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Leaf acclimation strategies to contrasting light conditions in saplings of different shade tolerance in a tropical cloud forest

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Abstract. To study the acclimation responses of the leaves of saplings of six tree species when changed to low or high levels of irradiance, we carried out a light exposure experiment. Species representative of contrasting shade tolerance groups were identified across a light gradient in the understorey of a Venezuelan Andean cloud forest. Measured traits included gas exchange, chlorophyll fluorescence, and morphoanatomical, biochemical and optical properties. Saplings were grown for 6 months in a shade-house receiving 20% photosynthetic photon flux (PPF) of full sunlight. Plant samples were then moved to shade-houses receiving low PPF (4%) or high PPF (65%). A factorial model (species × PPF), with repeated measurements (0, 15 and 120 days) was designed. Our results showed that morphological and anatomical traits were more plastic to PPF changes than photosynthetic traits. All species were susceptible to photoinhibition (15 days): shade-intolerant species showed dynamic photoinhibition (120 days), whereas shade-tolerant species presented chronic photoinhibition and the consequent inability to increase C assimilation rates under high PPF. The partially shade-tolerant species showed mixed responses; nonetheless, they exhibited larger adjustments in morphoanatomical and optical properties. Thus the acclimation responses of these species when subject to contrasting light conditions could help to explain their distribution along the light gradient in the understorey.

Additional keywords: chlorophyll fluorescence, gas exchange, leaf morphoanatomical properties, leaf optical properties, photoinhibition.

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Introduction

Photosynthesis is the key process in plants and can be affected by many environmental factors (Allakhverdiev 2011). Of these factors, light is the most critical resource for maintaining adequate performance of the photosynthetic apparatus, and ultimately determines the success or failure of plant growth and reproduction in the understorey of humid tropical forests. A light gradient occurs from the highly shaded understorey under the closed canopy to more illuminated large gaps (Chazdon and Fetcher 1984; Endler 1993). As forest succession proceeds, this light gradient generally becomes increasingly steeper (Zhang *et al*. 2015). In tropical cloud forests, light limitations can be even greater because of a persistent cloudiness and abrupt topography (Schwarzkopf *et al*. 2011). As a response to sudden or gradual changes in light availability in the understorey caused by the opening and closing of gaps, plants can modify their physiology and morphology (i.e. acclimation and plastic responses), enhancing their photosynthetic performance. However, not all species show the same acclimation capacity. The photochemical reactions of photosynthesis are sensitive to high levels of photosynthetic photon flux (PPF) by reducing the maximum quantum yield and quantum yield of PSII. These events reduce the electron flow throughout the photosystems, leading to reduced ATP and NADPH formation, and thus reducing CO_2 fixation. In some species, an excess of absorbed light energy can significantly reduce photosynthetic performance in the process known as photoinhibition (Allakhverdiev *et al*. 1997;

Baker 2008; Takahashi and Badger 2011; Cendrero-Mateo *et al.* 2015). In addition to biochemical changes in the photosynthetic apparatus and other plant traits to cope with light stress, plants have developed photoprotection mechanisms that include coordinated changes in their biochemical, morphoanatomical and optical properties. Several studies have found acclimation patterns in leaves to the light environment (Ribeiro *et al.* 2005; Castro-Esau *et al.* 2006). High PPF can damage the photosynthetic apparatus, especially in leaves growing in shaded environments or in leaves in which photoinhibition has occurred because of other types of stress, such as extreme temperatures or water stress (Matthew *et al.* 2012). In contrast, low light intensities impose stresses on plants because this limits photosynthesis and thus net C gain and plant growth. In the mountain cloud forest in the Venezuelan Andes, light limitations occur in the forest understorey because of a dense canopy cover and an almost continuous cloudiness. Large and sudden increases in light availability occur as a result of gap formation. Thus in order to survive and grow to reach the canopy, juveniles of tree species should have developed a series of mechanisms to cope with the spatial-temporal heterogeneity of the light environment. In effect, depending on the tree species, juveniles growing in this usually low light environment show a differential distribution along the light gradient, which allows them to be categorised into functional groups according to their light requirements (Quevedo *et al.* 2015, 2016). As reported in several studies (e.g. Chazdon and Pearcy 1991; Reich *et al.* 1995), species of the same functional group show similar regeneration patterns, as well as similar morphological and physiological responses, such as photosynthetic performance. For example, the fall of a tree can cause a gap, producing a sudden increase in PPF in the understorey and causing light stress leading to a transitory or permanent reduction in light use efficiency in shade-tolerant plants (Krause and Weis 1991), or favouring the photosynthesis of the shade-intolerant ones. Following this observation, we hypothesised that if the distribution of saplings (30–150 cm in height) of tree species in the forest understorey is the result of their light requirements, it is expected that traits related to the photosynthetic responses of leaves should change when plants are exposed to experimental conditions of contrasting light incidence. Our main objective was to study the acclimation strategies of the photosynthetic apparatus and related traits to cope with the stress caused by sudden changes in the light environment in juvenile plants of each functional group growing under controlled light conditions in shade-houses (4%, 20% and 65% of full sunlight). This issue is a key factor in explaining tree species' responses to disturbances and hence to understand successional patterns in forest ecosystems.

