

A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*

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ABSTRACT

A process-based leaf gas exchange model for C₃ plants was developed which specifically describes the effects observed along light gradients of shifting nitrogen investment in carboxylation and bioenergetics and modified leaf thickness due to altered stacking of photosynthetic units. The model was parametrized for the late-successional, shade-tolerant deciduous species *Acer saccharum* Marsh. The specific activity of ribulose-1,5-bisphosphate carboxylase (Rubisco) and the maximum photosynthetic electron transport rate per unit cytochrome f (cyt f) were used as indices that vary proportionally with nitrogen investment in the capacities for carboxylation and electron transport. Rubisco and cyt f per unit leaf area are related in the model to leaf dry mass per area (M_A), leaf nitrogen content per unit leaf dry mass (N_m), and partitioning coefficients for leaf nitrogen in Rubisco (P_R) and in bioenergetics (P_B). These partitioning coefficients are estimated from characteristic response curves of photosynthesis along with information on leaf structure and composition. While P_R and P_B determine the light-saturated value of photosynthesis, the fraction of leaf nitrogen in thylakoid light-harvesting components (P_L) and the ratio of leaf chlorophyll to leaf nitrogen invested in light harvesting (C_B), which is dependent on thylakoid stoichiometry, determine the initial photosynthetic light utilization efficiency in the model. Carbon loss due to mitochondrial respiration, which also changes along light gradients, was considered to vary in proportion with carboxylation capacity. Key model parameters – N_m , P_R , P_B , P_L , C_B and stomatal sensitivity with respect to changes in net photosynthesis (G_f) – were examined as a function of M_A , which is linearly related to irradiance during growth of the leaves. The results of the analysis applied to *A. saccharum* indicate that P_B and P_R increase, and G_f , P_L and C_B decrease with increasing M_A . As a result of these effects of irradiance on nitrogen partitioning, the slope of the light-saturated net photosynthesis rate per unit leaf dry mass (A^m_{max}) versus N_m relationship increased with increasing growth irradiance in mid-season. Furthermore, the nitrogen partitioning coefficients as

well as the slopes of A^m_{max} versus N_m were independent of season, except during development of the leaf photosynthetic apparatus. Simulations revealed that the acclimation to high light increased A^m_{max} by 40% with respect to the low light regime. However, light-saturated photosynthesis per leaf area (A^a_{max}) varied 3-fold between these habitats, suggesting that the acclimation to high light was dominated by adjustments in leaf anatomy ($A^a_{max} = A^m_{max} M_A$) rather than in foliar biochemistry. This differed from adaptation to low light, where the alterations in foliar biochemistry were predicted to be at least as important as anatomical modifications. Due to the light-related accumulation of photosynthetic mass per unit area, A^a_{max} depended on M_A and leaf nitrogen per unit area (N_a). However, N_a conceals the variation in both M_A and N_m ($N_a = N_m M_A$), and prevents clear separation of anatomical adjustments in foliage structure and biochemical modifications in foliar composition. Given the large seasonal and site nutrient availability-related variation in N_m , and the influences of growth irradiance on nitrogen partitioning, the relationship between A^a_{max} and N_a is universal neither in time nor in space and in natural canopies at mid-season is mostly driven by variability in M_A . Thus, we conclude that analyses of the effects of nitrogen investments on potential carbon acquisition should use mass-based rather than area-based expressions.

Key-words: acclimation to growth irradiance; cytochrome f; leaf morphology; light use efficiency; nitrogen content; nitrogen partitioning model; nitrogen use efficiency; Rubisco; shade tolerance.

INTRODUCTION

The above-ground physiological activity of temperate forest trees occurs in long-term equilibrium with a moderate supply of nutrients and infrequent temperature and water stress. Thus, productivity is affected annually by changes in canopy light interception (Linder 1985; Cannell *et al.* 1987; McMurtrie *et al.* 1992). Carbon acquisition also depends on the manner in which leaf structure and physiology are modified along light availability gradients, a regulated response that determines the gas exchange performance of the canopy (Tooming 1967; Gutschick & Wiegel 1988; Baldocchi & Harley 1995; Sands 1995b).

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Consistently greater light-saturated photosynthesis rates per leaf area (A_{\max}^a) are reported for leaves growing at higher quantum flux densities in the sun crown as compared to shade leaves (e.g. Björkman 1981; Jurik 1986b; Ellsworth & Reich 1993; Harley & Baldocchi 1995). As maximum photosynthesis rates decrease with decreasing light availability, a linear relationship is also observed between growth irradiance and leaf thickness or leaf dry mass per area (M_A) (Ellsworth & Reich 1992a; Kull & Niinemets 1993; Niinemets 1995; Niinemets & Kull 1995). Furthermore, a strong positive correlation is commonly observed between photosynthetic activity under ambient conditions and leaf nitrogen content, because as much as three-quarters of foliar nitrogen may be invested in photosynthetic function (Field & Mooney 1986; Evans 1989b). However, the way in which nitrogen content and photosynthetic activity are coupled is open to interpretation and depends on whether nitrogen is expressed on an area (N_a) or a mass (N_m) basis (Ellsworth & Reich 1992a; Reich & Walters 1994; Reich *et al.* 1995).

If biochemical potentials of leaf cells remained constant along the light gradient, i.e. if the light-saturated photosynthesis rate on a mass basis (A_{\max}^m) were constant, then a one-to-one dependence of A_{\max}^a on M_A would also be anticipated. However, nitrogen partitioning between the thylakoid proteins regulating reductant supply and Calvin cycle enzymes determining CO_2 fixation depends on the local light climate during leaf development (Chow *et al.* 1988; Evans 1989b, 1993a; Evans & Seemann 1989), and influences the relationship between A_{\max}^a and M_A . N_m and M_A may also be correlated, further complicating matters (Reich & Walters 1994; Schulze *et al.* 1994; Niinemets 1995, 1997a,b; Reich *et al.* 1995). While there is a broad consensus that leaf nitrogen content provides a useful basis for standardizing the biochemical potentials of foliage, contemporary models of photosynthesis and canopy gas exchange have only considered the effects of variation in N_a (Hirose & Werger 1987; Harley *et al.* 1992; Chen *et al.* 1993; Schulze *et al.* 1994; Leuning *et al.* 1995; Sands 1995a). In so far as variability of N_a within the canopy is dominated by leaf anatomy (Walters & Field 1987; Ellsworth & Reich 1992a, 1993; Harley & Baldocchi 1995; Niinemets 1995, 1997a,b), it is doubtful whether existing model analyses of 'nitrogen' effects on annual carbon gain have really added concretely to theoretical analyses based only on M_A (e.g. Gutschick & Wiegel 1988), and there is much to be achieved in understanding canopy photosynthetic production as affected by light availability, nitrogen availability, and nitrogen partitioning, if N_a is separated, allowing independent consideration of N_m and M_A .

This paper examines the potential consequences of variation in leaf structural (M_A) and physiological characteristics (N_m partitioning) for canopy carbon acquisition with the aid of a process-based simulation model. The task undertaken here was to develop a model explicitly separating the qualitative effects of partitioning of nitrogen resources between various pools of the photosynthetic machinery from the cumulative effects of having more

photosynthetic machinery per unit leaf area. *Acer saccharum*, a North-American late-successional, shade-tolerant deciduous species, was chosen for the model-based characterization of nitrogen investments because extensive information exists on its leaf biochemistry and structure as well as on its ecophysiological response along light gradients (Jurik 1986a,b; Reich, Walters & Ellsworth 1991; Ellsworth & Reich 1992a,b; Ellsworth & Liu 1994; Kloeppel & Abrams 1995; Tjoelker *et al.* 1995). From this synthesis with *A. saccharum*, we hope to draw conclusions on the basic relationships amongst leaf structure, chemical composition and photosynthesis in temperate deciduous forest species, and on how these relationships are altered by foliage acclimation to long-term irradiance conditions in the canopy, and to reconcile the inconsistencies between mass- and area-based expressions for net photosynthesis, M_A and leaf nitrogen content (as pointed out by e.g. Ellsworth & Reich 1992a; Reich & Walters 1994; Reich *et al.* 1995). The second objective of the current study was to classify existing information for temperate forest trees and to consider the best way to carry out future experiments related to differences in nitrogen partitioning and distribution patterns along light gradients in species with differing abilities to tolerate shade. We expected our analysis to provide important insight into how canopy carbon gain is optimized in relation to foliar nitrogen investments but also in response to other variables that are modified during the course of succession.

METHODS

Biochemical model of leaf photosynthesis specifying nitrogen investments

According to the model of Farquhar *et al.* (Farquhar, von Caemmerer & Berry 1980; Farquhar & von Caemmerer 1982; Harley & Tenhunen 1991), photosynthetic carbon dioxide uptake, A_n , is limited either by ribulose-bisphosphate (RUBP) concentration, or at saturating RUBP by the activity of ribulose-bisphosphate carboxylase/oxygenase (Rubisco) or the concentrations of CO_2 and O_2 . Considering that the rate of RUBP carboxylation in photosynthesis is either equal to the potential rate allowed by the concentration of RUBP (W_j , RUBP-limited rate), or to the potential rate limited by the activity of Rubisco and the concentrations of CO_2 and O_2 (W_c , Rubisco-limited rate), the rate of net photosynthesis is:

$$\left\{ \begin{array}{l} A_n = V_{\max} \frac{C_i - \Gamma^*}{C_i + K_c \left(1 + \frac{O}{K_o} \right)} - R_d, \quad \text{if } W_c < W_j \\ A_n = J \frac{C_i - \Gamma^*}{4(C_i + 2\Gamma^*)} - R_d, \quad \text{if } W_c > W_j \end{array} \right. \quad (1)$$

where V_{\max} is the maximum rate of carboxylation allowed by Rubisco, J is the potential electron transport rate, C_i is

the intercellular concentration of CO_2 and O that of O_2 , K_c is the Michaelis-Menten constant for carboxylation and K_o that for oxygenation, and R_d is the mitochondrial respiration rate continuing in the light. Γ^* , the CO_2 compensation point in the absence of R_d (Laisk 1977), is equal to $0.5O/\tau$, where τ is the substrate specificity factor of Rubisco (Jordan & Ogren 1984). Implicit in Eqn 1 is the assumption that transport of 4 mol electrons through the photosynthetic electron transport chain produces sufficient chemical energy to regenerate 1 mol RUBP in the Calvin cycle (Harley & Tenhunen 1991; Harley *et al.* 1992), i.e. that NADPH^+ is limiting RUBP regeneration (Farquhar & von Caemmerer 1982). Light dependence of potential electron flow is given by the empirical equation of Smith (Tenhunen *et al.* 1976):

$$J = \frac{\alpha Q}{\sqrt{1 + \frac{\alpha^2 Q^2}{J_{\max}^2}}}, \quad (2)$$

where α is the quantum utilization efficiency [$\text{mol electrons} (\text{mol quanta})^{-1}$] at saturating C_i and on an incident light basis, Q is incident quantum flux density ($\text{pmol m}^{-2} \text{s}^{-1}$), and J_{\max} is the light-saturated value of J .

To calculate C_i for a given ambient CO_2 concentration (C_a), the biochemical photosynthesis model is coupled with an empirical model of stomatal conductance (g_s) (Ball, Woodrow & Berry 1987; Harley & Tenhunen 1991):

$$g_s = G_f A_n \frac{h_s}{C_s}, \quad (3)$$

where C_s is the CO_2 concentration ($\mu\text{mol mol}^{-1}$), h_s the relative humidity (decimal fraction) at the leaf surface, and G_f a constant representing stomatal sensitivity to these factors (Tenhunen *et al.* 1994; Sala & Tenhunen 1996). Boundary layer conductance, which determines C_s and h_s , is calculated according to Nobel (1983). C_i , which is dependent on both g_s and A_n according to Fick's law ($C_i = C_a - 56A_n/g_s$), is found iteratively.

To model leaf gas exchange along light gradients, where both leaf morphology (e.g. Ellsworth & Reich 1993; Kull & Niinemets 1993; Niinemets 1995; Niinemets & Kull 1995) and chemical composition (e.g. Chow *et al.* 1988; Evans 1989b; Evans & Seemann 1989; Niinemets 1995) are modified in response to the prevailing radiation regime, we formulate simple but explicit rules which explain the effects of leaf nitrogen investments in the Calvin cycle and electron transport processes on potential flux rates W_c and W_j , i.e. on the basic biochemical characteristics of leaf gas exchange. We also consider the effects of stacking multiple units of a particular biochemical nature (changing leaf thickness). Given the large investment of leaf nitrogen in the photosynthetic machinery, specific nitrogen contributions to Calvin cycle and electron transport constituents provide an effective means by which coordinated regulation of canopy gas exchange may be achieved by plants (Chen *et al.* 1993).

