

# Increases of chlorophyll *a/b* ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light

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## ABSTRACT

According to the theory of optimal nitrogen partitioning within a leaf, the chlorophyll (Chl) *a/b* ratio is expected to increase when leaf N content decreases. Here, we report the first empirical support for this prediction. The Chl *a/b* ratio increased while Chl content decreased in response to N limitation in photosynthetic cotyledons and leaves of seedlings of four tropical woody species in the Bignoniaceae. The responses of all four species were in the same direction, but differed in magnitude. For *Tabebuia rosea*, the species that exhibited the greatest increase in Chl *a/b* ratios (up to values of 5.9), detailed photosynthetic characteristics were also examined. Light and N availability were positively correlated with the light- and CO<sub>2</sub>-saturated photosynthetic O<sub>2</sub> evolution rate, as well as with leaf carboxylation capacity ( $V_{\text{cmax}}$ ) and electron transport rate ( $V_j$ ). Severe N limitation and high light did not cause chronic photo-inhibition (i.e. no change in quantum yield or in dark-acclimated  $F_v/F_m$ ). The observed change in the ratio of  $V_{\text{cmax}}$  to leaf N in response to N availability was consistent with likely functional reasons for change in the Chl *a/b* ratio. Adjustment of the Chl *a/b* ratio was apparently an integral feature of acclimation to high light conditions and low N availability.

**Key-words:** *Callichlamys latifolia*; *Pithecoctenium crucigerum*; *Tabebuia guayacan*; *Tabebuia rosea*; Bignoniaceae; carboxylation; fluorescence; nitrogen allocation; photo-inhibition; photosynthesis.

## INTRODUCTION

The ability to acclimate to contrasting light environments is particularly important for tropical woody seedlings that initially establish in the deep shade and that later must take advantage of sudden openings in the canopy to grow to larger sizes (Whitmore 1989; Newell *et al.* 1993). Although maternal plants endow each seedling with sufficient N for initial development in shade (Milberg, Pérez-Fernández, &

Lamont 1998; Walters & Reich 2000; Kitajima 2002), low soil N may substantially constrain subsequent growth in gaps (Bungard, Press & Sholes 2000; Coomes & Grubb 2000). High irradiance in tropical latitudes can cause chronic photo-inhibition through impairment of photosystem II (PSII) reaction centres in leaves of plants that have experienced gap openings (Mulkey & Pearcy 1992; Araus & Hogan 1994; Lovelock, Jebb & Osmond 1994). Photo-damage is especially pronounced in plants with low N, although leaves may acclimate to higher light regimes by enhancing photoprotective mechanisms after several weeks (Bungard *et al.* 2000).

Acclimation to a change in irradiance involves optimal N allocation at whole plant, leaf and cellular levels (Hirose & Werger 1987; Evans 1989; Hikosaka & Terashima 1995). Evans (1989, 1993) suggested that shade-leaf chloroplasts should increase the ratio of N invested in thylakoid proteins to that in soluble proteins in order to balance energy capture and energy transfer. Hikosaka & Terashima (1995) further developed a theory of optimal N partitioning in chloroplasts by simulating effects of variable N partitioning to five chloroplast-protein components.

The Chl *a/b* ratio can be a useful indicator of N partitioning within a leaf, because this ratio should be positively correlated with the ratio of PSII cores to light harvesting chlorophyll-protein complex (LHCII) (Terashima & Hikosaka 1995). LHCII contains the majority of Chl *b*, and consequently it has a lower Chl *a/b* ratio (1.3–1.4) than other Chl binding proteins associated with PSII (Evans 1989; Green & Durnford 1996). Thus, the Chl *a/b* ratio is predicted to respond to light and N availability in the following ways (Hikosaka & Terashima 1995);

- 1 Chl *a/b* ratios should increase with increasing irradiance at a given N availability.
- 2 Chl *a/b* ratios should increase with decreasing N availability, especially under high light conditions.

This second prediction has received much less attention than the first prediction. The logic underlying the second prediction can be explained as follows (Hikosaka & Terashima 1995). When N supply becomes limiting under high light, the proportional allocation to PSII should increase at the cost of decreased N allocation to Rubisco, whereas N

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allocation to LHCII is maintained at a similar level. Consequently, the ratio of PSII to LHCII (and the Chl *a/b* ratio) should increase with decreasing N availability.

The first prediction for the relationship between the Chl *a/b* ratio and irradiance has been supported by many studies at the leaf and vegetation-stand levels (e.g. Terashima & Evans 1988; Dale & Causton 1992). However, published data are equivocal regarding the second prediction. The Chl *a/b* ratio and the ratio of PSII to Chl are independent of N availability for spinach (Terashima & Evans 1988). Both leaf N and Chl *a/b* ratios decreased slightly with leaf age in *Ipomoea tricolor* even when self-shading was prevented (Hikosaka 1996). For tropical rainforest tree species, Bunnard *et al.* (2000) found little response in Chl *a/b* ratios to light or N, whereas Thompson, Huang & Kriedemann (1992) found lower Chl *a/b* ratios in low N plants, a response opposite to the theoretical prediction.

Here we report the first empirical support for the prediction of increasing Chl *a/b* ratios in response to N limitation, a result found during an experimental study that examined whole-plant growth responses to light and nitrogen availability for seedlings of four Bignoniaceae species (Kitajima 2002). In order to assess whether the observed increases in Chl *a/b* ratios were the result of optimal acclimation or were due to impaired physiology under high light in N-deficient leaves, we quantified photosynthetic characteristics including quantum yields and chlorophyll fluorescence for the species (*T. rosea*) that showed the strongest response in Chl *a/b* ratios. Changes in allocation to different components of photosynthetic system were examined indirectly via the ratios of various photosynthetic parameters to overall N and Chl content.