Understanding the variability in physiology, morphology and structure is important for knowing how a plant species is able to grow in different habitats, which ultimately will also guide the selection of suitable habitats for the possible reintroduction of the species (Liang *et al.* 2010).

Materials and methods

Study area

The study was carried out in the experimental station San Eusebio University Forest in the Venezuelan Andes (8°37′00″N, 71°21′00″W) in Mérida State, Venezuela. The elevation ranges from 2200 to 2500 m above sea level and the region receives an annual precipitation between 1400 and 1560 mm annually with a short dry season (December–February) and a long wet season (March–November). The annual average temperature is 14.9°C. This forest grows on soils derived from the Colón Cretacean formation characterised by stratified, massive, black, noncalcareous lutites with conchoidal fractures. The landscape consists of rounded hills, with shallow to steep slopes (Márquez 1990). It comprises several forest communities from low, two-layered, low density forests (15–20 m height) to dense high forests with a complex structure formed by three layers and a canopy height of at least 30 m, with emergent coniferous trees (*Retrophyllum rospigliosii* (Pilg.) de Laub.) being more than 40 m high. The main tree families are Lauraceae, Melastomataceae, Guttiferae, Euphorbiaceae, Myrtaceae and Podocarpaceae. The forest is thick and rich in evergreen tree species densely covered by epiphytes, mosses and lichens.

Species selection and experimental design

We designed an experiment to determine the photosynthetic acclimation response of selected species from different functional groups (shade-tolerant, partially shade-tolerant and shade-intolerant) to contrasting light regimes. Species were selected according to their abundance along a light gradient (Quevedo *et al.* 2015): *Aegiphila ternifolia* (Kunth) Moldenke and *Myrcianthes karsteniana* (O. Berg) McVaugh were selected as shade-tolerant species, *Beilschmiedia sulcata* (Ruiz & Pav.) Kosterm. and *Casearia tachirensis* Steyerm. as partially shade-tolerant, and *Miconia meridensis* Triana and *Tetrorchidium rubrivenium* Poepp. as shade-intolerant species.

Seedlings were collected in the forest and transplanted to a 1 : 1 mix of sand and forest soil in polyethylene bags (diameter = 17 cm, height = 25 cm), and placed on metallic platforms (40 cm height) to avoid contact with the ground.

Three shade-houses were built in areas exposed to direct sunlight and covered with a black shade mesh from the top and sides to 40 cm above the ground to allow for air circulation. The first house used mesh, letting 20% of incident PPF pass (moderate irradiation, MPPF). The mesh of the second let only 4% of PPF pass (low irradiation, LPPF) and the third allowed 65% PPF (high irradiation, HPPF). All plants were grown in the 20% PPF shade-house for 6 months until they reached at least 30 cm in height. Plants were irrigated regularly for the whole duration of the experiment to avoid water stress.

At the start of the treatments (Day 0), 15 plants of each species were randomly selected. Five of them remained in the 20% PPF treatment, five plants were moved to the 4% shade-house and the remaining five were placed in the 65% shade-house. Plants were randomly distributed on the platforms and randomly relocated every 30 days to reduce any effects of microclimate unevenness. The time lapses were chosen to evaluate initial photosynthetic status (Day 0), to detect short-term photosynthetic responses (15 days) such as signs of acute photoinhibition, and to identify photosynthetic acclimation responses (120 days). By Day 120, all species had produced mature new leaves. For each treatment (species × PPF × time),

measurements were performed on five ($n = 5$) mature fully expanded leaves with no evidence of disease or mechanical damage from healthy plants.

Microclimate variables in shade-houses and under the open sky

For each shade-house, micro-station H21–002 data loggers (HOBO) recorded inside and outside irradiance at 10-min intervals with pyranometer sensors (S-LIB-M003, HOBO), and air temperature with 12-bit S-TMB-M002, HOBO sensors. Irradiance data (W m^{-2}) was transformed to approximate PAR (μmol $m^{-2}s^{-1}$) following Thimijan and Heins (1983).

Gas exchange and chl a fluorescence

We carried out measurements on Day 0 of the experiment under 20% PPF. Afterwards, the plants were moved to the corresponding shade-houses and measurements were repeated on Days 15 and 120.