Rubisco-limited rate of carboxylation

Rubisco is the dominant protein component of Calvin cycle enzymes, incorporating as much as 30% of total leaf nitrogen (Evans 1989b). We assume that Calvin cycle activity is proportional to Rubisco activity and define the index P_R ['nitrogen in Rubisco'; g N in Rubisco (g total leaf N) $^{-1}$], which in effect defines the overall capacity for the reaction W_c . Since relative changes in the parameter P_R along light gradients are of interest, expression of rates in terms of Rubisco is adequate and offers the long-term possibility of examining responses in relation to measured Rubisco activity from leaf material harvested in the field. With knowledge of the specific activity of Rubisco, i.e. the maximum rate of RUBP carboxylation per unit Rubisco protein [V_{cr} , $\mu\text{mol CO}_2 (\text{g Rubisco})^{-1} \text{s}^{-1}$], and provided all leaf Rubisco is in the fully activated state, the maximum rate of carboxylation per unit leaf area (V_{cmax} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) is given by

$$V_{cmax} = 6.25 V_{cr} M_A P_R N_m, \quad (4)$$

where M_A is leaf dry mass per area (g m^{-2}), N_m is leaf nitrogen content per leaf dry mass (g g^{-1}), and 6.25 [g Rubisco (g nitrogen in Rubisco) $^{-1}$] converts nitrogen content to protein content. Of course, the proportion of leaf nitrogen invested in carboxylation is actually greater than P_R . However, since other Calvin cycle enzymes are likely to change in concert with Rubisco, use of an alternative index for Calvin cycle activity would only mean that alternative values for V_{cr} would also be required when fitting the model to experimental data. Friend (1991, 1995) and Amthor (1994b) also invoke P_R , but their models do not separate the different components of N_a (N_m and M_A).

Potential rate of photosynthetic electron flow

Depending on light availability, the protein composition of chloroplast thylakoids varies to maintain efficiency in the balance between light harvesting and light energy conversion to photoproducts (Chow *et al.* 1988; Evans 1989b; Evans & Seemann 1989). Evans & Seemann (1989) divided thylakoid nitrogen investments between light harvesting ('nitrogen in light harvesting'; P_L) and bioenergetic pools ('nitrogen in bioenergetics'; P_B). Nitrogen investment in light harvesting determines the content of chlorophyll *a/b* protein complexes associated with photosystems I (PSI) and II (PSII), while investment in bioenergetics determines the protein content limiting the capacity for photosynthetic electron transport and photophosphorylation. Both P_B , influencing J_{\max} (in $\mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$), and P_L , influencing quantum conversion efficiency on an incident light basis (α ; see Eqn 2), determine W_j .

There is a wide consensus that the rate-limiting step of the light-saturated rate of thylakoid electron transport resides between the two photosystems (e.g. Foyer 1993; Harbinson 1994). Thus, we consider the index P_B to reflect changes in content of cytochrome *f*, ferredoxin NADP reductase (FNR), and the coupling factor (CF) which are

the primary proteins determining the overall rate of electron transport (Evans & Seemann 1989). Considering a constant 1:1:1.2 molar ratio for cyt f:FNR:CF, the investment in bioenergetics is at least 0.124 g N ($\mu\text{mol cyt f}$) $^{-1}$ [or 8.06 $\mu\text{mol cyt f}$ (g N in bioenergetics) $^{-1}$; Evans & Seemann 1989]. As in the case of P_R , P_B expressed in the units of g leaf nitrogen in cyt f, FNR, and CF per g total leaf N is an adequate index for nitrogen investments in electron transport, if the activity of electron transport, J_{mc} , is standardized in mol electrons (mol cyt f) $^{-1}$ s $^{-1}$. Standardizing the biochemical component of electron transport enables us to distinguish the determinants of J_{max} that result from modifications in leaf structure:

$$J_{max} = 8.06 J_{mc} M_A P_B N_m \quad (5)$$

Friend (1991, 1995) calculated the proportional investment of leaf nitrogen for a given potential electron transport rate per unit leaf chlorophyll. However, the latter basis is not straightforward, because J_{max} per unit chlorophyll is highly variable (Fig. A1). Understanding this, Hikosaka & Terashima (1995) used cyt f (as in the current model) as a representative protein to scale proteins of electron transport chain with leaf nitrogen. However, they, as well as Amthor (1994b) and Amthor *et al.* (1994), assume a strictly coordinated change in the capacities for RUBP carboxylation and electron transport. Though J_{max} and V_{cmax} determined from net photosynthesis versus C_i curves are generally correlated (Wullschleger 1993), the ratio of J_{max}/V_{cmax} varies by almost a factor of 3 for 59 species measured at 25 °C in the data set of Wullschleger (1993) – the minimum is 1.43 ± 0.27 and the maximum 3.90 ± 0.70 (averages \pm SE of three lowest and three largest estimates, respectively). In the light of this evidence, we consider that a model examining nitrogen partitioning strategies should be flexible enough to change J_{max} and V_{cmax} independently. Recent findings do show that a separate examination of J_{max} and V_{cmax} across light environments is relevant, because the adaptation to growth irradiance may shift the balance between electron transport and Rubisco activities (Sukenik, Bennett & Falkowski 1987; Ögren 1993).

In our model formulation, the specific activity of Rubisco, V_{cr} , and the potential rate of photosynthetic electron transport per unit cytochrome f, J_{mc} , are considered as essentially conserved among C₃ species. Temperature dependences of the activities of Rubisco and potential electron flow used in the model are scaled to results from a number of species as described in Appendix A.

Initial quantum yield

The quantum yield of photosynthesis at CO₂ saturation and at low light of 0.073 mol CO₂ (mol quanta) $^{-1}$ is extremely constant for all C₃ species (Ehleringer & Björkman 1977). The assumption that 4 mol electrons is sufficient to regenerate enough RUBP for the carboxylation of 1 mol CO₂ gives an equivalent value of 0.292 mol e⁻ (mol quanta) $^{-1}$. However, C₃ leaves functioning in natural environments utilize light with a lower efficiency due to species-specific

structural attributes which reduce light availability at the chloroplasts, for example leaf thickness, density, pubescence, or reflectiveness due to waxy coatings. These attributes are included in the initial light utilization efficiency at saturating CO₂ (α) calculated on an incident irradiance basis in our model (Eqn 2). The change in α along light gradients within the canopy of temperate broad leaf trees is a function of changes in leaf absorptance (ξ). We initially hypothesize that ξ depends on leaf chlorophyll content only, and express α using an empirical equation as a function of leaf absorptance and chlorophyll content (Evans 1993b):

$$\alpha = 0.292\xi = 0.292 \left(1 + \frac{0.076}{N_m M_A P_L C_B} \right)^{-1}, \quad (6)$$

where 0.076 (mmol Chl m $^{-2}$) is an empirical coefficient estimated from an analysis of chlorophyll versus absorptance relationships in a broad range of species (Evans 1993b), and C_B [mmol Chl (g N) $^{-1}$], ‘chlorophyll binding’, determines how efficiently the nitrogen invested in thylakoids participates in light harvesting. C_B depends on long-term irradiance conditions, which alter the stoichiometry of major thylakoid protein complexes (Chow *et al.* 1988; Evans 1989b; Evans & Seemann 1989). Therefore, nitrogen in light-harvesting apparatus should be divided between PSI, PSII and light-harvesting chlorophyll protein complexes of PSII (LHCII) (Evans & Seemann 1989; Hikosaka & Terashima 1995). N_L and C_B are calculated from leaf chlorophyll concentration (C_C) and J_{max} as summarized in Appendix B.

Day respiration

Though the interplay between mitochondrial respiration and photosynthesis in chloroplasts is fairly complex (Amthor 1994a), positive relationships between light-saturated photosynthesis and respiration in the night, R_n , are often observed (Tooming 1967; Walters & Field 1987; Ceulemans & Saugier 1991). Assuming that the respiratory costs for leaf maintenance scale directly with foliage physiological activity, the respiration rate of leaves at different levels in the canopy is taken as a proportion of V_{cmax} (e.g. Farquhar, von Caemmerer & Berry 1980; Collatz *et al.* 1991):

$$R_n = R_{coe} V_{cmax}, \quad (7)$$

where R_{coe} is a species-specific proportionality coefficient. Thus, an increase in leaf biochemical potentials leads to balanced increases in the costs for leaf maintenance. Since it is currently unclear to what extent R_n is inhibited in light, we arbitrarily set day respiration, R_d , as $R_d = 0.5 R_n$.

R_n depends on leaf temperature according to Eqn A2.

Parameter derivation for *A. saccharum*

The model described above provides a simple means of linking biochemistry with canopy response, but parameter derivation requires a unique combination of physiological, ecophysiological and morphological information. The

required information has seldom, if at all, been collected in an individual examination of leaf morphological and physiological properties along light gradients. An extensive literature search indicated that foliage functional and structural characteristics along light gradients are particularly well characterized for *A. saccharum*. Thus, we have attempted an initial parametrization of the model for this temperate tree species. Given that parameters must be estimated from a number of independent studies of *A. saccharum*, we followed a stepwise procedure, sequentially fixing those parameters which could be best determined, and using these fixed values to limit the range in possible values for variables yet undetermined. Despite the breadth of information available for this species, a number of assumptions were required in order to complete the parameter derivation.

Several studies provided values of R_n along with information on light-saturated net photosynthesis rate (A_{nmax}). The analysis of these data revealed that at a leaf temperature (T_l) of 25 °C R_n was strongly related to A_{gmax} (gross photosynthesis rate, $A_{nmax} + 0.5R_n$) with a proportionality coefficient of 0.145 (Fig. 1a). Whenever R_n , A_{nmax} and stomatal conductance were measured, V_{cmax} could also be calculated as described in Appendix C. Correlation analysis fixed R_{co} (Eqn 7) at 0.065 (Fig. 1b). The activation energy of R_n [$\Delta H_a(R_n)$; 42000 J mol⁻¹] was obtained from the observations of Weber *et al.* (1985). The scaling constant $c(R_n)$ (Eqn A2) was chosen for all leaves such that estimated V_{cmax} and R_n at $T_l = 25$ °C remained compatible with the regression line shown in Fig. 1b.

In general, responses of photosynthesis to leaf internal CO₂ concentration have been used to assess carboxylase and electron transport capacities of leaves, utilizing the initial slope of the response curve and saturation levels to estimate V_{cmax} and J_{max} , respectively (Farquhar, von Caemmerer & Berry 1980; Harley, Tenhunen & Lange 1986; Tenhunen *et al.* 1990; Harley & Tenhunen 1991; Harley *et al.* 1992). In *A. saccharum*, we faced a different situation of having information mainly on the relation-

ships between net photosynthesis and light at current ambient CO₂ concentrations (Ellsworth & Reich 1992a, 1993; Kloeppe & Abrams 1995; Tjoelker *et al.* 1995) and a few photosynthesis versus CO₂ response curves (Ellsworth & Liu 1994). To use the broad knowledge of the responses of net photosynthesis to irradiance in this species we developed a special routine for obtaining V_{cmax} and J_{max} from A_{nmax} , R_n , C_i and saturating quantum flux (Appendix C). In this analysis, missing R_n values had to be calculated from A_{gmax} according to the regression line depicted in Fig. 1a. Since the equation of Evans for leaf absorptance (1993b; eqn 6) appeared slightly to overestimate absorptance (see 'Results'), only α values computed from measured absorptance (Eqn 6) went into the calculations in Appendix C. Having determined J_{max} and V_{cmax} , nitrogen investments responsible for the light-saturated value of photosynthesis, P_R and P_B , could be computed from Eqns 4 and 5. P_B was further employed in calculations of P_L and C_B (Eqn B1) from measured leaf chlorophyll concentrations as described in Appendix B. Finally, G_f was chosen to achieve leaf conductances compatible with observations.

Boundary layer conductance was computed for a constant wind speed of 1 m s⁻¹ and characteristic leaf width of 0.05 m where specific data on cuvette experiments were lacking. Intercellular partial pressure of O₂ was set to 210 mmol mol⁻¹ in all calculations, and C_a to 340 μmol mol⁻¹ in all theoretical model calculations.

