant negative correlation (−0.43, $P < 0.001$) between P_{net} and intercellular $CO_2(C_i)$. The cause and significance of this will be discussed further below.

Light, conductance and CO_2 responses

For any data set collected using infrared gas analysis under reasonably stable environmental and physiological conditions, light and conductance responses can be considered the basic descriptors of photosynthetic capacity and performance. Our next objective, therefore, was to use the data collected over the study period to characterize those responses.

The light response of P_{net} was analyzed using the top-of-the-canopy data set after further dividing it into temperature-defined subsets and restricting it to samples with high conductance ($g_{w.v.} > 0.16$ mol m^{-2} s^{-1}). The results shown in Fig. 1 include low (<29°C) and optimal (29–34°C) temperatures; the fitting parameters for exposed and interior leaves are summarized in Table 1. High leaf temperature data (>34°C) were omitted because of the lack of samples at either low light or high conductance.

Figure 1 indicates light saturation at or below 750 μmol m^{-2} s^{-1}, and that value was used in restricting the data set in later analyses; above that, the correlation between P_{net} and light was

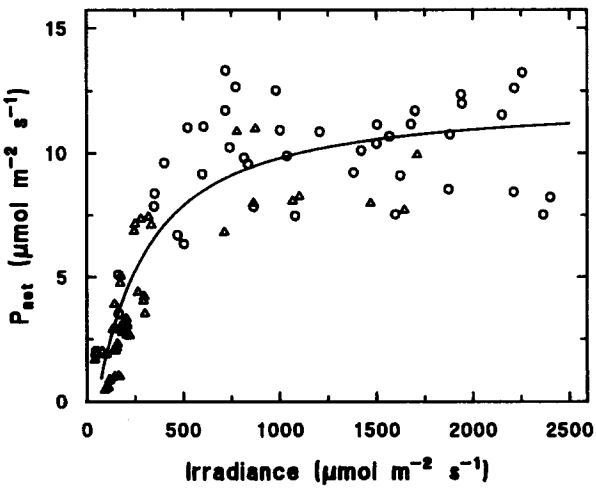

Fig. 1. Photosynthetic light response curve for top-of-the-canopy *B. parviflora* leaves at low (Δ) and optimal (O) temperatures. The curve was placed by non-linear regression using Eq. (1). The fitting parameters are summarized in Table 1.

Table 1. Summary of the fitting parameters for Eq. (1) determined by non-linear regression using light response data for top-of-the-canopy *B. parviflora* (shown in Fig. 1), interior *B. parviflora* and interior *B. gymnorrhiza*. Data were restricted to low and optimal T_{leaf} ranges and to conductance above 0.16 mol $m^{-2} s^{-1}$ for top-of-the-canopy results. No restrictions were imposed for analysis for interior leaves

	B. parviflora		*B. gymnorrhiza*
	Top	Interior	Interior
ϕ	0.050	0.092	0.101
Θ	0.367	0.439	0.186
P_{max}	14.5	7.4	7.9
R_d	2.3	0.4	0.4

not significant ($r = -0.13$, $P > 0.25$). Indeed, O_2 electrode measurements indicated a much lower saturation irradiance (see below). The estimates for quantum yield, ϕ, were within the range expected for C3 plants, though the low values of Θ (convexity) indicate that ϕ decreased sharply with increasing light. As expected, both P_{max} and dark respiration were lower for interior leaves.

The relationship between P_{net} and conductance was modeled in a similar manner using the conductance form of Eq. (1). The results are shown in Fig. 2. In this case, the data were restricted to the optimal temperature range and to saturating light. The analysis indicated a steep initial slope, and though there was pronounced convexity over the entire range of conductances, the relationship was reasonably linear to 0.16 mol $m^{-2} s^{-1}$ ($r = 0.75$, $P < 0.0001$). The availability of data at high conductances and high light made it possible to estimate P_{max} with some confidence; the value, 14.3 μmol $m^{-2} s^{-1}$, was very close to that based on the light response curve. Again, similar results were found for the interior leaves of both species (data not shown).

Finally, the biochemical control of photosynthesis was considered through the relationship of P_{net} to intercellular CO_2 (C_i) under ambient conditions. As noted above, in the data set as a whole, the correlation between these variables was unexpectedly negative. For this analysis, the data were restricted to the optimal temperature range, to saturating light levels, and to a range of conductance over which the photosynthetic response was linear. Figure 3 shows the relationship between P_{net} and C_i for that subset; the correlation was still negative and highly significant ($r = -0.58$, $P < 0.0001$).