We measured CO_2 assimilation rates (μmol $m^{-2}s^{-1}$) and dark respiration (R_D, μmol $m^{-2}s^{-1}$) with an open-mode gas exchange portable system (LCA-4 The Analytical Development Co. Ltd). CO_2 assimilation–light response curves were performed in the laboratory using a 1000-μmol $m^{-2}s^{-1}$ light source (1500-W lamp, Westinghouse); infrared radiation was filtered with water 10 cm in depth in a transparent tray (Plexiglas) with constant circulation to refresh the water temperature. Attenuation of PPF at the leaf surface was achieved by covering the top of the leaf chamber with mesh layers of neutral density. The measurements began in the dark, then the PPF was increased progressively by 20 μmol $m^{-2}s^{-1}$ to 100 μmol m^{-2} s^{-1}; from this point on, PPF was increased by 100 μmol $m^{-2}s^{-1}$ to 1000 μmol $m^{-2}s^{-1}$ as a final measurement. At each irradiance level, measurements were taken after CO_2 readings stabilised, which was usually achieved after 3 min. Since the leaf chamber had no temperature control, changes in leaf temperatures were unavoidable and ranged from 18°C to 25°C. A refrigerated bath with hose connections to a radiator and a fan was used to refresh the leaf chamber externally and consequently leaf temperatures within the gas exchange chamber (García-Núñez *et al*. 1995).

We measured the fluorescence of chl *a* (ChlF) with a pulse amplitude modulated system (PAM-2100, Heinz Walz GmbH). With this system, ChlF is excited with a light source that emits modulated light at regular intervals (Goltsev *et al.* 2016). The basal fluorescence (F_0) of dark-adapted leaves (for 12 h) was measured by applying a very weak modulated red light pulse (0.5 μmol m^{-2} s^{-1}, $\lambda_{max} = 650$ nm). Next, a saturating white light pulse (>6000 μmol $m^{-2}s^{-1}$) was applied for 0.8 s to measure the maximal fluorescence of dark-adapted leaves (F_m). After ChlF had returned to F_0, continuous actinic light averaging 165 μmol $m^{-2}s^{-1}$ was applied. The ChlF elevated to a peak and reached the steady-state level (F) within 3–5 min from the onset of the actinic light. A second saturating pulse was then applied to measure the maximum fluorescence of the light-adapted state (F_m'). At the end of measurement, a far-red light was turned on for 5 s to measure the minimal fluorescence of the light-adapted state (F_0'). To evaluate the photochemical activity, each sample leaf was exposed to a series of actinic light intensities at 25, 45, 65, 105, 170, 240, 380, 580, 860, 1340 and 1940 μmol $m^{-2}s^{-1}$ with a duration of ~3 min for each intensity (~33 min per sample). Thereafter, the following parameters (Maxwell and Johnson 2000; Baker 2008) were calculated: the maximum quantum efficiency of PSII ($F_v/F_m = (F_m - F_0) \div F_m$], the quantum yield of PSII ($\Phi PSII = (F_m' - F) \div F_m'$], the photochemical quenching coefficient ($qP = (F_m' - F) \div (F_m' - F_0')$) and the nonphotochemical quenching coefficient ($NPQ = (F_m - F_m') \div F_m'$). The electron transport rate (ETR) for PSII was calculated as ΦPSII × PPF × absorbance × the partition of the excitation energy (photons) between PSII and PSI (dII). We measured absorbance using an integrating sphere model 1800–12 (LI-COR). The dII is 0.5, assuming that both photosystems are equally involved in line electron transport (Krall and Edwards 1992, but see Miyake *et al*. 2005; Sukhov *et al*. 2015; Sukhova *et al*. 2017). However, for stressed plants, dII could be lower than 0.5. According to Sukhov *et al*. (2015), dII might depend on the duration of actinic light illumination, which could influence the results. In that study, the values of dII decreased from ~0.51–0.53 to 0.41–0.46 with increased light duration ($t^{1/2} = 20$ min) but remained essentially unchanged from the 60th to the 100th minute. Based on this evidence, we are aware that dII could have changed along the light saturation curve and maybe an overestimation of ETR could have been obtained by using a constant partition coefficient of 0.5.

Leaf optical, morphoanatomical and biochemical traits

To determine the optical, morphoanatomical and biochemical traits, five leaves per treatment (species × PFF × time) were selected. The specific leaf area (SLA) was determined by scanning the leaves at 300 pixels per cm, immediately after cutting to avoid size changes arising from dehydration. Leaf area was determined with Image J ver. 1.38× (http://rsbweb.nih.gov/ij/index.html, accessed 5 April 2018). Leaf dry weight (DW, g) was determined with a precision electronic scale (Model EW-N/EG-N, KERN & SOHN GmbH) after drying the leaves in a stove for 72 h at 57°C. SLA values were obtained by dividing fresh leaf area by leaf DW. Stomatal size and density were measured on epidermal impressions taken from both the adaxial and abaxial faces of at least three leaves per treatment. Impressions were obtained by applying transparent nail polish, then covering the paint with transparent tape. Imprints were examined with a 40× ocular graduated microscope (Zeiss Germany) to count stomata per field and calculate stomatal density per mm^2. In addition, the length and width of three stomata were measured to obtain stomatal area, assuming an ellipsoidal shape. The percentages of absorbance, transmittance and reflectance were determined with an integration sphere (LI-COR 1800–12), where absorbance = (1 – reflectance – transmittance). Total N was determined via the micro-Kjeldahl method (Müller 1961). Chl *a*, chl *b* and total chl concentrations were obtained following Arnon (1949) with the corrected equations described by Porra *et al*. (1989) and Porra (2002):