## METHODS

### Study site and species

All experiments and measurements were conducted on Barro Colorado Island (BCI), Panama, using four woody species in Bignoniaceae that are common in this seasonal moist tropical forest. *Tabebuia rosea* DC. and *Tabebuia guayacan* (Seem.) Hemsl. are canopy trees, whereas *Calli-chlamys latifolia* K. Schum. and *Pithecoctenium crucigerum* A. Gentry are lianas whose adults reach the forest canopy. These species differ in the degree of shade tolerance as measured by recruitment probability of seedlings per dispersed seed and by survival probability from the first to second year (Kitajima 2002; S. J. Wright unpublished results). *Calli-chlamys latifolia* is the most shade-tolerant, followed by *P. crucigerum*, *T. rosea*, and finally *T. guayacan*. Seedlings of *T. rosea* and *T. guayacan* germinate in the shaded understorey, but rarely survive until the end of the first year unless they are within treefall gaps. In contrast, >1-year-old seedlings of *C. latifolia* are common in the shaded understorey. The study species also differ in cotyledon morphology. Cotyledons of *T. rosea* and *T. guayacan* expand to become leaf-like photosynthetic organs, whereas green cotyledons of *C. latifolia* remain thick and have just

enough photosynthetic capacity to compensate for the cotyledons' own dark respiration (Kitajima 1992, 2002). Cotyledons of *P. crucigerum* remain inside the seed coat and serve only as storage organs. Additional ecological and taxonomic information for these species is available in Croat (1978).

### Growth conditions

Details of the growth conditions and results of functional growth analysis are given in Kitajima (2002). Summarized here is the relevant background information for the current study focusing on acclimation responses at the leaf level. At the time of radicle emergence, germinating seeds were transplanted singly into pots filled with sand and vermiculite (1 : 1 ratio), and were randomly assigned to light and N treatment combinations (40–120 plants per treatment combination for each species, reflecting differences in seed availability). Pot position was changed daily, and pots were rotated among three benches within each light treatment. Plants in the high light treatment received 27% of total daily photon flux density (PFD) above the canopy (6.77 mol m<sup>-2</sup> d<sup>-1</sup> averaged over 12–24 d; daily maximum PFD up to 1100 μmol m<sup>-2</sup> s<sup>-1</sup>), whereas plants in the low light treatment were located beneath layers of shade cloth and received 1.2% of the total daily PFD (0.29 mol m<sup>-2</sup> d<sup>-1</sup>; daily maximum PFD below 100 μmol m<sup>-2</sup> s<sup>-1</sup>), corresponding to deep shade in the forest understorey. Nitrogen treatments were created by saturating each pot daily with 20 mL of one of three nutrient solutions. One-fifth strength modified Johnson solution containing all macro- and micro-nutrients including 2.2 mM NO<sub>3</sub><sup>-</sup> and 0.42 mM NH<sub>4</sub><sup>+</sup> (Epstein 1972) was used for the high N treatment. This N concentration is comparable to the highest N concentration recorded in stream water on BCI (M. Keller and R. Stallard, pers. comm.). The low N solution had the same composition as the high N solution, except that 90% of the NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> was replaced with Cl<sup>-</sup> and K<sup>+</sup>, respectively, whereas all N ions were replaced with Cl<sup>-</sup> and K<sup>+</sup> in the zero N solution.

Cotyledons of all species expanded fully by 10–14 d after radicle emergence, whereas the first pair of opposite leaves expanded 10–28 d after radicle emergence. In all species, leaf area and seedling biomass growth responded to N supply in high light, but not in shade. In the two *Tabebuia* spp., seed N reserves supported the seedlings' N demands for 60 d in shade, but for only 14 d in high light. Plants grown in high light with zero N supply, and plants in shade at all nitrogen levels, did not develop the second pair of leaves. Subsequent growth in zero and low N treatment in high light was accompanied by dilution of tissue nitrogen concentration. In the other two species (which have storage cotyledons) the response of seedling biomass to light occurred later (11–14 d) than in *Tabebuia* spp. (7 d), whereas the response to N occurred either at a similar time (12 d in *P. crucigerum*) or much later (> 35 d in *C. latifolia*, which exhibits a high nitrogen concentration in its seed).

## Chl and N measurements

For all species, Chl was extracted in 90% acetone to determine Chl contents and Chl *a/b* ratios of fully expanded cotyledons and the first pair of leaves. For *T. rosea*, we sampled fully expanded cotyledons (of 30-day-old seedlings), the first pair of leaves (of 30-day and 50-day-old seedlings), and leaves used in physiological measurements. For the other three species, the seedling age at sampling varied (24 d and 50 d for leaves of *C. latifolia*; 50 d for leaves of *P. crucigerum*; 65 d for cotyledons and leaves of *T. guayacan*). Seedling age at sampling was older for *T. guayacan* because of differences in the timing of growth experiments and in the sample size, but its leaves and cotyledons appeared similar to those of 50-day-old *T. rosea*. None of these leaves or cotyledons exhibited colours that would indicate anthocyanin or delayed greening. Each sample (a whole cotyledon or a 10-cm<sup>2</sup> leaf disc) was ground with a chilled mortar and a pestle in 1 ml of pure acetone with a pinch of MgCO<sub>2</sub> to prevent pheophytin formation (Linder 1974), and the pigment extract in 90% acetone was analysed for Chl *a* and Chl *b* following Jeffrey & Humphrey (1975). The other sample from each pair of cotyledons or leaves was dried at 60 °C for subsequent determination of dry mass per area (for all species). Total Kjeldahl N was determined only for *T. rosea*, by using Nessler colorimetry (Allen 1974).