This analysis was refined using a multiple linear regression to eliminate the effects of con-

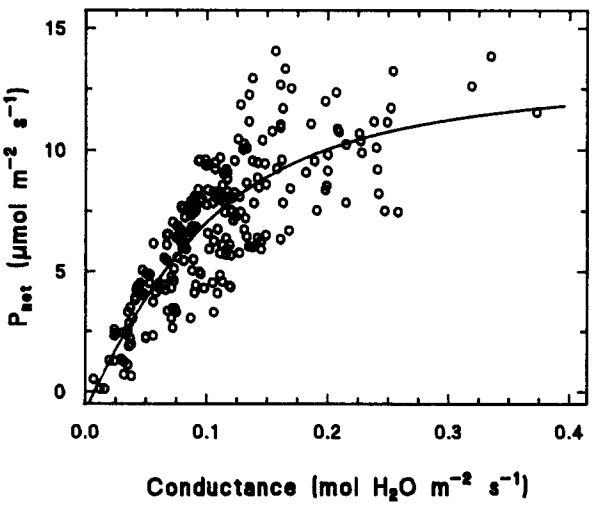

Fig. 2. Photosynthetic responses to leaf conductance for top-of-the-canopy *B. parviflora* leaves at optimal temperatures and saturating irradiances. The curve was placed by non-linear regression using the conductance form of Eq. (1). Parameter values were: initial slope, 109; Θ, 0.686; P_{max}, 14.3; *y*-intercept, −084; $n = 225$.

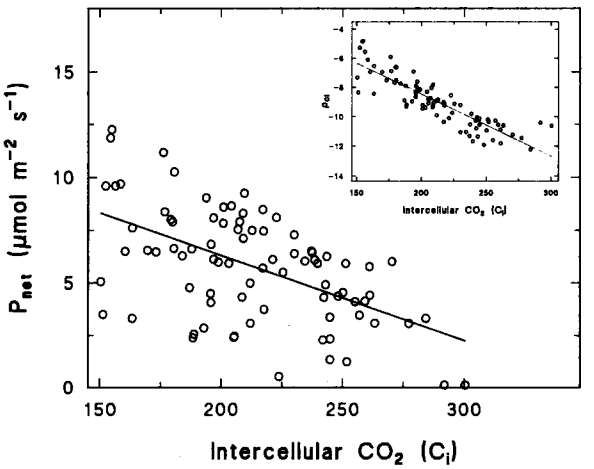

Fig. 3. The relationship between P_{net} and C_i for top-of-the-canopy *B. parviflora* leaves. Data were restricted to the optimal temperature range, saturating light, and water vapor conductances in the linear response range (<0.16 mol $m^{-2} s^{-1}$). *Inset*: partial residual plot visualizing the effect of C_i on P_{net} in a multiple linear model including C_i and conductance. The partial correlation coefficient was −0.82. ρ_{C_i} = residual $+\beta C_i$ where β is the regression coefficient for C_i and the residual is the Predicted P_{net}–Actual P_{net}.

ductance on the results shown in Fig. 3. Conductance and C_i were included as the independent variables. No correlations with vapor pressure, vapor pressure deficit, or time of day suggested their inclusion here or at any other point in the analyses, and the effects of irradiance were removed by the prior restriction of the data to saturating light conditions. The partial correlation coefficient between P_{net} and C_i indicates the strength of their relationship without the complication of the conductance response, and it was even less ambiguously negative (−0.87). This more complete representation of the P_{net} vs. C_i relationship was visualized using a partial residual plot (see Larson and McCleary 1972, Cheeseman and Wickens 1986) as shown in the inset in Fig. 3.

Despite the continued discrepancy between these results and the biochemical model (von Caemmerer and Farquhar 1981), we were reluctant either to fault the latter or to discard the field data as contradictory and artefactual. Using the same data sets, therefore, we performed an alternative analysis.

Boundary and deviation analysis

With sufficient numbers of samples, the relationship of P_{net} to light and conductance should indicate the maximal photosynthetic potential within a canopy zone regardless of other environmental limitations. Thus, while the use of statistical curve fits in Figs. 1 and 2 presupposes an average performance about which variation occurs, an alternate interpretation would be that it is the upper boundary of the data sets which is of primary importance. Because all reductions (deviations) from that level must then represent secondary limiting effects, we could use such an analysis to address our first two objectives simultaneously.