$$\text{chl}b = 22.90 \times D645 - 4.68 \times D663; \quad (1)$$

$$\text{chl}a = 12.70 \times \text{D}663 - 2.69 \times \text{D}645; \quad (2)$$

$$\text{chl}a + b = 20.21 \times D645 + 8.02 \times D663, \quad (3)$$

where D is the optical density for spectrophotometric measurements.

Statistical analysis

The experimental design was treated as a factorial model with repeated measures in time (species × PPF × time). A mixed-effects ANOVA model was used to analyse the response traits in terms of the simple effects and their interactions (Schabenberger and Pierce 2002). Combinations of species and PPF were evaluated at selected times on the same seedlings (subjects), which induced autocorrelation among observations. The MIXED procedure (SAS ver. 9.1, SAS 2004) was used to estimate the effects and make the desired comparisons among treatments. Species and PPF were considered to be fixed effects, whereas time was considered to be an effect representing the autocorrelation among measures taken at different times. To obtain the best fitting model, several structures of the residual's variance–covariance matrices were tested, including a model in which no correlation existed among repeated measures on the same subject (i.e. a classic ANOVA model). To compare the model's goodness of fit, we used Akaike's Information Criterion (AIC) (Schabenberger and Pierce 2002).

The best model was chosen for analysis. Statistical significance was determined for interactions and simple effects. The model allowed the determination of significant differences in the response variables among light levels at the same time and changes in the response through time. The SAS SLICE option allowed a correct estimation of the critical differences among the responses for single factors and interactions. Mean comparisons were calculated in terms of least squared means. A modified Tukey's test (Schabenberger and Pierce 2002) was used for multiple comparisons among light levels and along times (e.g. LPPF at Day 15 *v.* HPPF at Day 120). Variable response transformations were carried out as needed to meet the model's assumptions (e.g. normality, homoscedasticity). For example, variables measured as percentages such as absorbance, transmittance and reflectance were transformed via the arcsine function for ensuring data normality.

Results

Microclimate

The records indicated that under the open sky, the maximum PPF occurred at ~1030–1330 hours, with values between 1070 and 1350 µmol m^2 s^{-1} and temperatures between 21°C and 24.7°C. Inside the 20% shade-house, PPF varied between 214 and 270 µmol m^2 s^{-1} and temperatures averaged 18.2 C. Within the 4% PPF shade-house, PPF varied between 42.8 and 55.2 µmol m^2 s^{-1} (a maximum was reached at ~11:30 a.m.) with a temperature of 18.2°C. Inside the 65% shade-house, maximum PPF varied between 695 and 877 µmol m^2 s^{-1} and the average temperature was 20.1°C.

Statistical significance of the treatment effects and their interactions

A mixed model with a compound symmetry residual variance–covariance matrix produced the best fit as indicated by the lowest AIC value for all response variables. The highest AIC (the worst model structure) was for the classic fixed effects ANOVA model, wherein no correlation among repeated measures was considered. The statistical significance of interactions and single factors was obtained from ANOVA tables (Table 1).

Initial ANOVA analysis did not show any statistically significant differences on Day 0 for any variable among the groups of each species assigned to each light level; thus, for each species on Day 0, the data were pooled and only one average and s.e. was used ($n = 15$).

The final ANOVA analysis showed that all photosynthetic gas exchange traits differed significantly ($P < 0.01$) among species for varying levels of PPF and time, except R_D. All variables showed PPF × time and species × time interactions. However, the interaction between species and PPF levels was not significant for light compensation point (LCP) or R_D (i.e. the relative differences among species of both R_D and LCP remained more or less constant).

With respect to chlorophyll fluorescence traits, F_v/F_m was statistically significant for all types of interactions ($P < 0.01$). No significant species × PPF × time interactions appeared, implying that at least one of the double interactions was not significant. All traits showed significant, species × time interactions, indicating that responses differed among species with time. Species × PPF interactions were significant ($P < 0.05$) for all ChlF traits, indicating that responses to changes in PPF differed among species. Similar interpretations can be done for the remaining variables (e.g. the chl *a*/*b* ratio was affected only by PPF). Overall, for the MPPF level, the values of all variables for species across time remained fairly constant (i.e. no significant differences between Days 0 and 120 for this light level) or resembled those observed for LPPF. Therefore, in the figures below (Figs 1–5), the MPPF level is presented only for Day 0 (the control point) since the plants grew for 6 months under MPPF. Thus, significant differences for MPPF *v.* LPPF or HPPF were obtained from comparisons among time–light levels (e.g. MPPF–Day 0 *v.* HPPF–Day 120).