## Gas exchange measurements

Photosynthetic gas exchange characteristics were examined only for *T. rosea*, the species that showed the strongest response in Chl *a/b*. Photosynthetic light curves were determined with a leaf-disc O<sub>2</sub> electrode (Model LD2; Hansatech, Norfolk, England) for the first true leaves of 50-day-old-seedlings. Light was supplied to the adaxial side with a computer-controlled array of light-emitting diodes (LS3; Hansatech). After induction at 100 μmol m<sup>-2</sup> s<sup>-1</sup> with a continuous supply of air containing 5% CO<sub>2</sub> for 5–10 min to reach a steady state, the chamber was closed and O<sub>2</sub> evolution rates were measured in steps from 100 up to 400 μmol m<sup>-2</sup> s<sup>-1</sup>, and then down from 100 to 0 μmol m<sup>-2</sup> s<sup>-1</sup>. At each step, the rate was recorded as soon as it reached quasi-steady state (60–200 s). Light saturation occurred around 200 μmol m<sup>-2</sup> s<sup>-1</sup>, and the linear region of the light curves at 0–70 μmol m<sup>-2</sup> s<sup>-1</sup> was used to calculate the apparent quantum yield (= initial slope; mol O<sub>2</sub> mol<sup>-1</sup> incident photons) and dark respiration (the intercept). Gross photosynthetic rates at 400 μmol m<sup>-2</sup> s<sup>-1</sup> are reported as light- and CO<sub>2</sub>-saturated photosynthetic rates ( $P_{max}$ ). Absorbance of PFD by cotyledons and leaves was determined with a LI-1800 spectroradiometer (Li-Cor, Lincoln, NE, USA) equipped with an integrating sphere to calculate true quantum yields.

The  $F_v/F_m$  ratio of dark-acclimated leaves of 55-day-old-seedlings was measured with a pulse amplitude modulated fluorometer (PAM-101; Waltz, Effeltrich, Germany) as described by Schreiber (1986). Plants were brought into the

laboratory before dawn and kept in dark at a high humidity until measurements were made in the morning.

Using an open-system gas analyser (LCA3; ADC, Hoddesdon, UK), carboxylation capacity was estimated from measurements of net CO<sub>2</sub> assimilation rates ( $A$ ) at different CO<sub>2</sub> concentrations under saturating light provided by a metal halide lamp and adjusted by neutral filters (650 and 430 μmol m<sup>-2</sup> s<sup>-1</sup> for high- and low-light grown plants, respectively). On the day of measurements, plants were brought into a growth chamber in which air temperature and relative humidity were kept constant (28 °C and 75%). The ambient CO<sub>2</sub> was adjusted so that the estimated intercellular CO<sub>2</sub> pressure ( $p_i$ ) was increased from 34 to 70–80 Pa, and then was decreased from 34 to 5 Pa in 10–15 Pa intervals. During measurements, conductance to water vapour was between 50 and 200 mmol m<sup>-2</sup> s<sup>-1</sup>, whereas leaf temperature averaged at 29 °C. Carboxylation capacity ( $V_{cmax}$ ) and electron transport rates ( $V_j$ ) were estimated from non-linear curve fitting to the  $A-p_i$  relationship as described in Kirschbaum & Farquhar (1984) and Sims & Pearcy (1989), with the following parameters for Rubisco at 29 °C:  $K_m$ , the Michaelis–Menten constant for CO<sub>2</sub> by Rubisco after taking the competitive inhibition of O<sub>2</sub> into account = 86.7 Pa;  $K_a$ , Michaelis–Menten constant for activation of Rubisco = 4.0 Pa; and  $GSTAR$ , the CO<sub>2</sub> compensation point in the absence of non-photorespiratory respiration = 4.1 Pa.

Statistical analysis of the effects of N and light availability on N contents and physiological characteristics were examined using analysis of covariance (ANCOVA) implemented in JMP software (SAS Institute, Cary, NC, USA).