Figure 4 illustrates the results of such boundary and deviation analyses using the data for top-of-the-canopy leaves of *B. parviflora*. The response surfaces were, for the most part, clear, and the associated parameters for the three canopy divisions are summarized in Table 2. For light responses, both ϕ and P_{max} were relatively unaffected by the method of analysis. The upward shift (negative R_d) in the boundary analysis

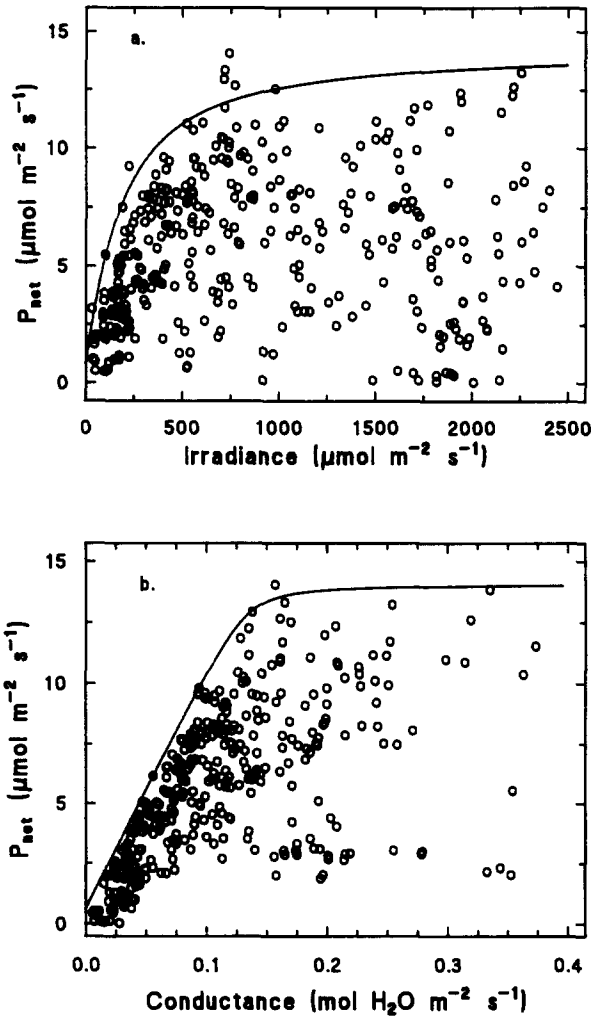

Fig. 4. Light and conductance responses of P_{net} in top-of-the-canopy *B. parviflora*. The curves are boundary lines of the form given by Eq. (1) with the parameters adjusted visually (see Materials and methods). For boundary and deviation analysis, the deviation is the difference between the actual and predicted values relative to the predicted value. Actual values above the boundary were omitted from the deviation analysis. The parameters for all canopy data groups are summarized in Table 2.

suggests a possible low-light sensitivity of R_d. The primary difference otherwise was in the apparent degree of convexity. For conductance responses, the fact that the curves passed through (or very near) the origin indicated that there was no effect of cuticular conductance and implied a constant C_i over the linear phase of the conductance response (Bethenod et al. 1988, Bethenod and Tardieu 1990).

Table 2. Boundary curve parameters for the light and conductance responses illustrated in Fig. 4, and for interior leaves of both species. Note: negative values of R_d in the light responses indicate a y-intercept greater than zero

	B. parviflora		*B. gymnorrhiza*
	Top	Interior	Interior
Light response			
ϕ	0.058	0.088	0.058
Θ	0.415	0.960	0.940
P_{max}	13.5	7.5	9.5
R_d	−0.8	−0.6	−1.6
Conductance response			
Initial slope	100	105	80
Θ	0.99	1.0	0.99
P_{max}	13.5	7.3	9.5
y-intercept	0.6	0	0

The curves in Fig. 4 represent maximal values of P_{net} from which deviations can be analyzed. For each sample, a predicted value was taken as the lower of the values from the two boundary curves, and a deviation was calculated as the difference between that prediction and the measured P_{net}. To allow comparisons across the full range of conditions, regardless of the limitation of P_{net} by light or conductance, these deviations were expressed relative to the predicted values. For all three canopy data sets, the mean relative deviations were 0.35 ± 0.25, i.e., on average, performance was reduced from potential by ~35%.