Gas exchange

The maximum photosynthetic rate (A_{max}) for all species varied between 1.2 and 5.3 µmol·$m^{-2}s^{-1}$ with corresponding light saturation points between 100 and 400 µmol $m^{-2}s^{-1}$. All species showed statistically significant differences ($P < 0.001$) for A_{max} between HPPF and LPPF after 15 days of exposure (Fig. 1*a–f*). There was a significant decrease in A_{max} for all species for HPPF but it was particularly strong for *C. tachirensis*, *M. meridensis* and *T. rubrivenium* (Fig. 1*c*,*e*,*f*). On Day 120 under LPPF, all species significantly reduced A_{max}, suggesting a downregulation of the photosynthetic apparatus as an acclimation mechanism to low light. Conversely, under HPPF, the A_{max} of most species did not recover, except in *T. rubrivenium*, which showed significant increases in A_{max} with respect to MPPF (Fig. 1*f*).

The light compensation point (LCP) decreased for all species under LPPF at end of the experiment (Day 120) (Fig. 1*g–l*). On the other hand, under HPPF, all species maintained or increased LCP after 120 days. *M. karsteniana* (a shade-tolerant species),

Table 1. Statistical significance of factors and their interactions from the ANOVA for measured parameters of six tree species in the San Eusebio University Forest

Factors: Sp, species; PPF, photosynthetic photon flux; T, time. Parameter abbreviations: A_{max}, maximum photosynthetic rate; LCP, light compensation point; Φ_a, apparent quantum yield; R_D, dark respiration rate; F_v/F_m, maximum quantum efficiency PSII; ETR, electron transport rate; ΦPSII, quantum yield of PSII; qP, photochemical quenching coefficient; NPQ, nonphotochemical quenching coefficient. Significance levels: **, $P < 0.01$; *, $P < 0.05$, ns, not significant ($P > 0.05$)

Properties	Parameters	Transformation	Sp	PPF	T	PPF × T	Sp × PPF	Sp × T	Sp × PPF × T
Photosynthetic (gas exchange)	A_{max} (μmol $m^{-2}s^{-1}$)	–	**	**	**	**	**	**	**
	LCP (μmol $m^{-2}s^{-1}$)	–	**	**	*	**	ns	**	**
	Φ_a	–	**	**	**	**	**	**	**
	R_D (μmol $m^{-2}s^{-1}$)	–	ns	*	**	**	ns	*	ns
Phothosynthetic (fluorescence)	F_v/F_m	–	**	**	**	**	**	**	**
	ETR (μmol $m^{-2}s^{-1}$)	–	**	*	*	ns	ns	**	ns
	ΦPSII	–	**	*	*	ns	ns	**	ns
	qP	–	**	*	*	**	ns	**	*
	NPQ	–	**	**	**	**	*	**	ns
Optical	Reflectance (%)	Arcsine	**	**	*	**	*	**	ns
	Transmittance (%)	Arcsine	**	**	ns	**	*	**	ns
	Absorbance (%)	Arcsine	**	**	ns	**	*	**	ns
Biochemical	Chl *a* (mg g^{-1})	Log	**	**	**	**	**	**	ns
	Ch *b* (mg g^{-1})	Log	**	**	**	**	**	ns	ns
	Chl *a*/*b* ratio	Log	ns	**	ns	ns	ns	ns	ns
	Total chl (mg g^{-1})	Log	**	**	**	**	**	**	ns
	N (mg g^{-1})	–	**	**	**	**	**	**	**
	Total chl : N	–	**	**	**	**	**	**	**
Morphological	Specific leaf area (cm^2 g^{-1})	Log	**	**	**	**	**	**	**
	Stomatal density (mm^2)	–	**	**	**	**	**	**	**
	Stomatal size (μm^2)	–	**	**	**	**	**	**	**

and *T. rubrivenium* and *M. meridensis* (shade-intolerant species) showed the largest LCP after 120 days under HPPF.

The dark respiration rate (R_d) decreased for all species under LPPF compared with HPPF by Day 120 (Fig. 1*m–r*), except for *M. meridensis*. There were significant differences ($P < 0.01$) for all species between LPPF and HPPF at 15 and 120 days of exposure, indicating early adjustment of the photosynthetic response for both sudden and gradual changes in the light environment. In general, R_D rates were low for all species; however, *M. meridensis* and *T. rubrivenium* had the highest respiration rates on Day 120 under both LPPF and HPPF. *M. karsteniana*, a shade-tolerant species, significantly increased its R_D under HPPF after 120 days.