RESULTS

Model parametrization for *A. saccharum*

Despite some difficulties in assessing the *in situ* integrated light climate for leaves, we can accept that foliar morphology, for example the parameter M_A , is linearly related to intercepted quantum flux density in *A. saccharum* (Fig. 2a). As expected, the data of Ellsworth & Reich (1993) and Tucker, Lassoie & Fahey (1993) further indi-

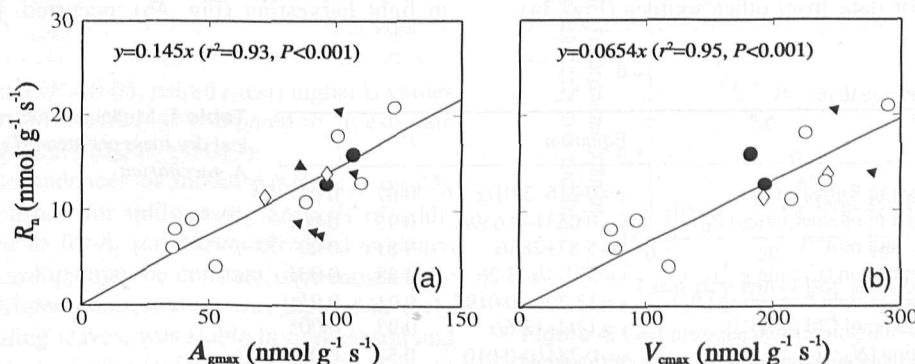


Figure 1. Correlation of dark respiration rate (R_n , nmol g⁻¹ s⁻¹) in *A. saccharum* with (a) the light-saturated gross photosynthesis rate, A_{gmax} (nmol g⁻¹ s⁻¹), at an ambient CO₂ of 350–360 μmol mol⁻¹ and for a leaf temperature (T_l) of 25 °C, and with (b) V_{cmax} at 25 °C. A_{gmax} was calculated as light-saturated net photosynthesis rate (A_{nmax}) + 0.5 R_n , whereas V_{cmax} was derived from A_{gmax} at a given intercellular CO₂ concentration (C_i) as described in Appendix C. Data are from Kloeppe & Abrams (1995) (●), Tjoelker *et al.* (1993) (◇), Tjoelker *et al.* (1995) (▼), Volin *et al.* (1993) (○) and Walters, Kruger & Reich (1993) (▲).

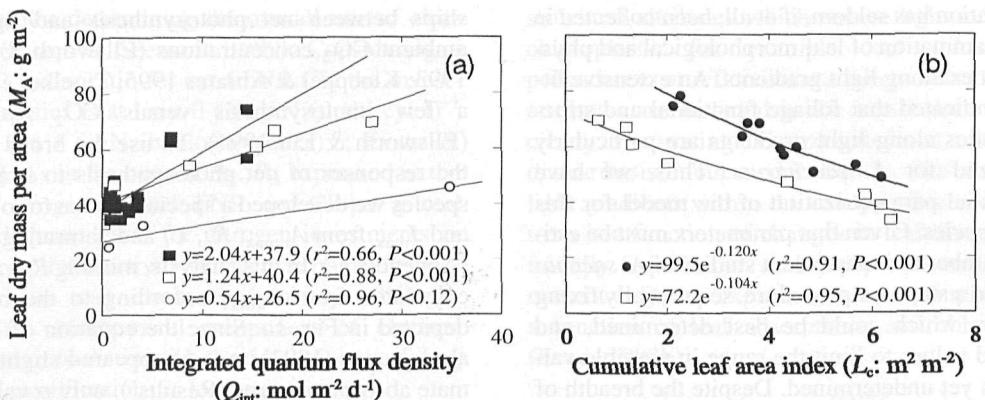


Figure 2. Leaf dry mass per area (M_A) in *A. saccharum* in relation to (a) growth irradiance (Q_{int}) and (b) cumulative leaf area index (L_c). Momentary Q at a given value of L_c is approximated by Lambert-Beer's equation as $Q = Q_0 e^{(-k L_c)}$, where Q_0 is Q above the canopy and k is the canopy extinction coefficient. Thus, the data depicted in (b) essentially express the same linear relationship between growth irradiance and M_A . Data are from Ellsworth & Reich (1992a) (○), Ellsworth & Reich (1993) (□), Tjoelker *et al.* (1995) (■) and Tucker, Lassoie & Fahey (1993) (●); measurements for shaded understory saplings excluded.

cate that M_A is exponentially related to the cumulative leaf area index (L_c) in situations with a relatively homogeneous canopy, underscoring the strong response to light in *A. saccharum* (Fig. 2b). Given the tight positive relationships between M_A and irradiance (Kull & Niinemets 1993; Niinemets & Kull 1995; Niinemets 1997a,b), and that M_A is easily and more accurately measurable than long-term irradiance conditions at the leaf, we established the relationships of model variables to M_A . This offers considerable advantages to canopy models for non-homogeneous stands where light climate is differentiated in three dimensions.

The dependences of model parameters on M_A in mid-season were curvilinear (Figs 3 & 4), and were fitted with hyperbolic functions (Table 1). P_R and P_B , determining the capacity for CO₂ carboxylation, increased (Figs 3a & b), while G_f , characterizing the efficiency with which the biochemical potentials are realized in carbon gain, decreased with increasing M_A (Fig. 3c). Recalculation of the data of Volin *et al.* (1993) provided a similar relationship between P_R and M_A to that for data from other sources (Fig. 3a).

However, the *in vitro* Rubisco activity they reported was too small by about a factor of 5 to allow the photosynthetic rates measured in their study. P_R ($r^2 = 0.62$, $P < 0.01$) and P_B ($r^2 = 0.85$, $P < 0.01$) also increased hyperbolically with increasing leaf nitrogen content per leaf area (N_a). However, this was wholly attributable to changes in M_A ($N_a = M_A N_m$), since P_R ($P > 0.2$) and P_B ($P > 0.4$) were independent of leaf nitrogen concentration. P_R and P_B were strongly correlated with each other ($r^2 = 0.88$, $P < 0.01$).

α , taken to be directly proportional to leaf absorptance (ξ), was independent of M_A (Fig. 4a). However, given the asymptotic relationship between ξ and leaf chlorophyll content (Eqn 6), and that the leaves became thinner with decreasing irradiance (Fig. 2), the proportion of absorbed light per unit leaf area could not be maintained at a high level with declining M_A , unless strong increases in the allocation of leaf nitrogen in light-harvesting compounds (Figs 4a & b) and modifications in thylakoid stoichiometry, improving the light absorptance per unit nitrogen invested in light harvesting (Fig. 4b), occurred. Equation 6 esti-

Dependent variable	Equation	r^2	P
Proportion of leaf nitrogen in Rubisco (P_R)	$y = 0.216 - 3.01/x$	0.67	0.01
Proportion of leaf nitrogen in bioenergetics (P_B)	$y = 0.0531 - 0.659/x$	0.95	0.001
Stomatal coefficient (G_f ; mol mol ⁻¹)	$y = 5.87 + 286/x$	0.81	0.02
Chlorophyll ($a+b$) concentration (C_C ; mg g ⁻¹)	$y = 571.3/x - 3.26$	0.88	0.001
Proportion of leaf nitrogen in light harvesting (P_L)	$y = 13.79/x - 0.0197$	0.91	0.01
Chlorophyll binding [C_B ; mmol Chl (g N) ⁻¹]	$y = 1.94 + 12.6/x$	0.97	0.005
Leaf nitrogen concentration (N_m ; g g ⁻¹)	$y = 0.284/x + 0.010$	0.54	0.001

Table 1. Model parameters in relation to leaf dry mass per area (M_A ; g m⁻²) in *A. saccharum**

* The relationships are depicted in Figs 3 and 4, except for N_m , which is based on Ellsworth & Reich (1992a). Though in the case of N_m the hyperbolic fit improved insignificantly the explained variance ($r^2 = 0.53$ for the linear and 0.54 for the hyperbolic relationship), in light of further studies (Ellsworth & Reich 1993; Tjoelker *et al.* 1995) it gave more realistic estimates of N_m at higher M_A than a linear fit.

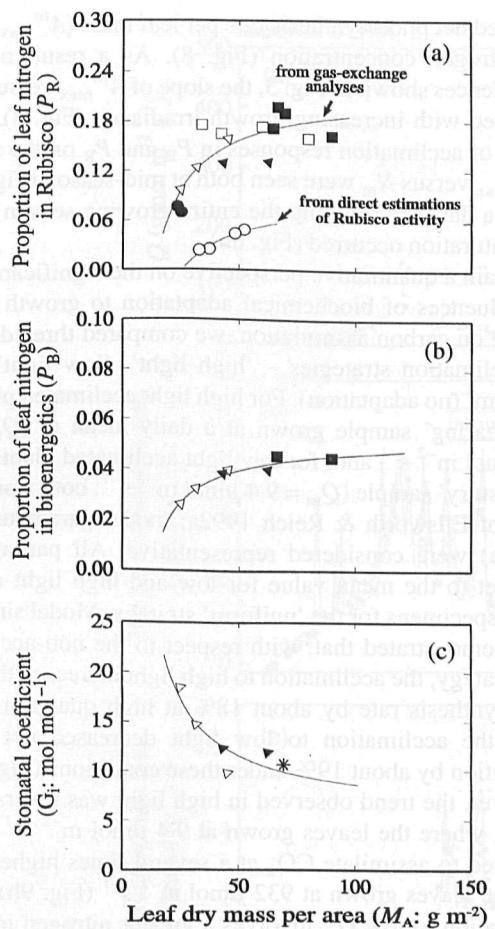


Figure 3. Results of model analysis showing relationships between M_A and (a,b) leaf nitrogen partitioning into (a) Rubisco and (b) bioenergetics, and (c) stomatal sensitivity correlated with changes in net photosynthesis in *A. saccharum*. Data are from Ellsworth & Liu (1994) (■), Ellsworth & Reich (1992a) (▽), Ellsworth & Reich (1993) (□), Ellsworth *et al.* (1994) (*), Kloeppe & Abrams (1995) (●), and Tjoelker *et al.* (1995) (▲). P_R values obtained from laboratory estimates of Rubisco activity (assuming a Rubisco assay temperature of 25 °C) of Volin *et al.* (1993) (○; $r^2 = 0.95$, $P < 0.05$) are also plotted. Regression equations based on coefficients from gas exchange analyses are given in Table 1.

mated significantly ($P < 0.05$, paired t -test) higher α values (mean \pm SE = 0.250 ± 0.006) as compared to an estimate from measured ξ values (0.242 ± 0.013).

Though the dependences of model parameters on M_A were only established for mid-season, analysis of additional measurements for *A. saccharum* provided evidence that these relationships may be constant over most of the season. Leaf nitrogen concentration was highest in early season in expanding leaves, was stable in mid-season and declined consistently during leaf senescence (Fig. 5a). At the same time, the fraction of leaf nitrogen in Rubisco changed during the development of the leaf photosynthetic apparatus, but P_R was independent of season and leaf nitrogen status after leaf maturation (Fig. 5b).

Comparison of model predictions with measured gas exchange

Weber *et al.* (1985) provided an independent series of diurnal time courses of transpiration and net photosynthesis for understory seedlings of *A. saccharum* (Fig. 6). Since no structural information was available from this study, M_A had to be determined first to calculate model parameters from the equations in Table 1. This was accomplished using the following routine: (1) total daily Q values were calculated from the data in Figs 6a and b; (2) these values were corrected according to Gates (1980) to yield mean seasonal daily Q (Q_{int}), and (3) M_A values (27.6 and 27.5 g m⁻² for 12-09-1981 and 13-09-1981, respectively) were calculated according to the regression line of the data of Ellsworth & Reich (1992a) in Fig. 2a, using Q_{int} as the x value. All model parameters except G_f were subsequently calculated from M_A (Figs 3 & 4; Table 1). G_f was adjusted so as reasonably to describe measured water use and held constant at 10.5 mol mol⁻¹ for both days. Nevertheless, the photosynthetic rate was insensitive to different values of G_f (9–14) at the air temperatures and irradiances observed during the simulated period. Even though we derived the model parametrization for the understory seedlings from the light measurements on only two consecutive days, the correspondence between measured and simulated values of gas exchange was striking (Figs 6c–f), signifying the robustness of the current modelling scheme.

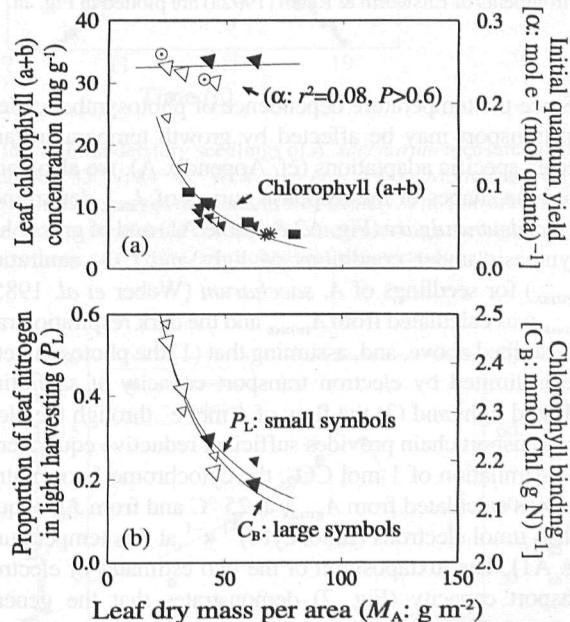


Figure 4. Leaf nitrogen partitioning into light-harvesting apparatus in *A. saccharum* and associated changes in initial quantum conversion efficiency on an incident light basis (α) as affected by M_A . All symbols are as in Fig. 3, except for ○—St-Jacques, Labrecque & Bellefleur (1991) (M_A was calculated from the values of leaf fresh mass per unit area assuming an appropriate leaf water content given in Schultz, Nothnagle & Baldwin 1982). Regression equations are given in Table 1.