Examination of the correlation between the deviations and other parameters and variables again pointed to C_i, and consistent with the results of Fig. 3, the lower the C_i level, or the greater its deviation from ambient, the more closely P_{net} corresponded to the predicted value. Or alternately, the higher the rate of photosynthesis at any combination of light and conductance, the lower was C_i. Similar results were found for interior leaves of both species (data not shown).

Intuitively, these results suggest that the underlying basis for the reduction of photosynthetic performance was variability of photosynthetic capacity; at any given light and conductance, a higher capacity should result in a greater reduction of C_i from the ambient level. Variations in capacity might, in turn, reflect photoinhibition or down regulation of photosynthetic performance by other means. Therefore, to address our third objective – to explore the underlying basis for the reduction of performance from potential – we examined (1) the light responses of leaf disks in an O_2 electrode system, and (2) the light and CO_2 responses of single leaves under canopy conditions.

O_2 electrode studies

Discs were taken from *B. parviflora* leaves throughout the canopy and throughout the day over a period of four clear, hot days. The light responses are shown in Fig. 5 for the top of the canopy. For interior leaves, the maximum O_2 evolution and dark respiration rates were reduced approximately 30%, but the light saturation points and photon yields were relatively unaffected. The time of leaf disc harvest through the day had no effect on either efficiency or maximum photosynthetic performance in either exposed or interior leaves, suggesting that the deviations from P_{max} discussed above were not indicative of photoinhibition in exposed leaves.

The possibility of photoinhibition was explored further using leaves which had been tagged for repeated IRGA measurements in situ and whose CO_2 exchange behavior had been followed over a period of several days. At the top of the canopy, there was a tendency, particularly apparent through repeated measurement, toward very low photosynthesis and conductance after mid-morning. Figure 6 illustrates the light response curve of O_2 evolution for one of those leaves after several consecutive hot, cloudless

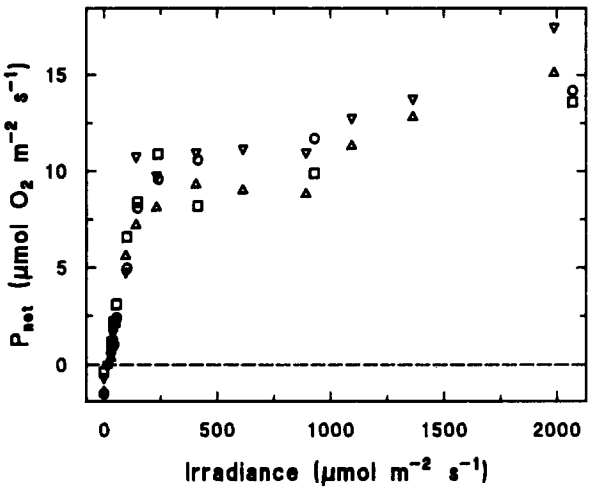

Fig. 5. Photosynthetic light responses of leaf disks taken from the top of the canopy zone and measured at 5% CO_2 with the polarographic O_2 electrode. The combined data are shown for four disks taken throughout the day at early morning (□), mid-morning (○), early afternoon (◇), and late afternoon (△). Similar low light responses and low saturation irradiances were found for interior leaves.

days. On the day of this analysis, P_{net} and stomatal conductance were low and peaked before 0800 h at 3.2 μmol CO_2 m^{-2} s^{-1} and 0.038 mol H_2O m^{-2} s^{-1}, respectively. At 1100 h, the leaf temperature had reached 40°C and incident radiation was above 2500 μmol m^{-2} s^{-1}. From then until harvesting (1320 h), P_{net} and stomatal conductance were essentially zero.

Even under these conditions, Fig. 6 indicates that normal photosynthetic capacity was retained. We concluded, therefore, that photoinhibition was not the major cause of the deviation of photosynthetic performance from maximal levels. If low photosynthesis reflected low capacity in situ, it recovered within the few minutes required to harvest the leaf and prepare it for the O_2 electrode measurements.

Thus, we explored the alternative hypothesis, that low performance reflected down-regulation of photosynthetic capacity, with in situ analyses of light and CO_2 responses of single leaves under canopy conditions.