## RESULTS

### Chl response to light and nitrogen

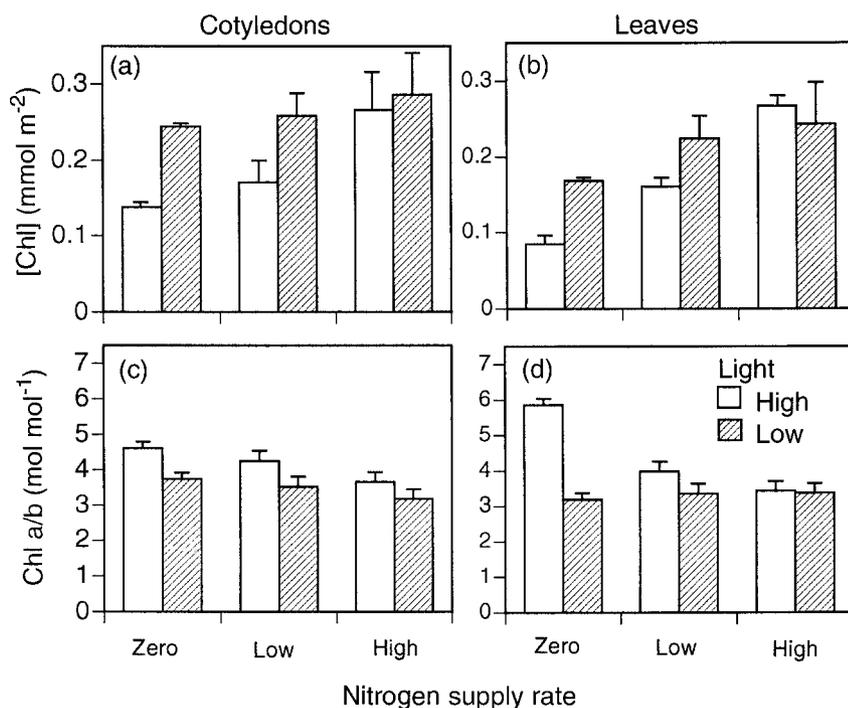
In *T. rosea*, leaf N content was positively correlated with N supply within any given light treatment (Table 1). Leaves under low and zero N supply in high light were yellow-green and had lower PFD absorption relative to other treatments (Table 1). N limitation decreased the total Chl per unit leaf area, [Chl], and Chl per unit mass, but increased the Chl *a/b* ratio in cotyledons and leaves (Fig. 1, Table 1). Under low and zero N supply, [Chl] was lower whereas the Chl *a/b* ratio was higher for plants grown in high light than for those grown in low light (Fig. 1). In contrast, [Chl] and the Chl *a/b* ratio were similar between the two light treatments under high N supply (Fig. 1). Shade leaves of *T. rosea* had a higher [Chl] than did sun leaves for a given leaf N per unit area (Fig. 2a), and [Chl] was positively correlated with leaf N within each light environment. The Chl *a/b* ratio was negatively correlated with leaf N in sun, but not in shade (Fig. 2b).

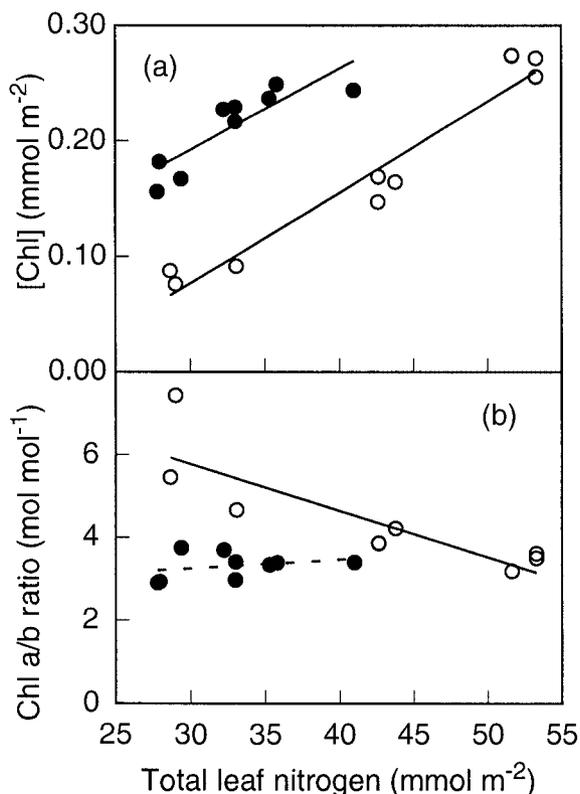
Figure 3 shows the relationship between the Chl *a/b* ratio and [Chl] for the four study species. On average, shade leaves had higher [Chl] and correspondingly lower Chl *a/b* ratios than did high-light leaves. The slope of the correlation between [Chl] and the Chl *a/b* ratio did not differ

**Table 1.** Physiological characteristics of the first pair of true leaves from 55-day-old seedlings of *Tabebuia rosea* grown under six treatment combinations, including photosynthetic light-curve characteristics determined with a leaf-disc oxygen electrode with saturating CO<sub>2</sub>, and dark acclimated  $F_v/F_m$  determined by chlorophyll fluorescence