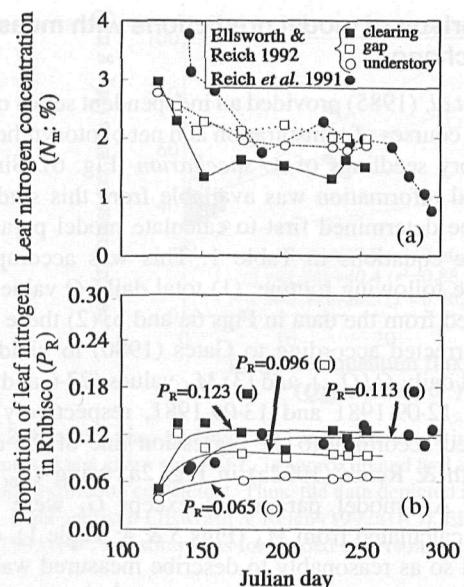


Figure 5. Leaf developmental effects on leaf nitrogen concentration and proportional investment of leaf nitrogen in Rubisco in *A. saccharum*. (a) Seasonal variability in leaf nitrogen concentration. (b) Leaf nitrogen partitioning into Rubisco. P_R was calculated from light-saturated net photosynthesis at a given C_i as described in Appendix C. A mean seasonal C_i/C_a ratio of 0.792 obtained from Jurik (1986a) was used for the data of Reich *et al.* (1991). C_i , calculated from seasonal mean light-saturated net photosynthesis and stomatal conductances, was also held constant for each of the light environments of Ellsworth & Reich (1992a). Curves are fitted by hand, whereas the P_R values depicted in the curves refer to the plateau regions of P_R versus season dependences. Light environments of Ellsworth & Reich (1992a) are plotted in Fig. 2a.

Since the temperature dependence of photosynthetic electron transport may be affected by growth temperature and species-specific adaptations (cf. Appendix A), we also compared the shapes of the response curves of J_{\max} determined for *Hordeum vulgare* (Fig. A2 & Table A1) and of gross photosynthesis under conditions of light and CO_2 saturation (A_{gmax}) for seedlings of *A. saccharum* (Weber *et al.* 1985). A_{gmax} was calculated from A_{nmax} and the dark respiration rate as described above, and, assuming that (1) the photosynthetic rate is limited by electron transport capacity at saturating CO_2 and light and (2) the flow of 4 mol e^- through the electron transport chain provides sufficient reductive equivalents for assimilation of 1 mol CO_2 , the cytochrome f concentration was calculated from A_{gmax} at 25 °C and from J_{mc} [equal to 156 $\mu\text{mol cyt f}^{-1} \text{s}^{-1}$ at this temperature; Fig. A1]. The juxtaposition of the two estimates of electron transport capacity (Fig. 7) demonstrates that the general response of J_{\max} agreed over a wide temperature range with the measured A_{gmax} versus temperature relationship.

Adaptation to growth irradiance: the case of *A. saccharum*

Changes in the nitrogen partitioning coefficients P_B and P_R with M_A (Fig. 3) influence correlations between light-

saturated net photosynthesis rate per leaf mass (A^{m}_{\max}) and leaf nitrogen concentration (Fig. 8). As a result of the dependences shown in Fig. 3, the slope of A^{m}_{\max} versus N_m increased with increasing growth irradiance (Fig. 8). The effects of acclimation responses in P_B and P_R on the slope of A^{m}_{\max} versus N_m were seen both at mid-season (Fig. 8a) and in a data set covering the entire growing season after leaf maturation occurred (Fig. 8b).

To gain a quantitative perspective on the significance of the influences of biochemical adaptation to growth light climate on carbon assimilation, we compared three different acclimation strategies – ‘high light’, ‘low light’ and ‘uniform’ (no adaptation). For high light acclimated plants, the ‘clearing’ sample grown at a daily mean Q (Q_m) of 932 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and, for low light acclimated plants, the ‘understory’ sample ($Q_m = 9.4 \mu\text{mol m}^{-2} \text{s}^{-1}$; both from the study of Ellsworth & Reich 1992a; model parameters as Fig. 8a) were considered representative. All parameters were set to the mean value for low and high light acclimated specimens for the ‘uniform’ strategy. Model simulations demonstrated that, with respect to the non-acclimation strategy, the acclimation to high light increased the net photosynthesis rate by about 18% at high quantum flux, while the acclimation to low light decreased net CO_2 acquisition by about 19% under these conditions (Fig. 9a). However, the trend observed in high light was reversed at low Q , where the leaves grown at 9.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were predicted to assimilate CO_2 at a several times higher rate than the leaves grown at 932 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 9b). The acclimation to low Q_m involves a greater nitrogen investment in light harvesting which improves the initial quantum utilization efficiency for a given nitrogen concentration (Fig. 4). A greater respiratory requirement for maintenance of the biochemical potentials of photosynthesis also reduces the efficiency of high light-grown leaves in low light conditions.

N_m was not constant amongst various light microenvironments in mid-season. Instead, it increased with decreasing M_A (Ellsworth & Reich 1992a; Table 1). As the model calculations depicted in Fig. 9b suggest, this investment pattern is beneficial for the leaves developed under low Q_m , since it results in an improvement of the light use efficiency for an incident irradiance. Differences in N_m in mid-season also meant that when A^{m}_{\max} values for low and high light-grown leaves were examined at the respective mean leaf nitrogen concentrations (0.0194 g g^{-1} for ‘understory’ and 0.0154 g g^{-1} for ‘clearing’; Ellsworth & Reich 1992a), A^{m}_{\max} was only c. 11% higher in the ‘clearing’ than in the ‘understory’ (cf. Figs 8a & 9a).

Anatomical adjustments in foliar structure also played a major role in acclimation to growth irradiance in this species. Given that M_A varies by more than 3-fold across light gradients (Fig. 2), and A^{m}_{\max} at a common nitrogen concentration by roughly 40% (Fig. 9a), it is clear that, in mid-season, the variability in light-saturated net photosynthesis rate per unit area ($A^{\text{a}}_{\max} = A^{\text{m}}_{\max} M_A$), which increases strongly with M_A (data not shown; see Ellsworth & Reich 1992a, 1993), is mostly governed by plastic

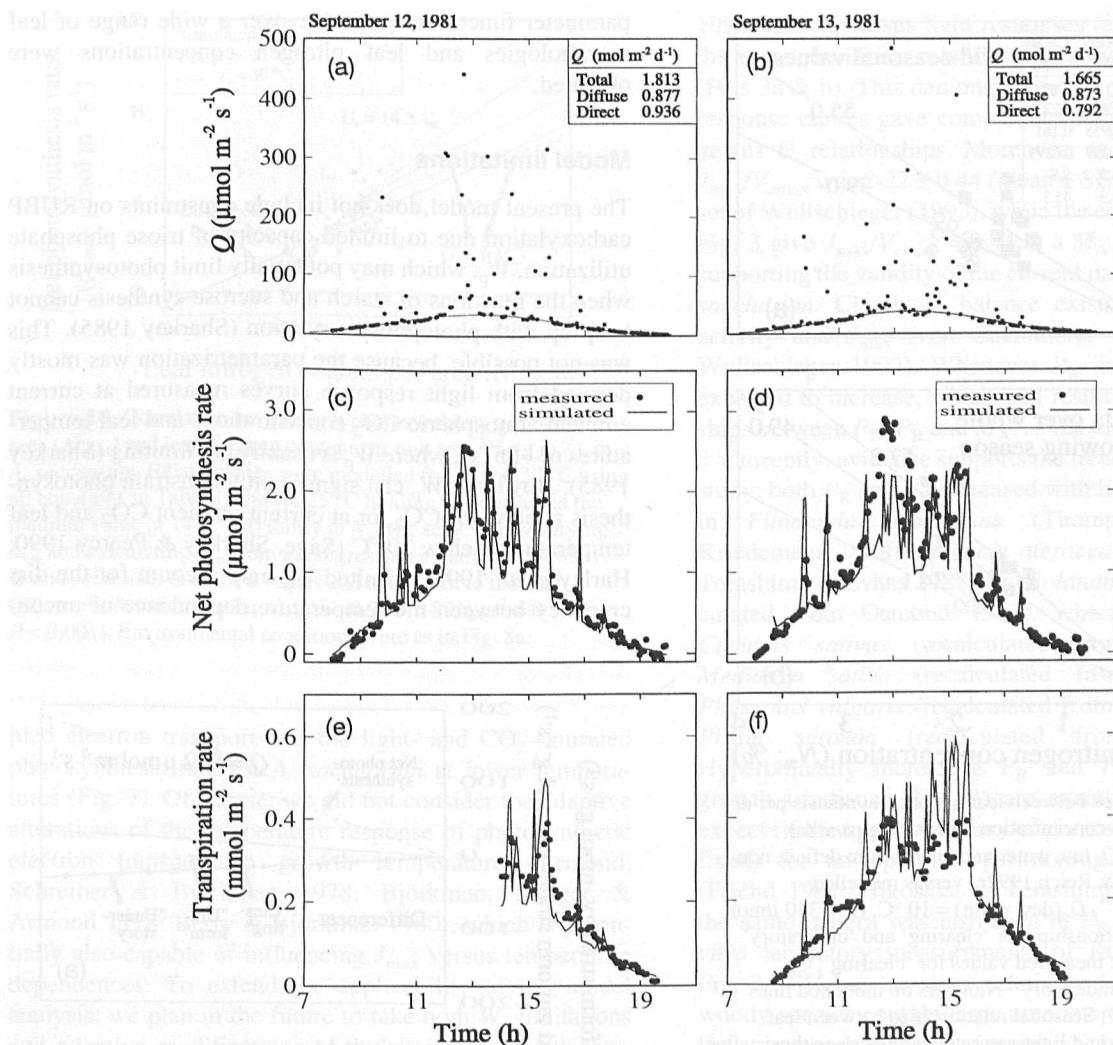


Figure 6. Observed and predicted photosynthesis and transpiration rates for leaves of understory seedlings of *A. saccharum* measured on two days at the University of Michigan Biological Station near Pelleston, Michigan ($45^{\circ} 33' \text{N}$, $84^{\circ} 42' \text{W}$; Weber *et al.* 1985). Momentary values of Q , T_l , C_a and air humidity are used to predict leaf photosynthesis rates. (a,b) Diurnal changes in quantum flux density. The line drawn through the data represents diffuse Q and was found by fitting a sinusoidal function to the diurnal course of Q after removing the higher light intensities apparently affected by direct flux. (c,d) Daily courses of net photosynthesis rate. (e,f) Daily courses of transpiration rate.

changes in leaf anatomy rather than in biochemistry. Variability in M_A with irradiance (Fig. 2) also provides an explanation as to why leaf nitrogen content per unit area increased strongly with irradiance and M_A ($N_a = N_m M_A$; data not shown; see Ellsworth & Reich 1992a, 1993), even when N_m decreased significantly with increasing M_A (Table 1). Acclimation of M_A to growth irradiance is also the major factor governing the positive correlation between A_{\max}^a and N_a when all measurements across the light gradient are pooled in mid-season (Fig. 10). However, the role of nitrogen and nitrogen partitioning on A_{\max}^a is not directly evident in this relationship, because the same N_a may be determined from different paired values of N_m and M_A . Accordingly, N_a alone contains no information concerning the biochemical light-adaptation state of foliage, and therefore the correlation between A_{\max}^a and N_a lacks universality.

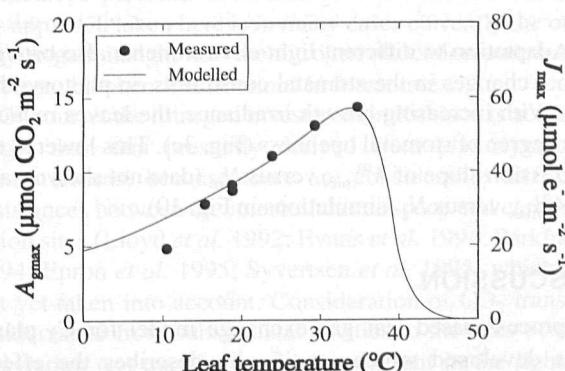


Figure 7. Comparison of the shapes of temperature-response curves for light- and CO_2 -saturated gross photosynthesis rate (A_{\max} ; $A_{\max} + 0.5R_n$) for *A. saccharum* (recalculated from Weber *et al.* 1985) and J_{\max} , established by temperature constants of J_{mc} (Table A1) and a cyt f concentration of $0.287 \mu\text{mol m}^{-2} \text{s}^{-1}$.