Single leaf light and CO_2 responses

The photosynthetic light responses of individual leaves were studied in the 0 to 200 μmol m^{-2} s^{-1} light range using the field portable integrating sphere in conjunction with the IRGA. A sample result is shown in Fig. 7. This study began at 1100 h and required 65 min for completion. The initial photosynthetic rate was low and 'light

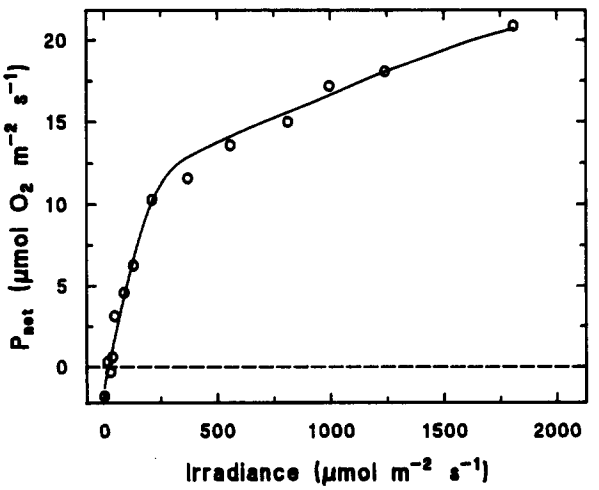

Fig. 6. The photosynthetic (O_2 evolution) response of a *B. parviflora* leaf disk taken from the top of the canopy after prolonged exposure to high T_{leaf} and irradiance concomitant with low P_{net} and stomatal conductance. The apparent incident quantum yield was 0.072; $P_{max} > 20$ μmol O_2 m^{-2} s^{-1}.

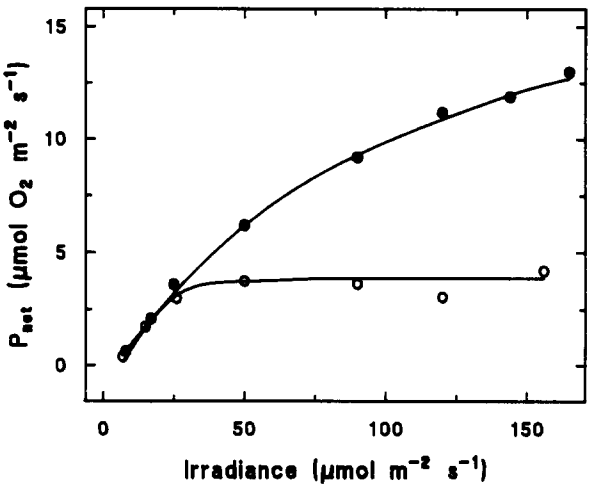

Fig. 7. The photosynthetic light response of an attached *B. parviflora* leaf determined in situ with the IRGA/integrating sphere configuration. P_{net} was calculated using the modified form of the LI-COR system equation (Eq. (2)). Open circles represent the 'down' series; closed circles are the increasing light ('up') series. The down series was performed first. Duplicate samples were taken at each irradiance.

saturated' at 25 μmol $m^{-2} s^{-1}$ based on the decreasing light series. When the irradiance was increased again, however, an increase in photosynthetic capacity was indicated with no change in the initial quantum yield. Though it is not possible to discount stomatal effects completely in the absence of leaf temperature and conductance data, a major nonstomatal contribution in Fig. 7 was suggested (1) by the lack of recovery during the 'down' phase and (2) by the difference in rates by 25 μmol $m^{-2} s^{-1}$ in the 'up' phase. To account for these results, substantial stomatal opening would have had to occur in less than 15 min (the period between the 25 μmol $m^{-2} s^{-1}$ points) and at very low light levels.

Finally, using the normal LI-COR configuration, CO_2 responses of single leaves were studied using repeated measurements in a closed chamber. A typical result is shown in Fig. 8. Stomatal conductance was initially high and both P_{net} and conductance decreased as C_i dropped. When conductance fell to approximately 0.1 mol H_2O $m^{-2} s^{-1}$, P_{net} continued to fall while C_i increased toward ambient. This response cannot be predicted using a steady state biochemical model (Cheeseman, unpublished results), and again suggests a rapid adjustment of photosynthetic capacity.