The apparent quantum yield (Φ_a) was larger for all species under LPPF (Fig. 1*s–x*), but only in *C. tachirensis* (Fig. 1*u*) differed significantly between LPPF and HPPF on Day 15. All species decreased their Φ_a on Day 15 after being moved from MPPF (Day 0) to HPPF; by Day 120, Φ_a had not recovered, except in *T. rubrivenium* (Fig. 1*x*), which showed a stable Φ_a over time.

Chlorophyll a fluorescence

The maximum quantum yield (F_v/F_m) varied significantly among species, PPF levels and time (Fig. 2*a–f*). For the shade-tolerant species, *A. terniflora* (Fig. 2*a*) and *M. karsteniana* (Fig. 2*d*), F_v/F_m decreased with time under HPPF and did not recover its previous values after 120 days. Conversely, both *C. tachirensis* (Fig. 2*c*) and *B. sulcata* (Fig. 2*b*) (partially shade-tolerant) showed a partial recovery in F_v/F_m under HPPF from Day 15 to Day 120. In contrast, *M. meridensis* (Fig. 2*e*) and *T. rubrivenium* (Fig. 2*f*), the shade-intolerant species, were the only species that totally recovered their former F_v/F_m values under HPPF by Day 120.

In general, the PSII quantum efficiency(ΦPSII) showed low variation among species and was slightly affected by changes in PPF (Fig. 3*a–f*). Only *A. terniflora* (Fig. 3*a*) showed significant differences between HPPF and LPPF on Day 120, ΦPSII being higher under LPPF. On Day 120, all species reduced their ΦPSII with respect to Day 0 except for *T. rubrivenium*, in which ΦPSII remained stable and *B. sulcata* for which showed a significant increment.

The ETR varied significantly among species, with values between 40 and 90 μmol m^2s^{-1} (Fig. 3*g–l*). Only the shade-tolerant species (Fig. 3*g*,*j*) showed significant differences for ETR between LPPF and HPPF, with lower ETR recorded under HPPF.

Across the duration of the experiment, the Photochemical quenching (qP) (Fig. 3*m–r*) did not differ significantly for the shade-tolerant *A. terniflora* and *M. karsteniana* (Fig. 3*m*,*p*). However, for all species, qP was higher under HPPF than under LPPF. *B. sulcata* (Fig. 3*n*), which showed a significant increment in qP under LPPF on Day 15 but went down back to previous values by Day 120.

Overall, after 120 days, NPQ was larger under HPPF than under LPPF (Fig. 3*s–x*) but only *B. sulcata* (Fig. 3*t*), *M. karsteniana* (Fig. 3*v*) and *T. rubrivenium* (Fig. 3*x*) showed significantly higher NPQ. Under HPPF, except for *B. sulcata*, all species had a tendency to increase NPQ, but only *C. tachirensis* and *M. meridensis* (Fig. 3*u*,*w*) showed statistically significant