Light treatment	High light			Low light			Two-way ANOVA <i>P</i>	
	High	Low	Zero	High	Low	Zero	Light (interaction)	N
Nitrogen treatment								
N per leaf area (mmol m <sup>-2</sup> )	53.2 (1.6)	42.6 (6.9)	30.3 (2.5)	37.4 (3.2)	33.0 (0.9)	27.5 (27.5)	0.0001 (0.02)	0.0001
Leaf mass per area (g m <sup>-2</sup> )	29.9 (2.1)	30.4 (0.4)	29.5 (3.1)	12.8 (0.2)	10.8 (0.2)	11.8 (0.3)	0.0001 (NS)	NS
Chl per unit leaf area (mmol m <sup>-2</sup> )	0.267 (0.010)	0.161 (0.011)	0.085 (0.008)	0.243 (0.006)	0.224 (0.006)	0.169 (0.013)	0.0001 (0.0001)	0.0001
PAR absorption	0.877 (0.004)	0.808 (0.012)	0.706 (0.030)	0.852 (0.005)	0.852 (0.005)	0.815 (0.014)	0.0001 (0.001)	0.0001
Quantum yield (mol O <sub>2</sub> mol <sup>-1</sup> photon)	0.091 (0.004)	0.087 (0.001)	0.087 (0.001)	0.098 (0.009)	0.094 (0.009)	0.089 (0.006)	NS (NS)	NS
Dark respiration (μmol O <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	-1.26 (0.13)	-1.15 (0.24)	-1.52 (0.09)	-1.27 (0.07)	-1.26 (0.20)	-1.42 (0.36)	NS (NS)	NS
$P_{max}$ per unit leaf area (μmol O <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	8.94 (1.77)	5.76 (1.70)	3.91 (0.37)	3.96 (0.50)	3.67 (0.35)	2.69 (0.25)	0.0001 (0.02)	0.0009
$P_{max}$ per unit Chl (mmol O <sub>2</sub> mol Chl <sup>-1</sup> s <sup>-1</sup> )	38.2 (6.5)	43.3 (10.3)	64.1 (2.9)	21.5 (1.7)	22.0 (2.6)	24.4 (1.1)	0.001 (0.05)	0.002
$P_{max}$ per unit N (mmol O <sub>2</sub> mol N <sup>-1</sup> s <sup>-1</sup> )	0.194 (0.035)	0.160 (0.033)	0.180 (0.010)	0.140 (0.003)	0.150 (0.012)	0.145 (0.012)	0.007 (NS)	NS
$F_v/F_m$	0.78 (0.01)	0.78 (0.01)	0.76 (0.01)	0.76 (0.02)	0.77 (0.01)	0.76 (0.01)	NS (NS)	NS

Mean (standard deviation) is indicated for three plants. Statistical significance levels of treatment effects are indicated as *P*-values from a two-way ANOVA (NS: *P* > 0.05).

**Figure 1.** Effects of N supply on Chl content per unit area (a, b) and Chl *a/b* ratios (c, d) for cotyledons of 30-d seedlings (left side, a, c) and leaves of 50-d seedlings (right side, b, d) of *Tabebuia rosea* grown under high- and low-light treatments (open and closed symbols, respectively). Mean with standard deviation.

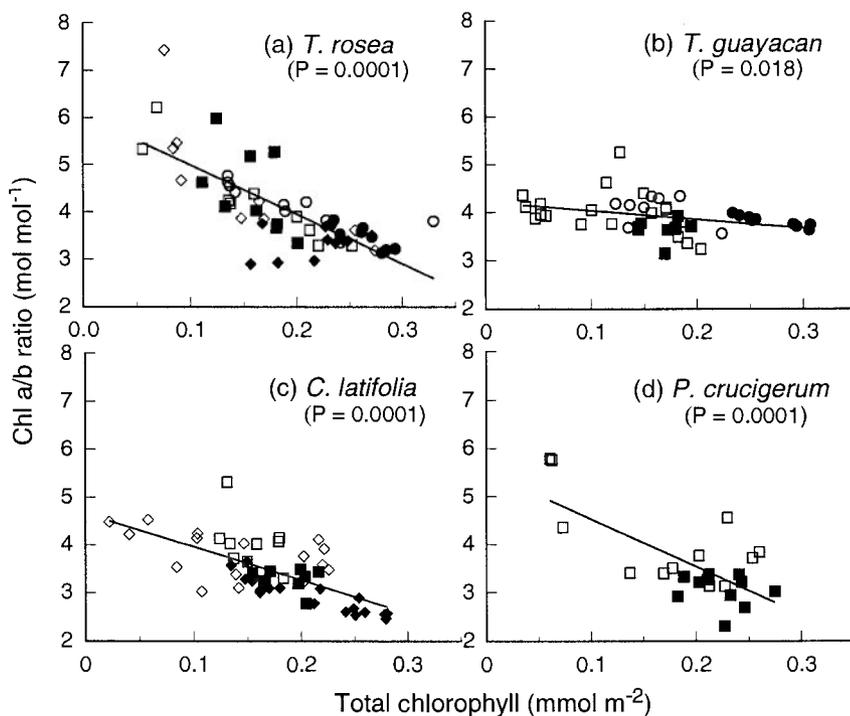


**Figure 2.** Relationship of N per unit area with Chl per unit area (a) and Chl *a/b* ratio (b) for leaves of 50-d seedlings of *Tabebuia rosea* grown under high- and low-light treatments (open and closed symbols, respectively). Significant regressions are shown as solid lines.

significantly between the two light treatments (no significant light-by-[Chl] interaction in ANCOVA with light as the main factor and [Chl] as the covariate;  $P > 0.05$ ). In all species, the Chl *a/b* ratio was negatively correlated with [Chl] across light treatments, although species differed in the strength of correlation, ranging from a strong negative correlation in *T. rosea* to a weak correlation in *T. guayacan* (Fig. 3, Table 2). In *T. guayacan*, [Chl] was not a significant covariate in the result of ANCOVA ( $P > 0.05$ ), but N treatment had a significant effect on Chl *a/b* ratio in a two-way analysis of variance (ANOVA) ( $P = 0.04$  for N treatment effect,  $P = 0.005$  for light effect). The negative correlation for *P. crucigerum* was due to the values of three plants under high light conditions and zero nitrogen supply (the three points with the lowest [Chl]). The relationship between the Chl *a/b* ratio and [Chl] was not significantly different between cotyledons and leaves for the two species with photosynthetic cotyledons, *T. rosea* and *T. guayacan* (ANCOVA, organ type as the main factor and [Chl] as the covariate,  $P > 0.05$ ).