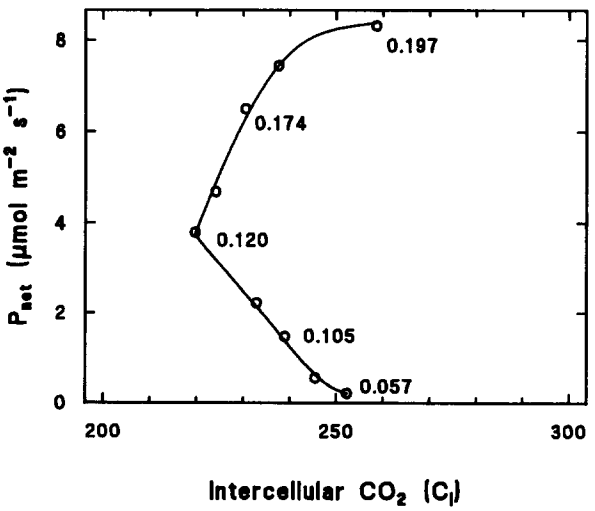

Fig. 8. The relationship between P_{net} and C_i determined for a single *B. parviflora* leaf by repeated measurements in a closed chamber. Numbers indicate the stomatal conductance associated with the points. P_{net} and conductance decreased throughout the series. T_{leaf} and irradiance were 35°C and ~1700 μmol $m^{-2} s^{-1}$, respectively.

Discussion

In this study, we have analyzed the photosynthetic gas exchange characteristics of leaves in a mature, closed mangrove canopy at the beginning of the period of most rapid growth. The data were collected mainly as rapid, spot measurements to give the best possible estimate of in situ performance.

Our first objective was to determine the photosynthetic potential of leaves as indicated by their responses to light and stomatal conductance. Regardless of the method of data analysis or the canopy zone, the estimates of P_{max} and R_d based on light responses (Tables 1 and 2) were consistent with expected differences between sun and shade leaves, and were reasonable based on literature values for C3 trees and on O_2 electrode studies using excised leaves (Fig. 7). The estimates of quantum yield at limiting light were indicative of healthy photosynthetic systems. Light compensation points were low, as expected for slow growing, stress tolerant plants.

On the other hand, the light saturation points indicated by both the CO_2 exchange data and the O_2 electrode studies were unexpectedly low. From the standpoint of photosynthetic performance, this limitation in capacity was probably not significant. Analysis of the photosynthetic conductance responses gave similar estimates of P_{max}, and indicated that in the majority of the measurements, P_{net} was conductance limited: in 80% of all the measurements at the top of the canopy, the data fell in the linear region of the response curve, and in the boundary and deviation analyses, the predicted value of P_{net} was based on the conductance surface in 60% of all cases.

For the second objective, to determine the extent to which photosynthetic performance was reduced from its potential, it is clear from Fig. 4 that performance was seldom equal to potential. The mean P_{net} at saturating light and optimal temperatures was only 52% of P_{max} (data not shown), and for all canopy levels, the boundary and deviation analysis indicated that perform-

ance averaged only 65% of potential over the full range of conditions.

With our third objective, to explore the underlying basis for and physiological significance of the difference between photosynthetic potential and performance, we initially expected to find that leaves exposed to high photon fluxes and high temperatures with their stomates nearly closed had suffered photoinhibitory damage. The O_2 electrode and in situ light and CO_2 responses, however, showed that the reductions were rapidly reversible (Figs. 5–8). Without this easy explanation of our results, and particularly of the correlation between P_{net} and C_i, we were forced to re-evaluate the relationship between the phenomena which we measure and the biochemical model on which we would expect to interpret them. In fact, we found no literature precedent for the applicability of the model, i.e., that developed by von Caemmerer and Farquhar (1981), to data sets produced by sampling large numbers of leaves or plants. The fundamental discrepancy appears to lie in the failure of the P_{net} vs. conductance relationship to show the type of curvilinearity predicted by the biochemical model (cf. Wong et al. 1979).

The literature precedents for linear conductance responses are numerous (e.g., Wong et al. (1979) and seven earlier reports cited there). As Bethenod and Tardieu (1990) have noted, the slope of this relationship is equal to $C_a - C_i$ (from Eq. (3)), and thus, linearity implies a constant C_i. Wong et al. (1985a,b) also demonstrated C_i to be nearly constant over a wide range of P_{net} in both C3 and C4 plants, even when influenced by nitrogen and phosphorous nutrition, by water stress (see also Di Marco et al., 1988) and by photoinhibition. Constant C_i has also been reported through variations in salinity (Bowman and Strain 1988) and midday stomatal closure (Tenhunen et al. 1984, Barthou et al. 1988), and independent of leaf temperature or v.p.d. effects on conductance (Andrews and Muller 1985).