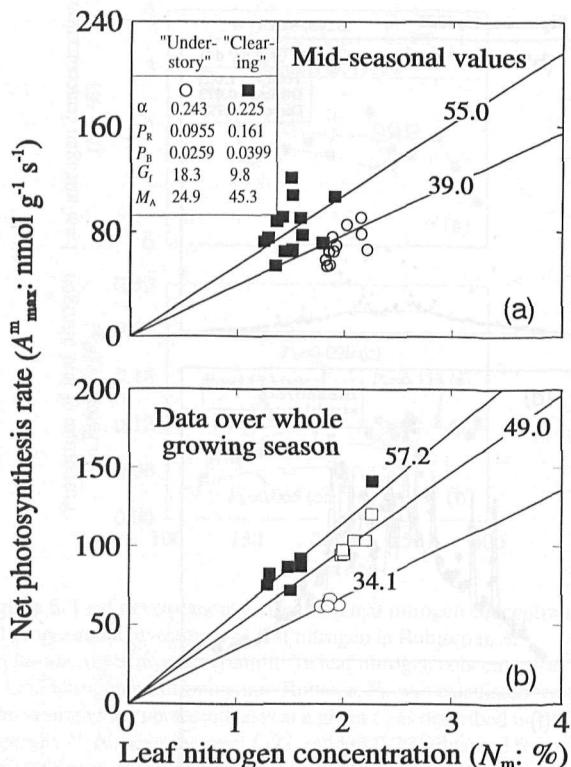


Figure 8. Relationships between leaf net photosynthesis per dry mass and leaf nitrogen concentration in *A. saccharum*. (a) Measured (saturating Q , low water vapour pressure deficit, non-limiting T_f ; Ellsworth & Reich 1992a) versus modelled [$Q = 1200 \mu\text{mol m}^{-2} \text{s}^{-1}$, D_p (dew point) = 10 °C, $C_a = 340 \mu\text{mol mol}^{-1}$; $T_f = 25^\circ\text{C}$] relationships for 'clearing' and 'understory' sites in mid-season. ■: measured values for 'clearing', ○: measured values for 'understory'. Numbers on modelled lines denote slope values. (b) Seasonal relationships between leaf nitrogen concentration and light-saturated net photosynthesis after leaf maturation. Environmental conditions were as in (a), and symbols as in Fig. 5. Modelled lines were derived using P_R values obtained from the plateau regions of P_R versus season dependences (constant values depicted in Fig. 5b), and the values of P_B , P_L and C_B calculated from seasonal mean M_A (Table 1). Early seasonal measurements during the development of foliage, when P_R was unstable (Fig. 5b), are excluded.

Adaptation to different light environments also brought about changes in the stomatal constraints on photosynthesis. With increasing growth irradiance, the leaves reduced the degree of stomatal openness (Fig. 3c). This lowered the regression slope of A_{max}^m versus N_m (data not shown), and of A_{max}^a versus N_a (simulations in Fig. 10).

DISCUSSION

A process-based leaf gas exchange model for C_3 plants was developed which specifically describes the effects observed along light gradients of shifting nitrogen investment in carboxylation and bioenergetics and modified leaf thickness due to altered stacking of photosynthetic units. The model was parametrized for the late-successional, shade-tolerant deciduous species *A. saccharum*, and

parameter functions applicable over a wide range of leaf morphologies and leaf nitrogen concentrations were obtained.

Model limitations

The present model does not include constraints on RUBP carboxylation due to limited capacity of triose phosphate utilization, W_p , which may potentially limit photosynthesis when the reactions of starch and sucrose synthesis cannot keep up with photophosphorylation (Sharkey 1985). This was not possible, because the parametrization was mostly derived from light response curves measured at current ambient atmospheric CO_2 concentrations and leaf temperatures of $\approx 25^\circ\text{C}$, where W_p is usually not limiting (Sharkey 1985). However, W_p can significantly constrain photosynthesis at elevated CO_2 , or at current ambient CO_2 and leaf temperatures below 20 °C (Sage, Sharkey & Pearcy 1990; Harley *et al.* 1992). Limited W_p may account for the discrepancy between the temperature dependence of uncou-

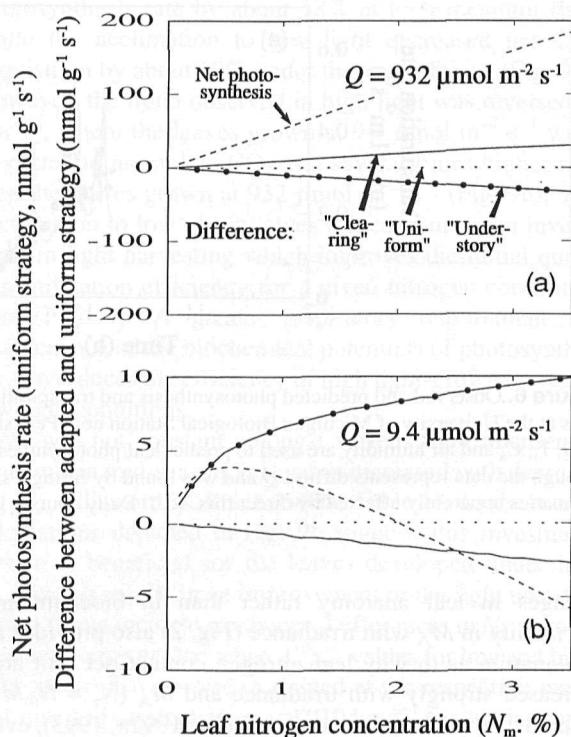


Figure 9. Modelled effects of acclimation to long-term incident light conditions on foliage photosynthetic properties in *A. saccharum*. (a) Differences in leaf photosynthesis with acclimation to incident light conditions ('clearing', 'understory'; model parameters as in Fig. 8a, except for G_f , which was set to a mean value of $14.3 \text{ mol mol}^{-1}$, and α , which was calculated according to Eqn 6, using the C_B and P_L values computed from the equations in Table 1) and without acclimation ('uniform'; all parameters set to a mean value). Environmental conditions were as Fig. 8a, except that Q was set to $932 \mu\text{mol m}^{-2} \text{s}^{-1}$, i.e. to a daily mean quantum flux density in 'clearing' (cf. Ellsworth & Reich 1992a). (b) As (a), except that Q is set to $9.4 \mu\text{mol m}^{-2} \text{s}^{-1}$, i.e. to a daily mean Q in 'understory'.

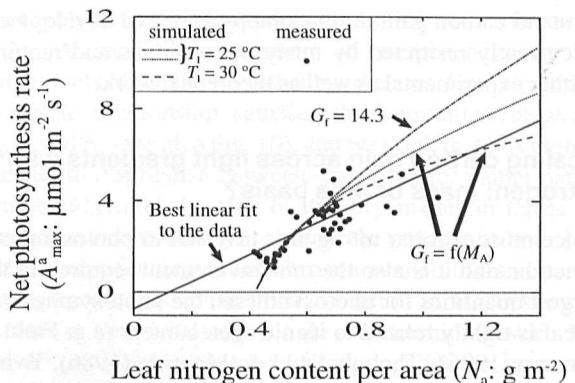


Figure 10. Correlation between net photosynthesis rate per unit area ($A_{\text{max}}^{\text{a}}$) and leaf nitrogen content per unit area, N_{a} (g m^{-2}), in *A. saccharum*. Relationships were modelled for 25 and 30 °C using all equations in Table 1, and for 25 °C holding G_f constant at the medium value of 14.3. Variability in N_{a} was achieved by varying M_{A} and calculating N_{m} from the regression equation in Table 1. ● and solid line: measured values and a linear fit to the data (Ellsworth & Reich 1992a; $A_{\text{max}}^{\text{a}} = -0.72 + 5.89N_{\text{a}}$; $r^2 = 0.51$, $P < 0.001$). Environmental conditions were as in Fig. 8a.

pled electron transport and the light- and CO_2 -saturated photosynthesis rate for *A. saccharum* at lower temperatures (Fig. 7). Of course, we did not consider the adaptive alterations of the temperature response of photosynthetic electron transport to growth temperature (Armond, Schreiber & Björkman 1978; Björkman, Badger & Armond 1978; Berry & Björkman 1980), which is potentially also capable of influencing J_{max} versus temperature dependences. To extend the applicability of the model analysis, we plan in the future to take both W_p limitations and adaptive modifications of thylakoids to growth temperature into account.

Since dark respiration (R_{n}) is dependent on V_{cmax} , the proportion of daily photosynthesis that is lost as day respiration (R_{d}) increases with decreasing Q as soon as Q drops below the value necessary to saturate photosynthesis. Therefore, it is especially important to know the fraction of R_{n} continuing in the light for understory plants exposed to relatively low diffuse Q , and how this proportion is affected as the light climate shifts. There is evidence that R_{d} and R_{n} are approximately the same (Azcón-Bieto & Osmond 1983) or that R_{d} is roughly 0.8 R_{n} (McCashin, Cossins & Canvin 1988). However, in other studies (Laisk 1977; Brooks & Farquhar 1985; Villar, Held & Merino 1995) $R_{\text{d}}/R_{\text{n}}$ decreases asymptotically with increasing Q to values between 0.2 and 0.5. This may mean that a constant $R_{\text{d}}/R_{\text{n}}$ ratio of 0.5 in our model may have underestimated R_{d} at low irradiances and overestimated it at high irradiances.

Model parametrization: effects of growth irradiance

Though V_{cmax} and J_{max} were mostly derived from photosynthetic light response curves (Appendix C), the model parameters calculated from A_{n} versus C_{i} (Ellsworth & Liu

1994) and A_{n} versus light responses (all other data) fitted the same curvilinear relationships between P_{R} , P_{B} and M_{A} (Figs 3a & b). This demonstrates that photosynthetic light response curves gave comparable parameter values to A_{n} versus C_{i} relationships. Moreover, at 25 °C the ratio of $J_{\text{max}}/V_{\text{cmax}}$ was 2.22 ± 0.44 (mean \pm SE, $N = 59$) in the data set of Wullschleger (1993), while the equations depicted in Fig. 3 give $J_{\text{max}}/V_{\text{cmax}} = 2.44$ at a M_{A} of 50 g m^{-2} , again supporting the validity of the current parametrization for *A. saccharum*. Clearly, a balance exists between Rubisco activity and J_{max} (von Caemmerer & Farquhar 1984; Wullschleger 1993). Whenever P_{R} increases, P_{B} is also expected to increase, which will result in similar relationships between P_{B} , P_{R} and M_{A} , as illustrated in Fig. 3.

Currently, evidence supports the trends suggested by our study: both P_{R} and P_{B} increased with increasing irradiance in *Flindersia brayleyana* (Thompson, Stocker & Kriedemann 1988), *Spinacia oleracea* (recalculated from Terashima & Evans 1988) and *Solanum dulcamara* (recalculated from Osmond 1983), whereas P_{B} did so in *Cucumis sativus* (recalculated from Evans 1989a), *Medicago sativa* (recalculated from Evans 1993a), *Phaseolus vulgaris* (recalculated from Evans 1989a) and *Pisum sativum* (recalculated from Evans 1987). Hyperbolically increasing P_{R} and P_{B} with increasing growth irradiance (Fig. 3) are exactly what one would expect (if the upper boundary of photosynthetic nitrogen is fixed) for an optimum partitioning of leaf resources (Friend 1991; Hikosaka & Terashima 1995). Moreover, the same pattern was also seen in P_{R} calculated from *in vitro* laboratory measurements of Rubisco activity (cf. Fig. 3a). However, since relatively long-lived leaves of woody species contain large amounts of defensive phenolic compounds such as tannins (Schultz, Nothnagle & Baldwin 1982; Coley 1983), direct determination of enzyme activities is in many cases seriously handicapped, and so Rubisco activities accounting for less than 20% of observed photosynthetic rates have often been reported for woody species (e.g. Fig. 3a, Tselenker, Chetverikov & Andreyeva 1983; Saied, Douglas & Fry 1994; Ü. Niinemets, personal observation). Thus, we believe that the approach taken here is in many cases currently the only way to gain insight into the nitrogen allocation patterns in different biochemical fractions in forest trees.

Of course, the nitrogen investment in Rubisco is actually larger than that presently derived from photosynthesis measurements, because there are considerable diffusive resistances between the intercellular airspace and carboxylation sites (Lloyd *et al.* 1992; Evans *et al.* 1994; Parkhurst 1994; Epron *et al.* 1995; Syvertsen *et al.* 1995) which are not yet taken into account. Consideration of CO_2 transfer conductance from substomatal cavities to the sites of carboxylation (g_{i}) may be especially relevant in the light of the recent study by Syvertsen *et al.* (1995), which showed that a component of g_{i} , an intercellular gas-phase conductance from the stomatal cavity to the outer surface of mesophyll cell wall (g_{ias}), was negatively correlated with M_{A} . Though g_{ias} is usually relatively large compared to g_{i}

(Evans *et al.* 1994; Syvertsen *et al.* 1995), this observation may indicate that the underestimation of P_R is greater for leaves at higher irradiance. On the other hand, it is well known that stomatal density increases, and the distribution of stomata shifts from hypostomy towards amphistomy with increasing irradiance (Oberbauer, Strain & Riechers 1987; Peat & Fitter 1994), effectively compensating for decreasing g_{ias} (Parkhurst 1994). However, changes in stomatal distribution with respect to adaxial and abaxial leaf surface with irradiance do not appear to play a role in *A. saccharum* (D. S. Ellsworth, Brookhaven National Laboratory, Upton, New York, personal communication). Be that as it may, further testing of the model and its utility for calculating the carbon balance of different canopy layers requires an examination of the dependences of parameters P_B and P_R on irradiance in the natural environment with a number of species.