### Gas exchange and fluorescence in *T. rosea*

Light and N treatments significantly influenced light- and CO<sub>2</sub>- saturated photosynthetic rates ( $P_{\max}$ ), but not quantum yields and dark respiration rates of *T. rosea* (Table 1).  $P_{\max}$  was positively correlated with leaf N in both light treatments (Fig. 4a  $P < 0.005$ ). Photosynthetic N use efficiency (= the ratio of  $P_{\max}$  to N) was not affected by N treatment, but was higher in the higher light treatment (Table 1).  $P_{\max}$  per mol Chl was higher at the higher light treatment (Fig. 4b, Table 1).  $P_{\max}$  per mol Chl increased



**Figure 3.** Relationships between the Chl *a/b* ratio and Chl per unit area [Chl] for seedlings of four species [(a) *Tabebuia rosea*, (b) *T. guayacan*, (c) *Callichlamys latifolia*, (d) *Pithecoctenium crucigerum*] grown under high (open symbol) and low (closed symbol) light treatments. Each point represents either a cotyledon (circle) or leaf (square for 24–30 d seedlings, and diamond for 50–65 d seedlings). As there is no significant light-by-[Chl] interaction in ANCOVA (Table 2), the regression line for the pooled data is shown for each species.

Variable	d.f.	F-values			
		<i>T. rosea</i>	<i>T. guayacan</i>	<i>C. latifolia</i>	<i>P. crucigerum</i>
Light	1	1.38 (NS)	2.87 (NS)	19.09***	4.64*
[Chl]	1	63.56***	0.37 (NS)	18.40***	13.0**

Light  $\times$  [Chl] interactions were non-significant for all four species. *F*-values are shown with the level of significance indicated as follows: \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.005$ ; \*,  $P < 0.05$ ; NS,  $P > 0.05$ .

with decreasing leaf N in high light ( $P < 0.005$ ) but not in low light ( $P > 0.1$ , Fig. 4b).

Dark-acclimated  $F_v/F_m$  was high and did not differ among light and N treatments (mean across treatments = 0.77; Table 1). Quantum yields were also high and did not differ among treatments (mean = 0.091, Table 1). Thus, chronic photo-inhibition was not observed even in severely N-deficient leaves at high light.

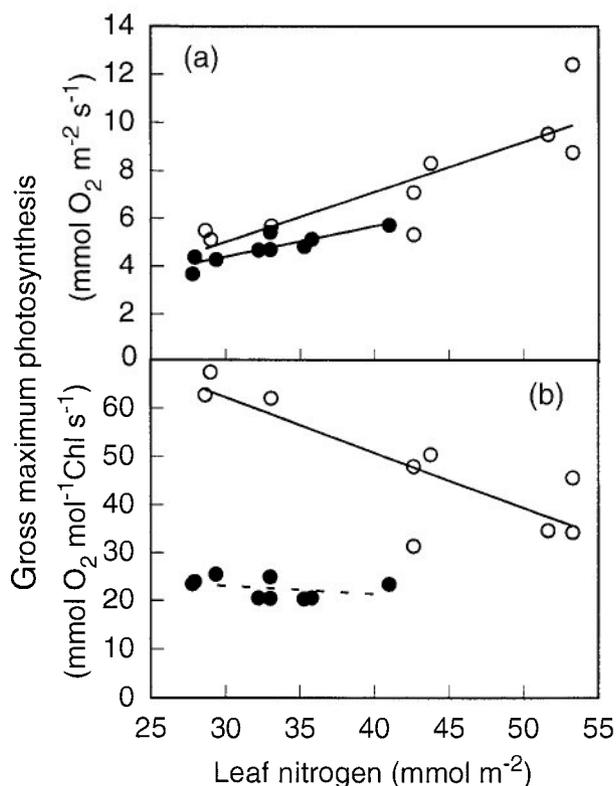
N treatment affected electron transport rate ( $V_j$ ) and carboxylation capacity ( $V_{cmax}$ ) estimated from the  $A-p_i$  relationships more strongly in high than in low light (Fig. 5, Table 3). Overall, shaded plants had lower  $V_j$  and  $V_{cmax}$ . A measure of N use efficiency,  $V_{cmax}$  per mol N, significantly increased with a decrease in N supply in high light, but an

opposite trend was observed in the low light treatment (Table 3).  $CO_2$  compensation point ( $p_i$  at which point *A* is zero) did not differ significantly among light and N treatments (Table 3).