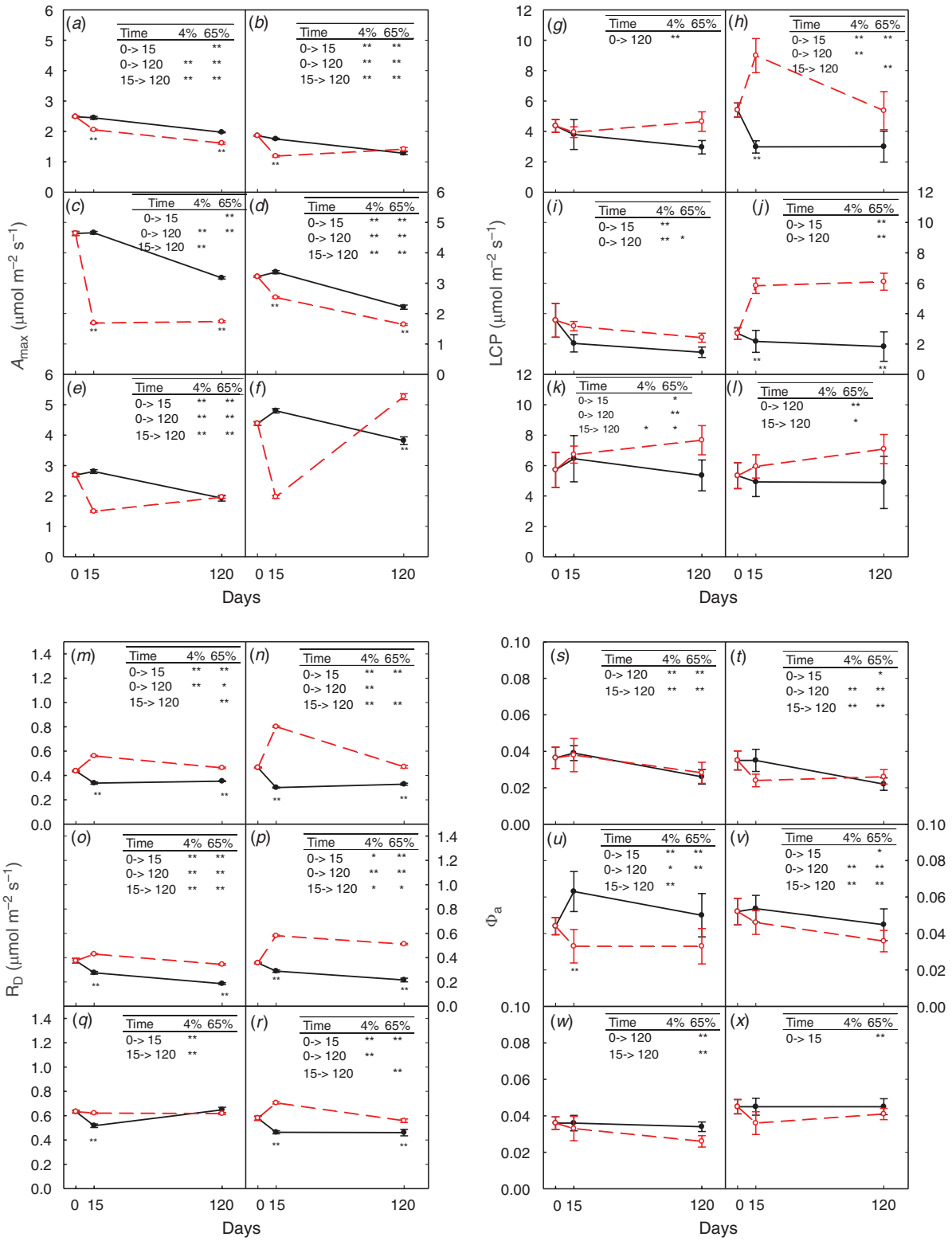

Fig. 1. Gas exchange traits:(*a–f*) Maximum photosynthetic rate (A_{max}); (*g–l*) light compensation point (LPC); (*m–r*) dark respiration rate (R_D); (*s–x*) apparent quantum yield (Φ_a). Species: (*a*, *g*, *m*, *s*) *A. terniflora*; (*b*, *h*, *n*, *t*) *B. sulcata*; (*c*, *i*, *o*, *u*) *C. tachirensis*; (*d*, *j*, *p*, *v*) *M. karsteniana*; (*e*, *k*, *q*, *w*) *M. meridensis*; (*f*, *l*, *r*, *x*) *T. rubrivenium*. Solid lines indicate a photosynthetic photon flux (PPF) of 4%; dashed lines, 65% PPF. Asterisks in tables indicate significant changes in the response with time at each light level (**, $P<0.01$; *, $P<0.05$).

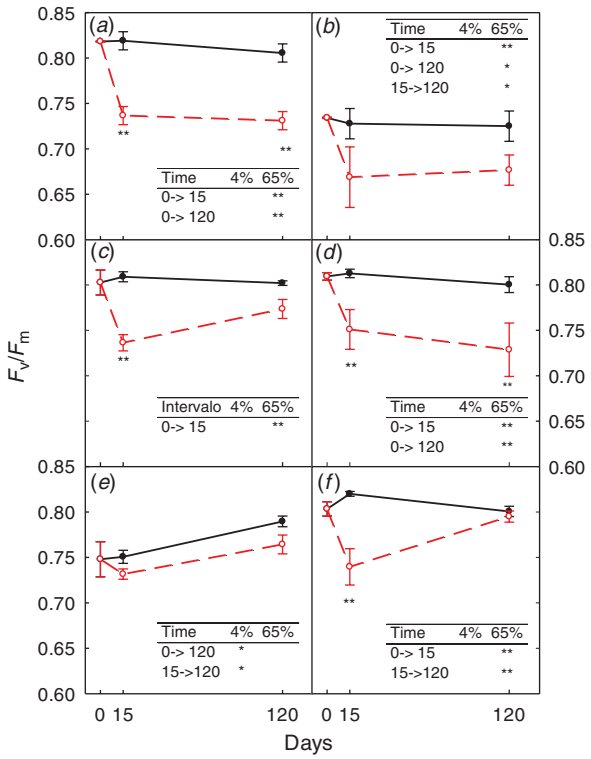

Fig. 2. Chlorophyll *a* fluorescence – maximum quantum efficiency of PSII (F_v/F_m): (*a*) *A. terniflora*; (*b*) *B. sulcata*; (*c*) *C. tachirensis*; (*d*) *M. karsteniana*; (*e*) *M. meridensis*; (*f*) *T. rubrivenium*. Solid lines, 4% photosynthetic photon flux (PPF); dashed lines, 65% PPF. Asterisks in tables indicate significant changes with time at each light level (**, $P < 0.01$; *, $P < 0.05$).

increments. On the other hand, under LPPF, no clear patterns were observed as some species increased NPQ (e.g. *M. meridensis*, Fig. 3*w*), whereas others decreased (e.g. *B sulcata*; Fig. 3*t*).