Initial quantum conversion efficiency was modelled based on the assumption that leaf absorptance is affected by changes in leaf chlorophyll concentration only (Eqn 6). However, ξ is also a function of leaf structure as it influences optical properties (Fukshansky 1981; Osborne & Raven 1986; Lee *et al.* 1990; Vogelmann 1993), and to account for the slight model overestimation of α we may need to modify Eqn 6 with a parameter describing how anatomical changes occurring across light gradients in leaves alter ξ for a given leaf chlorophyll content. Nevertheless, both model predictions and actual measurements agreed in that α is constant with irradiance in mid-season (Fig. 4a). In addition, the model also showed that this constancy is achieved at low irradiances with disproportionate investments of leaf nitrogen in light-harvesting apparatus. Non-linear effects of growth irradiance on thylakoid composition have also been observed previously (Leong & Anderson 1984a,b; Chow *et al.* 1988; Lee & Whitmarsh 1989), and, given that the upper boundary of photosynthetic nitrogen is fixed, represent the optimum strategy for leaf nitrogen partitioning (Hikosaka & Terashima 1995).

As it stands, the model appears also to be useful for handling seasonal data, because the relationship between P_R and M_A is largely independent of the phenological stage of leaves (Fig. 5). Further evidence indicates that leaf chlorophyll concentration declines in direct proportion to total leaf nitrogen during senescence (Zolg & Bornkamm 1981; Evans 1983; Hikosaka 1996), suggesting that the coefficients C_B and P_L at a given M_A may also be used to model the changes in α from leaf maturation till abscission. However, for early season data, changes in P_R , P_B and P_L associated with leaf maturation (Figs 5 & 8b; see also Reich, Walters & Ellsworth 1991) should be taken into account. Other data indicate that the limiting step of photosynthetic electron transport resides before plastohydroquinone reoxidation in developing leaves (Holloway, Maclean & Scott 1983), suggesting that PSII rather than cyt f may provide the appropriate basis for expressing the rates of photosynthetic electron transport during the development of photosynthetic apparatus. Consequently, modelling

of stand carbon gain during canopy leaf area development is currently restricted by many uncertainties and requires further experimental as well as theoretical work.

Scaling carbon gain across light gradients with nitrogen: mass or area basis?

Since most of foliar nitrogen is invested in photosynthetic function, and it is also the mineral element required in the largest quantities for photosynthesis, the photosynthesis of a leaf is tightly related to its nitrogen content (e.g. Field & Mooney 1986). Though Field & Mooney (1986), Evans (1989b) and Field (1991) suggest that this relationship is not fundamentally different on an area or a mass basis, recent studies demonstrate that the measurement basis may crucially alter $A_n - N$ relationships (Reich & Walters 1994; Reich *et al.* 1995). In general, N_a is more variable across the canopy and along light gradients (Ellsworth & Reich 1993; Kull & Niinemets 1993; Niinemets 1995, 1997a) than N_m , which tends to be relatively constant (Chazdon & Field 1987; Walters & Field 1987; Sims & Pearcy 1989; Ellsworth & Reich 1993; Harley & Baldocchi 1995; Tjoelker *et al.* 1995). Similarly, both N_m and A^m_{\max} in *A. saccharum* varied little in mid-season (Fig. 8a) – in marked contrast to A^a_{\max} and N_a (Fig. 10). Furthermore, acclimation to local light conditions, altering nitrogen partitioning between various components of photosynthetic apparatus (Figs 3 & 4) and thereby changing the slope of A^m_{\max} versus N_m dependences, resulted in a statistically insignificant relationship for a pooled set of data (Ellsworth & Reich 1992a; Fig. 8a). Thus, collectively the evidence outlined above seems to favour N_a for obtaining momentary estimates of canopy photosynthesis as is done in numerous models (cf. ‘Introduction’). However, area basis is the product of mass basis and M_A . Given that the acclimation of foliar biochemistry to growth irradiance altered A^m_{\max} at most by 40% (Figs 8 & 9a), and that M_A varied by more than 3-fold (Fig. 2), the positive relationship between A^a_{\max} and N_a in mid-season is primarily the product of plastic changes in leaf anatomy (Fig. 2), which alter the amount of photosynthetic tissue per unit area rather than anything intimately related to ‘nitrogen’. Gutschick & Wiegell (1988) and Baldocchi & Harley (1995) modelled canopy photosynthesis, accounting for the variability in M_A with canopy depth, and concluded that canopy photosynthesis is optimized for a given foliar biomass if M_A declines with increasing canopy depth. A similar conclusion is reached for ‘optimal nitrogen distribution’ on the basis of N_a (Hirose & Werger 1987; Leuning *et al.* 1995; Sands 1995a), and in all situations in which N_m remains constant throughout the canopy. Though the analyses of N_a include ‘nitrogen’, they do not really enhance the understanding based on M_A .

Nevertheless, nitrogen plays a prominent role in the deciduous forest canopy, and in a time dimension over which a canopy model should make useful predictions, N_m varies 4-fold (Figs 5a and 8b), but M_A varies much less (Reich, Walters & Ellsworth 1991). This leads seasonally

to a highly heterogeneous population of N_a values in terms of N_m , M_A and the partitioning of nitrogen between the components of the photosynthetic apparatus. Consequently, no single relationship satisfies the correlation between A^a_{\max} and N_a (see also Fig. 10), and we think that it is highly relevant to distinguish between structural dry matter partitioning (M_A) and the cost of this dry matter in terms of nitrogen concentration (N_m). N_a as the product of both M_A and N_m says nothing about nitrogen availability and partitioning unless the simultaneous variation in M_A is known. Of course, there is also no single relationship between N_m and A^m_{\max} ; rather the slope of this dependence increases with increasing growth irradiance (Fig. 8b). Given that light acclimation is related to M_A (Figs 3 & 4), M_A and N_m contain more information than the product variable N_a .

The shape of the nitrogen versus photosynthesis relationship: is there 'structural' nitrogen?

Scaling of carbon fluxes with nitrogen at the canopy level requires that the form of the relationship between N and A_{\max} be established explicitly. Often these dependences intersect the x -axis at a positive value of leaf nitrogen, N_0 (e.g. Field & Mooney 1986; Evans 1989b; Reich *et al.* 1994). Currently N_0 is interpreted as the basic nitrogen cost of 'structural' or non-photosynthetic components of leaves (Pons *et al.* 1990) or as the minimum leaf nitrogen for photosynthesis (Schulze *et al.* 1994; Leuning *et al.* 1995; Sands 1995a). Thus, one may claim that, if the leaf satisfies N_0 , any extra nitrogen will be invested in the photosynthetic apparatus. Yet, the data supporting the existence of a 'structural' nitrogen pool inherently different from an 'assimilative' pool are fragile. As the analysis of seasonal A^m_{\max} versus N_m relationships in *A. saccharum* revealed, P_R was independent of leaf nitrogen concentration over most of the season, except during the development of leaf photosynthetic apparatus in early spring (Fig. 5b). This pattern was exactly the same in *Oryza sativa* (Makino, Mae & Ohira 1984). Further studies show that P_B and P_R decline in a parallel manner during senescence (Evans 1983). Since the remobilization of 'structural' and 'assimilative' nitrogen proceeds with identical kinetic constants, we suggest that seasonal A^m_{\max} versus N_m relationships should go through the origin. Of course, heterogeneity in P_B and P_R values depending on light-acclimation state in the sample used to develop an A^m_{\max} versus N_m relationship may result in a non-zero x -intercept (cf. Figs 3 & 8), but this is certainly not 'structural' or 'non-photosynthetic' nitrogen.

Many fertilization experiments, where leaf nitrogen concentration has been changed over a broad range at approximately constant growth irradiances, also provide no strong evidence for the existence of N_0 . P_R was independent of nitrogen nutrition regime in *Triticum aestivum* (Evans 1983) and *Oryza sativa* (Makino, Mae & Ohira 1984), P_B was independent in *O. sativa* (Makino, Nakano & Mae 1994), and P_R and P_B were independent in *Flindersia brayleyana* (Thompson, Stocker & Kriedemann 1988); no

clear-cut differences were found between nitrogen availability treatments in the proportion of leaf nitrogen in photosynthetic components in four wild grasses (Pons, van der Werf & Lambers 1994). In contrast, P_R and P_B increased with increasing nitrogen availability in *Solanum dulcamara* (recalculated from Osmond 1983) and *Spinacia oleracea* (recalculated from Evans & Terashima 1987), and P_R did so in *O. sativa* (Makino, Nakano & Mae 1994), whereas P_R , P_B and P_L decreased with increasing nitrogen availability in *T. aestivum* (van den Boogaard *et al.* 1995), and so also did the proportion of enzymes responsible for triose phosphate utilization in *O. sativa* (Makino, Nakano & Mae 1994). Thus, the patterns are contradictory between and within species (cf. *O. sativa* and *T. aestivum*) and collectively do not contribute to the fundamental hypothesis of a basic nitrogen cost of leaf structure independent of nutrient availability. Moreover, in studies highlighting greater nitrogen partitioning into 'assimilative' compartments, investment in Rubisco far exceeds the investment in P_B (Osmond 1983; Evans & Terashima 1987; Makino, Nakano & Mae 1994) or proceeds simultaneously with decreasing nitrogen investments in enzymes responsible for sucrose and starch synthesis (Makino, Nakano & Mae 1994). The latter evidence should be tempered in the light of the opinion that Rubisco also functions essentially as a major storage protein (Millard 1988; Stitt & Schulze 1994), and that optimal P_R and P_B should vary little with nitrogen availability (Friend 1991). In fact, positive y -intercepts occasionally calculated for A^m_{\max} versus N_m relationships (e.g. Leuning, Cromer & Rance 1991; Reich *et al.* 1994, 1995) show that N_0 obtained from regression analysis is physiologically meaningless.

However, in contrast to A^m_{\max} versus N_m relationships, linear regressions of A^a_{\max} versus N_a should not go through origin. This is simply because the lowest values of N_a are determined by M_A (which is always larger than zero) rather than by N_m . In seedlings of *A. saccharum*, the most shaded leaves are expected to have an M_A of not less than 20 g m^{-2} (Fig. 2a), and assuming a realistic value of 0.02 g g^{-1} for N_m , minimum measurable N_a is at least 0.4 g m^{-2} in mid-season – exactly what one observes (Fig. 10). Yet, this minimum N_a is certainly not 'structural' nitrogen. In fact, the A^a_{\max} versus N_a relationship is truncated, having no x -intercept value, and it is important to recognize that N_0 obtained from a linear fit of A^a_{\max} versus N_a relations is a 'fudge factor' and has nothing to do with nitrogen.

Nitrogen partitioning in relation to shade tolerance and succession

Leaf nitrogen in sunny habitats is optimally partitioned if nitrogen is preferentially invested in Rubisco and electron transport components, and in shady conditions if it is directed towards effective light harvesting. However, shade-tolerant *A. saccharum* demonstrated a low potential for biochemical acclimation to high irradiance (Fig. 9a) relative to the adjustment to low irradiance (Fig. 9b). Thus, this species was able to achieve the goal of enhanced light

interception at low light, but failed to improve markedly the efficiency of nitrogen use in high light. Since improved light interception is expensive in terms of nitrogen (Fig. 4), greater N_m in low light (Table 1) may also be related to enhanced light capture in this species. Defining instantaneous nitrogen (E_N) and light (E_L) use efficiencies as A_n per unit leaf nitrogen and incident quanta, respectively, E_N and E_L were calculated as a function of N_a at different incident light values (Fig. 11). It is apparent that a trade-off occurs between E_N , which is higher in greater Q , and E_L , which is maximal at low irradiance. Maximization of E_L always requires somewhat greater N_a than the maximization of E_N at a particular light level. For growth at low irradiances, efficient light harvesting is a more important objective than efficient nitrogen use, and we speculate that the leaves of this shade-tolerant species are adjusted towards higher E_L . Furthermore, this may be valid more generally. Shade-tolerant species tend to have lower fractional investments of foliar nitrogen in Rubisco and photosynthetic electron transport components than intolerant ones. In six rainforest *Piper* species, the slope of the A^{max} versus N_m relationship was nearly 3 times greater for clearing than for understory species (Chazdon & Field 1987), and P_R ranged from 0.06 to 0.24 in the sun-adapted species *Phaseolus vulgaris*, but only from 0.04 to 0.12 in the shade-adapted plant *Alocasia macrorrhiza* (Seemann *et al.* 1987). An identical basic tendency in terms of P_B – early successional and cultured species having greater P_B than late-successional wild plants – is also seen among a

number of species studied under laboratory conditions (Evans 1993a). On the basis of this evidence and of the current synthesis with *A. saccharum*, we hypothesize that shade-tolerant late-successional species invest resources preferentially to improve light harvesting and optimize carbon gain at low Q , while intolerant species invest towards enhancing dark reactions of photosynthesis. However, no differences in biochemical investments in the various components of the photosynthetic apparatus were found between the forest herb *A. macrorrhiza* and the cultured plant *Colocasia esculenta* grown in five light environments (Sims & Pearcy 1989), questioning the generality of this hypothesis. For a rigorous test, more species should be examined for P_R , P_B and P_L functions with respect to growth irradiance as depicted in Figs 3 and 4.