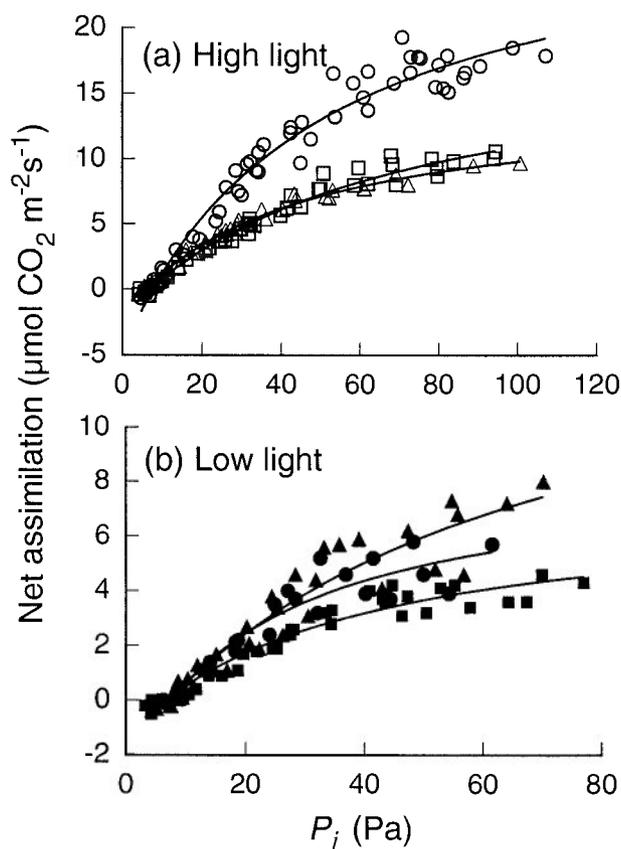
## DISCUSSION

The results of this study supported predictions made by theoretical models of optimal nitrogen partitioning among photosynthetic components (Evans 1989; Hikosaka & Terashima 1995). The unique finding in this study was that the Chl *a/b* ratio increases in response to N limitation, a theoretical prediction not previously demonstrated empirically. In other aspects, these results corroborate existing empirical studies (Terashima & Evans 1988; Evans 1989; Sims & Pearcy 1989; Hikosaka & Terashima 1996; Bungard *et al.* 2000) as well as certain theoretical predictions. For example, acclimation to high light was accompanied by increases in electron transport rate ( $V_j$ ), in Rubisco carboxylation capacity ( $V_{cmax}$ ) per unit leaf area and unit chlorophyll, and in the Chl *a/b* ratio. Within each light treatment,  $V_j$  and  $V_{cmax}$  both increased in response to N supply (Table 3).

The Chl *a/b* ratio should respond to N availability more strongly under high light than in shade (Hikosaka & Terashima 1995, 1996). In shade, where optimal  $P_{max}$  is constrained by photon availability, optimal nitrogen allocation strategy suggests a consistently low ratio of PSII to LHCII across leaf N content, because the N cost per mole of chlorophyll is almost three times as high for PSII than for LHCII (Hikosaka & Terashima 1996). The result is a uniformly low Chl *a/b* in shade leaves independent of leaf N content (Fig. 2b). In contrast, in high light environments, N availability may affect partitioning among Rubisco, PSII and LHCII. In high light environments, as long as N supply is not limited, leaves should achieve very high  $P_{max}$  through disproportionate investment of N into Rubisco relative to PSII, while maintaining sufficient [Chl] through a relatively low ratio of PSII to LHCII. However, when  $P_{max}$  is constrained by low N in the same high light environment, proportional N allocation to PSII should increase at the cost of decreased N allocation to Rubisco and LHCII (see Fig. 6 of Hikosaka & Terashima 1996). In support of this view, we observed a decrease in  $V_{cmax}$  per N at higher N availability in sun, but not in shade (Table 3). This difference between high- and low-N leaves exists because  $P_{max}$  is related linearly to PSII, but curvilinearly with Rubisco (i.e. less



**Figure 4.** Relationship of N per unit area with light- and  $CO_2$ -saturated gross photosynthetic oxygen evolution rate ( $P_{max}$ ) per unit area (a) and per unit mol Chl (b) for leaves of 50 d seedlings of *Tabebuia rosea*. Significant and non-significant regressions are shown as solid and broken lines, respectively.



**Figure 5.** Relationship between net CO<sub>2</sub> assimilation rate (*A*) versus intercellular CO<sub>2</sub> partial pressure (*P<sub>i</sub>*) for leaves of 50-day-old seedlings of *Tabebuia rosea* in high (a) and low light (b). Circles, high N; triangles, low N; squares, zero N. See Table 3 for the age of plants used in measurements. The scales differ in both axes between (a) and (b).

increase in *P<sub>max</sub>* per unit increase in Rubisco at higher Rubisco content). Ultimately, the curvilinear relationship of *P<sub>max</sub>* to Rubisco exists because the rate of internal diffusion of CO<sub>2</sub> can become limiting to photosynthesis when Rubisco content per unit leaf area is high (Evans 1999).

For all study species, the Chl *a/b* ratio was negatively correlated with [Chl]. Variation in [Chl] within a light treatment for a given species was presumably due to variation in leaf N content, as in *T. rosea* (Fig. 2). Despite the expected difference between sun and shade in responsiveness of the Chl *a/b* ratio to N limitation, as discussed in the preceding paragraph, the slope of the linear correlation between Chl *a/b* and [Chl] did not differ between high and low light treatments for any species. The responsiveness of the Chl *a/b* ratio to N limitation, however, differed among the four species, ranging from a strong response in *T. rosea* to a weak response in *T. guayaca* (Fig. 3). Studies of other tropical trees and temperate herbs reported either no response or a slight decrease in the Chl *a/b* ratio in response to N limitation under high light (Terashima & Evans 1988; Thompson *et al.* 1992; Hikosaka 1996). However, leaves of some Malaysian tree seedlings grown under lower N supply exhibited a slight but non-significant increase in Chl *a/b* ratio as they recovered from photodamage over the course of 20 d (Bungard *et al.* 2000). Comparison of a larger number of plant species from various habitats is necessary to understand which species characteristics, such as ecological habitat or life history traits, may be related to the responsiveness of the Chl *a/b* ratio to N limitation.