The present results indicate that in the *Bruguiera* mangroves, the control of photosynthetic capacity does not maintain a particular C_i. Rather, at any given conductance, capacity varies from a maximum value to essentially nil, and C_i varies inversely with P_{net} from ca. 150 $\mu L\,L^{-1}$ to ambient (Fig. 3). We are aware of only one other report in which P_{net} has been plotted against C_i in a collected data set of the sort we have used (Wise et al. 1990), and the relationship was similar to that reported here. Inverse relationships between P_{net} and C_i are, however, otherwise evident in the literature, associated with both slow and fast changes in photosynthetic capacity. Tichá et al. (1988), for example, reported ontogenic changes in C_i in bean leaves which mirrored changes in P_{net}, independent of conductance. Similarly, Ghashghaie and Saugier (1989) reported a decrease in mean daily P_{net} and an increase in C_i during the development of water stress in a study of tall fescue.

Kirschbaum (1988) reported a more rapid response of photosynthetic capacity in *Eucalyptus*. When water stressed trees were re-watered, there was an initial (30 min to 4 h) increase in conductance and photosynthetic capacity at any given C_i which suggested a relationship such as that illustrated in Fig. 8. Gsell et al. (1989) showed a transient inhibition of photosynthesis in *Helianthus* after cutting a petiole which far exceeded the reduction explicable by stomatal closure. The changes were transient – recovery began in 20–30 min – and associated with changes in activity of several Calvin cycle enzymes. On a time scale similar to that illustrated in Fig. 7, Kirschbaum and Pearcy (1988) showed an increase in capacity in *Alocasia* during induction when irradiance was increased from 10 to 500 μmol quanta $m^{-2}\,s^{-1}$.

In summary, it is clear that the measurement of photosynthesis under natural conditions reports a complex balance of biochemical and biophysical parameters in a highly dynamic overall system. Though each measurement must fall on some set of response curves, it is not clear that any of those curves can be known.

The nature of the photosynthetic balancing act has been discussed recently in a review by Foyer et al. (1990). On the one hand, it is of paramount importance to protect the photochemical apparatus from photodamage. This requires energy dissipation and the ability to reduce the quantum efficiency of PS I and PS II at high light. On the other hand, it must be accomplished while balancing ATP and NADPH production to their consumption through CO_2 re-

duction, without overreducing the photosystems and photoinactivating electron transport (Siebke et al. 1990).

Harbinson et al. (1990a) found a clear correlation between the flux of electrons through the photosystems and the activity of stromal enzymes when irradiance was varied, but evidence for the control of electron fluxes when carbon fixation was limited by CO_2 at constant light. The relationship between photosystem and CO_2 fixation quantum efficiencies was also variable, suggesting photosynthetic control of electron fluxes, at least when photosynthesis was limited on the biochemical side (Harbinson et al. 1990b).

The control of Calvin cycle activity, and particularly of rubisco activation, has been shown in a number of studies to correlate with irradiance or electron flow, even over relatively short periods (Brooks et al. 1988, Pearcy and Seemann 1990). A correlation of the induction state and initial rubisco activity with stomatal conductance is indicative of the balance between environmentally and biochemically determined photosynthetic potential (Pearcy and Seemann 1990). Woodrow et al. (1990) have shown that at high light, the sensitivity of photosynthesis to rubisco activity is the dominating factor determining net carbon fixation. Still, because the activation and deactivation of carbon assimilatory enzymes are slow compared with potential changes in ambient irradiance and electron flux, the photosystems must remain the primary control point for short-term adjustments (Siebke et al. 1990).

In conclusion, in mangroves as well as in less exotic species, photosynthesis at steady state requires a complex balancing act simply to coordinate electron flows, ATP production, NADPH reduction and the redox state of the chloroplast, carbon fixation/reduction and CO_2 supplies. Under any set of conditions, it is reasonable to doubt that there is a unique solution to the overall balance of activities, let alone that it should converge to a maximal value of P_{net}. Thus, the variability of the data summarized in Fig. 4 may well represent the expected outcomes for a non-linear model and a complex system. As overall performance in natural and agricultural conditions is determined by such a system, it should be a major focus of research attention.

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