Compromises between water use and growth light environment

G_f (Tenhunen *et al.* 1990, 1994; Sala & Tenhunen 1996) is an important empirical factor for estimating both water use and the C_i at which the leaf photosynthetic apparatus functions for a given C_s and h_s (Eqn 3). G_f decreases as drought-acclimated plants reduce water use. The parameter provides a useful measure of accommodation to water limitation. Decreasing G_f with increasing M_A (Fig. 3c) is in accordance with greater water deficit in saplings growing in open versus shaded habitats (Ellsworth & Reich 1992a,b), and with the lower C_i/C_a ratio of sun versus shade branches (Tjoelker *et al.* 1995) in trees of drought-intolerant (Bahari, Pallardy & Parker 1985) *A. saccharum*. Similarly, the degree of stomatal limitation of photosynthesis increased with increasing M_A in *Prunus persica* (DeJong & Doyle 1985), and a proportionally smaller increase in stomatal conductance as compared to mesophyll conductance was observed in *Liriodendron tulipifera* and *Cornus florida* following experimental creation of canopy gaps (Wallace & Dunn 1980).

Greater radiation loads and consequently higher evaporative demand at high light environments and in the upper canopy require more efficient control of water use. Therefore, the C_i/C_a ratio decreases with increasing irradiance and with height in the canopy (Leuning *et al.* 1995). Ellsworth & Reich (1992a,b), comparing the values of A_{max}^a for plants growing at intermediate ($A_{max}^a = 3.26 \mu\text{mol m}^{-2} \text{s}^{-1}$, $G_f = 14.7 \text{ mol mol}^{-1}$) and high irradiances ($A_{max}^a = 3.32 \mu\text{mol m}^{-2} \text{s}^{-1}$, $G_f = 9.8$), concluded that *A. saccharum* shows a limited potential for biochemical acclimation to higher light. However, apart from biochemical constraints, water relations also play a role, since restricted diffusive conductance decreases C_i and limits the carbon gain in sunny habitats. Greater risk of desiccation is simply a cost paid for growth at high light. Since the gradients of light and water availability are in general inversely related (Bazzaz & Wayne 1994), Abrams (1994) suggested that shade and drought tolerances of species should also vary in opposite directions. This expectation was nicely demonstrated in a comparison between late-successional

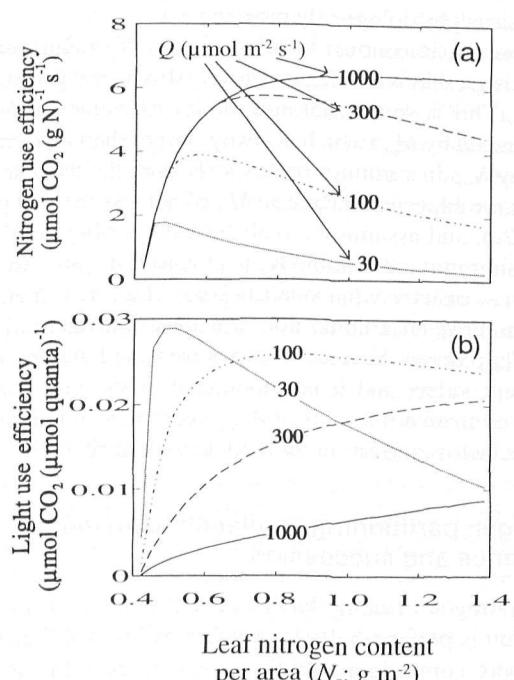


Figure 11. Predicted instantaneous nitrogen and light use efficiencies (E_N and E_L , respectively) for *A. saccharum* as a function of N_a at different incident quantum flux densities. $T_1 = 25^\circ\text{C}$. All model parameters were calculated according to Table 1, and N_a was found as in Fig. 10.

A. macrorrhiza and early-successional *C. esculenta* (Sims & Pearcey 1989); though there were no differences in biochemical acclimation to irradiance, *A. macrorrhiza* had greater diffusive resistances to CO₂ than *C. esculenta*, and this led to lower carbon gain at high irradiances in the late-successional species. As the latter study and the current synthesis with *A. saccharum* demonstrate, in addition to the economies of light harvesting and nitrogen partitioning, changes in water availability may significantly modify the success and distribution of species across the gap-understory continuum.

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APPENDIX A: PARAMETER DERIVATION FOR TEMPERATURE DEPENDENCE OF THE C₃ PHOTOSYNTHESIS MODEL

Rubisco kinetics

Temperature dependence of the specific activity of Rubisco, V_{cr}, is described as (cf. Johnson, Eyring & Williams 1942; Harley *et al.* 1992):

$$V_{\text{cr}} = \frac{e^{\left(c - \frac{\Delta H_a}{RT_k}\right)}}{1 + e^{\left(\frac{\Delta S T_k - \Delta H_d}{RT_k}\right)}}, \quad (\text{A1})$$

where R is the gas constant (8.314 J K⁻¹ mol⁻¹), T_k leaf temperature (K), ΔH_a an activation energy and ΔH_d a deactivation energy, ΔS an entropy term and c a scaling constant. The temperature dependence of light-saturated electron transport rate per unit cyt f is similarly fitted (see the following section), whereas other kinetic constants of Rubisco (cf. Eqn 1) – K_c, K_o, τ -, and R_d – are related to temperature according to:

$$\text{Parameter} = e^{-\frac{\Delta H_a}{RT_k}} \quad (\text{A2})$$

Temperature dependences of the kinetic parameters of Rubisco were obtained from Jordan & Ogren (1984) for purified enzyme from *Spinacia oleracea* (Table A1). Several experimental studies have found differing *in vivo* Rubisco specific activities among C₃ species (e.g. Seemann & Berry 1982). Minor differences in amino acid sequences and in specific activity at 25 °C of Rubisco purified from various C₃ species are described by Parry *et al.*

Table A1. Parameters describing temperature dependences (Eqns A1–A2) of the kinetic constants of Rubisco and photosynthetic electron transport for C₃ plants

Model parameter*	Parameter	Value
V _{cr} [μmol CO ₂ (g Rubisco) ⁻¹ s ⁻¹]	c(V _{cr})	32.9
	ΔH _a (V _{cr})	74000 J mol ⁻¹
	ΔH _d (V _{cr})	203000 J mol ⁻¹
	ΔS(V _{cr})	645 J K ⁻¹ mol ⁻¹
K _c (μmol mol ⁻¹)	c(K _c)	38.08
	ΔH _a (K _c)	80470 J mol ⁻¹
K _o (mmol mol ⁻¹)	c(K _o)	11.88
	ΔH _a (K _o)	14510 J mol ⁻¹
τ	c(τ)	-3.949
	ΔH _a (τ)	-28990 J mol ⁻¹
J _{mc} [μmol e ⁻ (μmol cyt f) ⁻¹ s ⁻¹]	c(J _{mc})	14.77
	ΔH _a (J _{mc})	24100 J mol ⁻¹
	ΔH _d (J _{mc})	564150 J mol ⁻¹
	ΔS(J _{mc})	1810 J K ⁻¹ mol ⁻¹

*Temperature constants of Rubisco were derived from Jordan & Ogren (1984; *Spinacia oleracea*), and of photosynthetic electron transport from Nolan & Smillie (1976; *Hordeum vulgare*; see also Fig. A2).

(1987). Apparently, the Rubisco specificity factor calculated for a given C_i may differ between species due to varying internal transport resistances and CO₂ concentrations at the carboxylation site (Evans *et al.* 1994; Epron *et al.* 1995).

Cytochrome f limitation of electron transport

Evans (1993a), considering the conservative structure of electron transport integrated within the thylakoid membranes, proposed a constant value of 148 mol e⁻ (mol cyt f)⁻¹ s⁻¹ for potential photosynthetic electron flow per unit cyt f, J_{mc}, at 25 °C in C₃ plants. Use of several new data sources provides an updated value of 156 mol e⁻ (mol cyt f)⁻¹ s⁻¹ at 25 °C (Fig. A1). Accepting the latter estimate, we assumed that the temperature dependence of J_{mc} was of the form found for 2,6-dichlorophenolindophenol (DCIP) reduction by uncoupled chloroplasts of *Hordeum vulgare* (Nolan & Smillie 1976; Fig. A2), and a scaling factor, μmol electrons (μmol cyt f)⁻¹ / [μmol DCIP (g chl)⁻¹], was found using a J_{mc} value of 156 μmol electrons (μmol cyt f)⁻¹ s⁻¹ at 25 °C (slope of the regression in Fig. A1) to derive the temperature parameters of cyt f limitation of electron transport (Table A1). The temperature stability of thylakoid membranes and consequently the shape of the response curve in Fig. A2 depend on growth temperature and the genetic potentials of species (Björkman, Badger & Armond 1978; Berry & Björkman 1980). These adaptive and regulatory phenomena, which may be associated with the temperature parameters of thylakoid electron transport (Table A1), relate to the response of plants under high- and

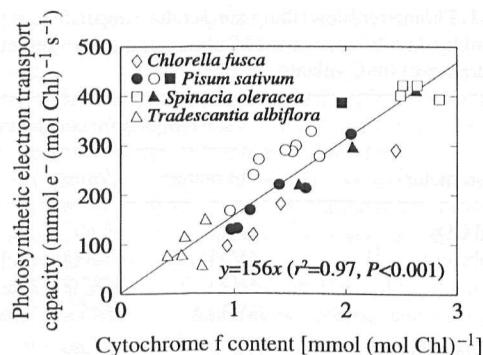


Figure A1. Relationships between the capacity of photosynthetic electron transport estimated from O_2 evolution at saturating irradiance and CO_2 concentration, and cytochrome f content at 25 °C. Rates of O_2 evolution were converted to rates of electron transport assuming that one molecule of O_2 is released per four electrons driven through the photosynthetic electron transport chain. Data were standardized to a common temperature using the temperature coefficients of photosynthetic electron transport given in Table A1. Data are from Wilhelm & Wild (1984) (◇), Evans (1987) (●, means per treatment), Chow & Anderson (1987a,b) (○), Lee & Whitmarsh (1989) (■), Evans & Terashima (1987) (□), Terashima & Evans (1988) (▲), and Chow, Adamson & Anderson (1991) (△).

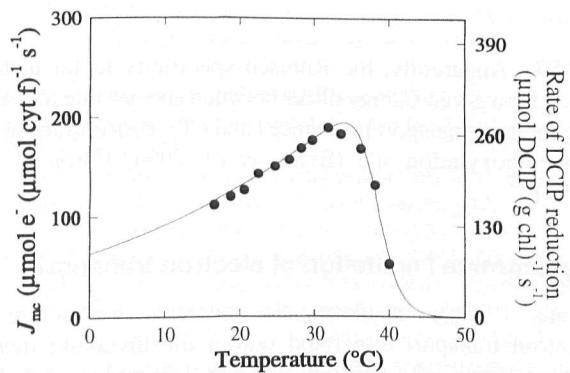


Figure A2. Temperature dependence of the reduction of 2,6-dichlorophenolindophenol (DCIP) of uncoupled chloroplasts of *Hordeum vulgare* (●: Nolan & Smillie 1976) and of photosynthetic electron transport rate per unit cytochrome f, J_{mc} (solid line, parameter values in Table A1), scaled as described in the text to results from a number of C₃ species (Fig. A1).

low-temperature stress. Such potential plant behaviour may be very important to characterize, but it is not considered in the current model version.