The Chl *a/b* ratios observed under the high light treatment in this study were higher than those reported in many other studies (Terashima & Evans 1988; Dale & Causton 1992; Turner, Ong & Tan 1995). This was partly because the

**Table 3.** Summary of leaf traits for leaves of *Tabebuia rosea* grown under six treatment combinations of light and nitrogen availability

Light treatment	High			Low			One-way ANOVA <i>P</i>	
	High	Low	Zero	High	Low	Zero	High (H,Z)	Low (H,L,Z)
N treatment	High	Low	Zero	High	Low	Zero		
Plant age (days)	42	56	42	54	55	55		
Sample size	3	3	3	3	3	3		
N per leaf mass (mmol g <sup>-1</sup> )	2.71 (0.15)	1.41 (0.31)	1.14 (0.23)	2.92 (0.23)	3.06 (0.03)	2.33 (0.12)	0.0004	0.0012
Leaf mass per area (g m <sup>-2</sup> )	26.0 (2.1)	28.0 (1.6)	26.3 (2.0)	11.4 (0.6)	12.0 (1.2)	11.2 (0.4)	0.04	NS
Initial slope (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> Pa <sup>-1</sup> )	0.36 (0.02)	0.20 (0.01)	0.19 (0.02)	0.18 (0.03)	0.18 (0.03)	0.12 (0.01)	0.002	0.04
<i>V<sub>i</sub></i> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	24.4 (4.5)	12.1 (0.4)	14.8 (1.1)	8.1 (1.5)	9.1 (1.6)	5.7 (0.7)	0.03	0.05
<i>V<sub>max</sub></i> (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	57.1 (7.5)	34.6 (0.4)	31.7 (3.3)	38.2 (2.9)	24.1 (4.2)	16.9 (1.2)	0.006	0.0004
<i>V<sub>max</sub></i> per Nitrogen (mmol CO <sub>2</sub> mol N <sup>-1</sup> s <sup>-1</sup> )	0.78 (0.10)	0.81 (0.01)	1.05 (0.11)	1.02 (0.08)	0.73 (0.13)	0.61 (0.04)	0.04	0.004
<i>P<sub>i</sub></i> compensation point (Pa)	7.05 (1.13)	4.66 (1.11)	5.94 (0.50)	7.04 (0.36)	6.86 (1.56)	6.99 (1.62)	NS	NS

Characteristics of assimilation (*A*) – internal CO<sub>2</sub> partial pressure (*p<sub>i</sub>*) relationships: initial slope (0 Pa < *p<sub>i</sub>* < 30 Pa), *V<sub>i</sub>*, *V<sub>max</sub>*, and CO<sub>2</sub> compensation point. Mean (standard deviation). Results of a one-way ANOVA for contrasting N levels within each light regime for those with same plant age (*P* > 0.05 for NS).

widely used Arnon's equations (Arnon 1949) tend to underestimate the Chl *a/b* ratio (Porra, Thompson & Kriedmann 1989). For example, the Chl *a/b* ratios of 2.7 and 4.8 reported in this study correspond to Chl *a/b* ratios of 2.1 and 3.4, respectively, according to Arnon's equations. But, many Chl *a/b* ratios observed in this study are high even in comparison to those reported for N-limited tropical tree seedlings in other studies that used equivalent spectrophotometric equations (Thompson *et al.* 1992) and pigment determination with high-performance liquid chromatography (HPLC; Bungard *et al.* 2000). These differences possibly arise because N limitation was more severe in our study ( $[\text{Chl}] < 0.1 \text{ mmol m}^{-2}$ ) than in the other two studies ( $[\text{Chl}] > 0.2 \text{ mmol m}^{-2}$ ).

An increase in the Chl *a/b* ratio for N-deficient leaves may be viewed as a response to higher intracellular light intensity, because thylakoids of N-deficient leaves experience greater PFD per PSII core for given incident light (Terashima & Hikosaka 1995). The PFD experienced by thylakoids depends on many factors, including the intraleaf light gradient, mesophyll cell arrangement, chloroplast orientation, and thylakoid stacking patterns (Terashima, Sakaguchi & Hara 1986; Evans 1999). Excess light per PSII core must be dissipated in order to avoid photodamage.

Chronic and severe N stress imposed upon seedlings in this experiment did not impair pre-dawn quantum yields or  $F_v/F_m$  ratios, suggesting that PSII reaction centres were largely intact. Xanthophyll cycle carotenoids, carboxylation capacity, and electron transport rates are all important in avoiding excess energy build-up in the PSII (Demming-Adams & Adams 1992; Horton, Ruban & Walters 1996; Hogan *et al.* 1997). Photodamage to the PSII reaction centre may be exacerbated under nutrient deficiency because of constraints on the carboxylation capacity (Bungard *et al.* 2000). Nitrogen-deficient plants increase non-photochemical dissipation of energy through an increase in xanthophyll cycle pigments per unit chlorophyll (Verhoeven, Demmig-Adams & Adams 1997; Bungard *et al.* 2000). It will be interesting to examine how the responses of the Chl *a/b* ratio of species to nitrogen stress under high light may be correlated with production of xanthophyll pigments with HPLC measurements of pigments.

More attention should be paid to N limitation in studies of growth and survival responses of tropical woody species relative to light availability. Interpretations of trenching experiments suggest that low soil N availability due to root competition significantly constrains seedling growth, especially in light gaps (Coomes & Grubb 2000; Lewis & Tanner 2000; Ed Tanner, personal communication). Chl *a/b* ratio can be easily measured in studies of plant response to N limitation as an indicator of N partitioning among different functional groups of photosynthetic components for both tropical and temperate plants.

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