APPENDIX B: ESTIMATION OF FOLIAR NITROGEN COST FOR LIGHT HARVESTING

Nitrogen investment in light harvesting depends on the concentration and stoichiometry of chlorophyll protein complexes associated with photosystems I (PSI) and II (PSII), and with light-harvesting chlorophyll protein complexes of PSII (LHCII) (Evans & Seemann 1989; Hikosaka & Terashima 1995). Apart from light-harvesting function,

certain thylakoid chlorophyll-protein complexes participate in electron transport, and according to the current model of electron transport (cf. Methods) their concentration should scale with cytochrome f. This holds in particular for the concentration of photosystem II reaction centres, which is strongly related to potential photosynthetic electron flow (Evans 1987; Hikosaka & Terashima 1995). In the light of this correlation, Hikosaka & Terashima (1995) took the content of PSII as being directly proportional to light-saturated net photosynthesis rate. However, a comparison of actually measured PSII contents (data in Fig. B1) with those calculated according to Hikosaka & Terashima revealed an overall poor fit ($r^2 = 0.55$). Furthermore, their formula appeared to consistently underestimate PSII content at low values of leaf photosynthesis and overestimate at higher values: for example, in *Pisum sativum* (Evans 1987) at a growth irradiance of 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ the mean oxygen evolution rate at saturating CO_2 concentration and light was 32.9 $\text{mmol O}_2 \text{ mol Chl}^{-1} \text{ s}^{-1}$, and the respective PSII content 1.94 $\text{mmol (mol Chl)}^{-1}$, but it was only 1.01 as based on the model of Hikosaka & Terashima. This discrepancy arises because, apart from electron transport function, the complexing of thylakoid membranes always requires a certain amount of PSII. Given this extra PSII, the slope of PSII versus electron transport activity should increase with decreasing capacity for photosynthetic electron transport. Therefore, a curvilinear relationship is appropriate to estimate PSII content from leaf electron transport potentials (Fig. B1). Taking the content of PSI as proportional to total

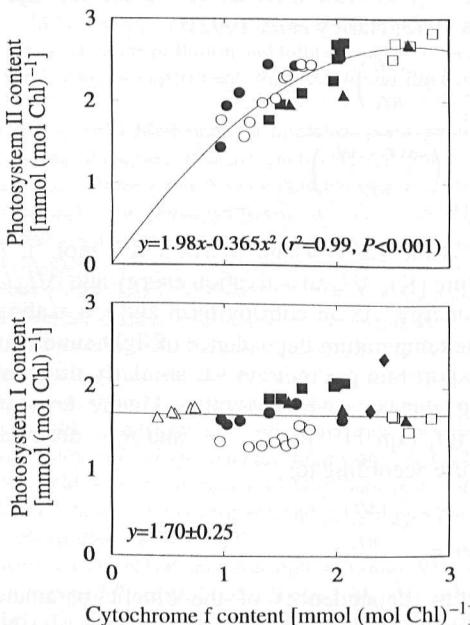


Figure B1. Dependence of thylakoid composition on cytochrome f content. Variability in thylakoid composition was achieved by growing plants under different irradiances or at various nutrient availabilities. In the case of photosystem II, the contents of total rather than functional photosystems are plotted (see e.g. Chow, Hope & Anderson 1989). All symbols are as in Fig. A1, except for ♦: *Beta vulgaris* (Spiller & Terry 1980).

leaf chlorophyll (Fig. B1; Hikosaka & Terashima 1995), LHCII content is calculated from the difference between total leaf chlorophyll and the chlorophyll associated with PSI and PSII (Hikosaka & Terashima 1995). Finally the fraction of leaf nitrogen in light harvesting, P_L , is given as:

$$P_L = \frac{C_C}{N_m} \left(\frac{P_{C(\text{PSI})}}{C_{B(\text{PSI})}} + \frac{P_{C(\text{PSII})}}{C_{B(\text{PSII})}} + \frac{P_{C(\text{LHCII})}}{C_{B(\text{LHCII})}} \right) = \frac{C_C}{N_m C_B}, \quad (\text{B1})$$

where C_C is leaf chlorophyll concentration (mmol g^{-1}), $P_{C(\text{PSI})}$, $P_{C(\text{PSII})}$ and $P_{C(\text{LHCII})}$ are the fractions of leaf chlorophyll associated with PSI, PSII and LHCII, respectively, and the chlorophyll bindings of the thylakoid protein complexes used in the current model [0.858 $\text{mmol Chl (g N)}^{-1}$ for PSII, 2.18 for PSI and 2.75 for LHCII] are those from Hikosaka & Terashima (1995). Since the chlorophyll binding of various thylakoid protein complexes is still not exactly known (Hikosaka & Terashima 1995), C_B for the whole leaf is defined in the model as the weighted average of chlorophyll bindings of PSI, PSII and LHCII. C_B and P_L may be estimated for a collection of leaves where light response curves have been determined, since estimates of P_B at light saturation provide values for cytochrome f content in each specific leaf which may be used with the relations from Fig. B1 to derive the fractions of chlorophyll associated with PSI, PSII and LHCII.

APPENDIX C: DERIVATION OF V_{cmax} AND J_{max} FROM PHOTOSYNTHESIS VERSUS LIGHT RESPONSE CURVES AT CURRENT AMBIENT CO₂ CONCENTRATIONS

We assume that at present ambient CO₂ concentrations the saturated portion of the light response curve is determined by Rubisco activity ($W_c < W_j$) (Fig. C1; see also Sukenik, Bennett & Falkowski 1987; Ögren 1993; Ögren & Evans 1993). Thus, calculating the C_i value from A_{max} and stomatal conductance according to Fick's law, correcting net photosynthesis for non-photorespiratory losses of CO₂ (R_n) to yield the gross photosynthesis rate (A_{gmax}), and considering that all Rubisco is in the fully activated state, one may directly compute V_{cmax} . In so far as calculated V_{cmax} was relatively insensitive to R_n correction, we assume that $A_{\text{gmax}} = A_{\text{max}} + 0.5R_n$.

Considering that the light saturation of net assimilation is reached at an incident quantum flux density, Q , at which $W_c = W_j$ (Fig. C1), the capacity for light reactions of photosynthesis may be calculated from W_c and saturating Q (Q_{sat} , Fig. C1). Writing

$$W_j = \frac{JC_i}{4(C_i + 2I^*)} \quad (\text{C1})$$

and

$$W_c = \frac{V_{\text{cmax}} C_i}{C_i + K_c \left(1 + \frac{O}{K_o} \right)} \quad (\text{C2})$$

(Farquhar, von Caemmerer & Berry 1980; Harley & Tenhunen 1991), substituting Eqn 2 in Eqn C1, and revealing Q at $W_j = W_c$ gives Q_{sat} as:

$$Q_{\text{sat}} = \frac{1}{\alpha} \left(\frac{1}{16W_c^2 \left(1 + \frac{2I^*}{C_i} \right)^2} - \frac{1}{J_{\text{max}}^2} \right)^{-\frac{1}{2}}. \quad (\text{C3})$$

The light-response curve of photosynthesis is abruptly truncated only if the gradients in the light absorption and photosynthetic potentials of leaf cells are identical across the leaf. However, recent experimental evidence shows that these gradients are not the same and that net photosynthesis saturates at higher Q values than those established by Eqn C3 (Evans, Jakobsen & Ögren 1993; Ögren 1993).

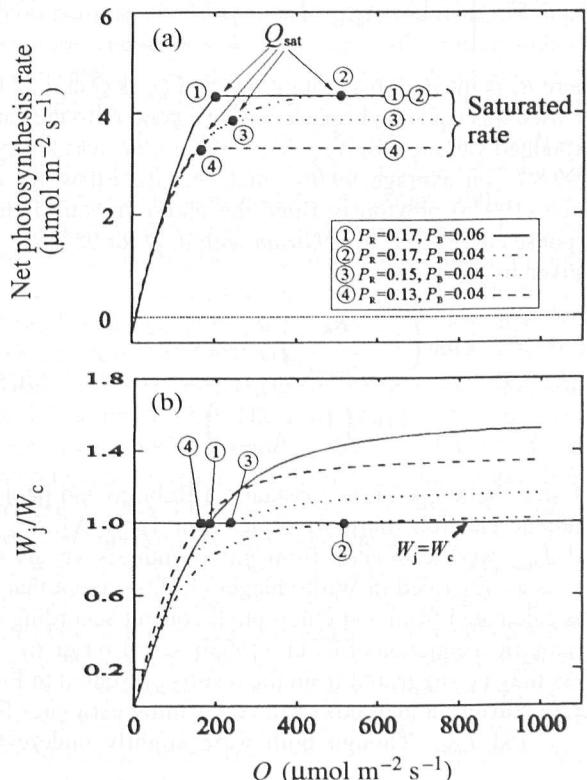


Figure C1. Illustration of the light response of (a) net photosynthesis and (b) the ratio of W_j to W_c in *A. saccharum* as affected by the nitrogen partitioning between bioenergetics and carboxylation (P_B and P_R). T_1 and intercellular CO₂ concentration (C_i) were fixed at representative values of 25 °C and 260 $\mu\text{mol mol}^{-1}$, respectively. Leaf nitrogen concentration per unit dry mass (N_m), leaf dry mass per unit area (M_A) and initial quantum conversion efficiency on an incident light basis (α) were held constant at 0.0154 g g⁻¹, 45.3 g m⁻² and 0.225 mol electrons (mol quanta)⁻¹, respectively. W_j equals W_c at the crossover point of photosynthetic light response, where photosynthesis becomes limited by W_c . Photosynthetic quantum flux density (Q) at $W_c = W_j$ is termed saturating quantum flux density (Q_{sat} , $\mu\text{mol m}^{-2} \text{s}^{-1}$; see Eqn C3). P_B , necessary for the same Q_{sat} , increases with increasing A_{gmax} (with increasing P_R). 1: $Q_{\text{sat}} = 210$; 2: $Q_{\text{sat}} = 500$; 3: $Q_{\text{sat}} = 255$; 4: $Q_{\text{sat}} = 165$.

This would inevitably lead to an underestimation of J_{\max} . Nevertheless, in the paper of Ellsworth & Reich (1992a) Q_{sat} was defined as the Q level required to reach 95% of the light-saturated value of net photosynthesis. Given the asymptotic nature of the photosynthesis versus quantum flux relationship, defining Q_{sat} in this way may give a better basis for determination of J_{\max} from experimentally established values of saturating quantum flux.

To test the applicability of the outlined method for calculation of V_{cmax} and J_{\max} from net photosynthesis versus light-response curves, the data of Sims & Pearcy (1989), which contain the dependences of photosynthesis on both intercellular CO_2 and incident Q , were re-analysed. Photosynthesis versus Q relationships were fitted by an empirical equation of Hanson *et al.* (1987):

$$A_n = A_{n\max} \left[1 - \left(1 - \frac{R_n}{A_{n\max}} \right)^{1 - \frac{Q}{Q_c}} \right], \quad (\text{C4})$$

where R_n is the dark respiration rate and Q_c is Q at $A_n = 0$. We used this equation because it gave a good fit to the data (explained variance for non-linear regressions was always $> 99.8\%$, on average 99.9%) and because Ellsworth & Reich (1992a) previously fitted the photosynthetic light-response curves of *A. saccharum* with it. Q for 95% $A_{n\max}$ is given by:

$$Q_{\text{sat}} = Q_c \frac{\log \left(1 - \frac{R_n}{A_{n\max}} \right) - \log (0.05)}{\log \left(1 - \frac{R_n}{A_{n\max}} \right)}. \quad (\text{C5})$$

Using the temperature constants of Rubisco and photosynthetic electron transport depicted in Table A1, V_{cmax} and J_{\max} were estimated from photosynthesis versus C_i curves as described in Wullschleger (1993), except that α was calculated from leaf chlorophyll content according to an empirical equation of Evans (1993b; see also Eqn 6).

As may be suggested from the results presented in Fig. C2, the different methods gave very similar estimates for V_{cmax} and J_{\max} . Though both were slightly underesti-

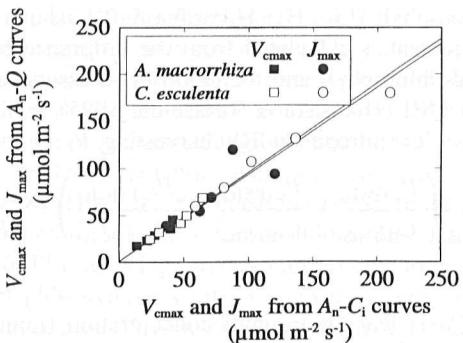


Figure C2. Relationships between V_{cmax} and J_{\max} obtained from photosynthetic light-response curves measured at an ambient CO_2 concentration of $340 \mu\text{mol mol}^{-1}$, and between V_{cmax} and J_{\max} calculated from photosynthesis versus intercellular CO_2 curves. Data are from Sims & Pearcy (1989) for *Alocasia macrorrhiza* and *Colocasia esculenta*. For V_{cmax} : $y = 1.04x$ ($r^2 = 0.99$, $P < 0.001$); for J_{\max} : $y = 1.06x$ ($r^2 = 0.98$, $P < 0.001$).

mated from light-response curves (Fig. C2), the ratios of J_{\max}/V_{cmax} from light- (2.62 ± 0.11) and CO_2 -response curves (2.62 ± 0.03) were not different (t -test, $P > 0.9$), signifying that the photosynthetic light-response curves measured at current ambient CO_2 concentrations may be used for derivation of the parameters of biochemical photosynthesis models. Although the definition of Q_{sat} as Q , which is necessary to reach 95% of $A_{n\max}$, resulted in an excellent agreement between the values of J_{\max} estimated from A_n versus Q and A_n versus C_i curves for the herbs *Alocasia macrorrhiza* and *Colocasia esculenta*, in the further development of the method one needs to define explicitly how Q_{sat} is related to leaf structure and the gradients of light and photosynthetic capacity across the leaves (e.g. Evans, Jakobsen & Ögren 1993; Ögren 1993). Nevertheless, given the similar range of leaf dry mass per area and growth light environments for *A. macrorrhiza*, *C. esculenta* (Sims & Pearcy 1989) and for the seedlings of *A. saccharum* (Ellsworth & Reich 1992a), we suggest that the above definition of Q_{sat} should give J_{\max} values from A_n - Q curves, which are in close agreement with those from A_n - C_i curves for *A. saccharum*.

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