Leaf optical, biochemical and morphoanatomical properties

For all species, SLA increased with time under LPPF and decreased significantly under HPPF from 0 to 120 days (Fig. 4*a–f*). *M. meridensis* (Fig. 4*e*) and *A. terniflora* (Fig. 4*a*) had the largest reduction in SLA under HPPF (43.7% and 33.5%) with respect to LPPF.

Under LPPF, the absorbance of the leaves of all species increased significantly with time, whereas under HPPF, the absorbance decreased or remained steady with respect to MPPF (Fig. 4*g–l*). Only *T. rubrivenium* (Fig. 4*l*) did not show significant differences between light regimes or time. The largest values of absorbance were shown by *M. karsteniana* (Fig. 4*j*) on Day 120 under LPPF. On the other hand, under HPPF, *C. tachirensis* (Fig. 4*i*) showed a noticeable drop in absorbance after 120 days. Transmittance under HPPF was larger than under LPPF or was nonsignificantly different for all species during the measurement period (Fig. 4*m–r*).

In general, the reflectance increased significantly with time for all species grown under HPPF, whereas under LPPF, the reflectance was relatively constant for all species (Fig. 4*s–x*). *M. karsteniana* (Fig. 4*v*) showed the lowest reflectance under LPPF on Day 120, whereas under HPPF, *C. tachirensis* (Fig. 4*u*) showed the highest reflectance.

Chlorophyll *a*, chl *b* and total chlorophyll showed statistically significant differences ($P < 0.01$) between HPPF and LPPF on Day 120. All species increased chl *a* concentrations with time under the LPPF treatment (Fig. 5*a–f*), except for *B. sulcata* (Fig. 5*b*) and *C. tachirensis* (Fig. 5*c*). In addition, chl *b* increased with time under LPPF for all species (Fig. 5*g–l*). Total chlorophyll was higher for all species under LPPF (Fig. 5*m–r*), except in *T. rubrivenium* (Fig. 5*r*). Chlorophyll *a*/*b* was higher for plants grown under HPPF than under LPPF (Fig. 5*s–x*), but the differences were not significant among species for each light regime. Only *T. rubrivenium* showed significant differences on Day 120 (Fig. 5*x*).

The relationship between total chlorophyll content per unit of area and absorbance for the pooled data of all species showed a good fit with a hyperbolic function ($r^2 = 0.68$; Fig. 6*a*). This relationship indicates a strong initial increase in absorbance with an increase in chlorophyll content up to a saturation point. On the other hand, the reflectance decreased with increases in chlorophyll content (a quadratic function, $r^2 = 0.65$; Fig. 6*b*).

On Day 120, foliar N content for all species grown under LPPF was significantly lower than under HPPF. In HPPF, all species increased foliar N over time from 0 to 120 days (Fig. 7*a–f*), except *M. meridensis* (Fig. 7*e*), which decreased foliar N by 8%. Conversely, *T. rubrivenium* (Fig. 7*f*) showed the highest N content (42.9 mg g^{-1}) under HPPF on Day 120, ~50% higher than the rest of the species. For all species, the chl : N ratio was larger under LPPF (Fig. 7*g–l*). *A. terniflora* (Fig. 7*g*) and *M. karsteniana* (Fig. 7*j*) showed the highest chl : N ratios on Day 120.

All species showed hypostomatic leaves. Stomatal density increased for all species under HPPF, though it was significantly higher for newly formed leaves on Day 120 (Fig. 7*m–r*). Increases were largest for *C. tachirensis*, *M. karsteniana* and *M. meridensis* (Fig. 7*o–q*), which showed significant differences with respect to LPPF. The remaining species showed smaller differences between the two light levels, suggesting a lower degree of acclimation.

Stomatal size increased significantly with time (Fig. 7*s–x*), for all species growing under LPPF with respect to those growing under HPPF except for *C. tachirensis* (Fig. 7*u*).

Discussion

All species showed low CO_2 assimilation rates independently of the treatment, although higher but not significant rates were observed in shade-intolerant species than in the partially shade-tolerant or shade-tolerant ones. Low assimilation rates are commonly observed for cloud forest juveniles according to their shade tolerance and the light environments in which they grow (García-Núñez *et al*. 1995; Letts and Mulligan 2005). In turn, the low R_D rates found under LPPF represent a basic adjustment allowing shade-tolerant plants to survive in shaded environments by minimising C losses via respiration to maintain a positive C balance (Reich *et al*. 2003). In the understorey of high dense cloud forests, most plants have low rates of R_D and low LCP. In these situations, R_D change is caused primarily by temperature changes (Way and Oren 2010), although LCP was

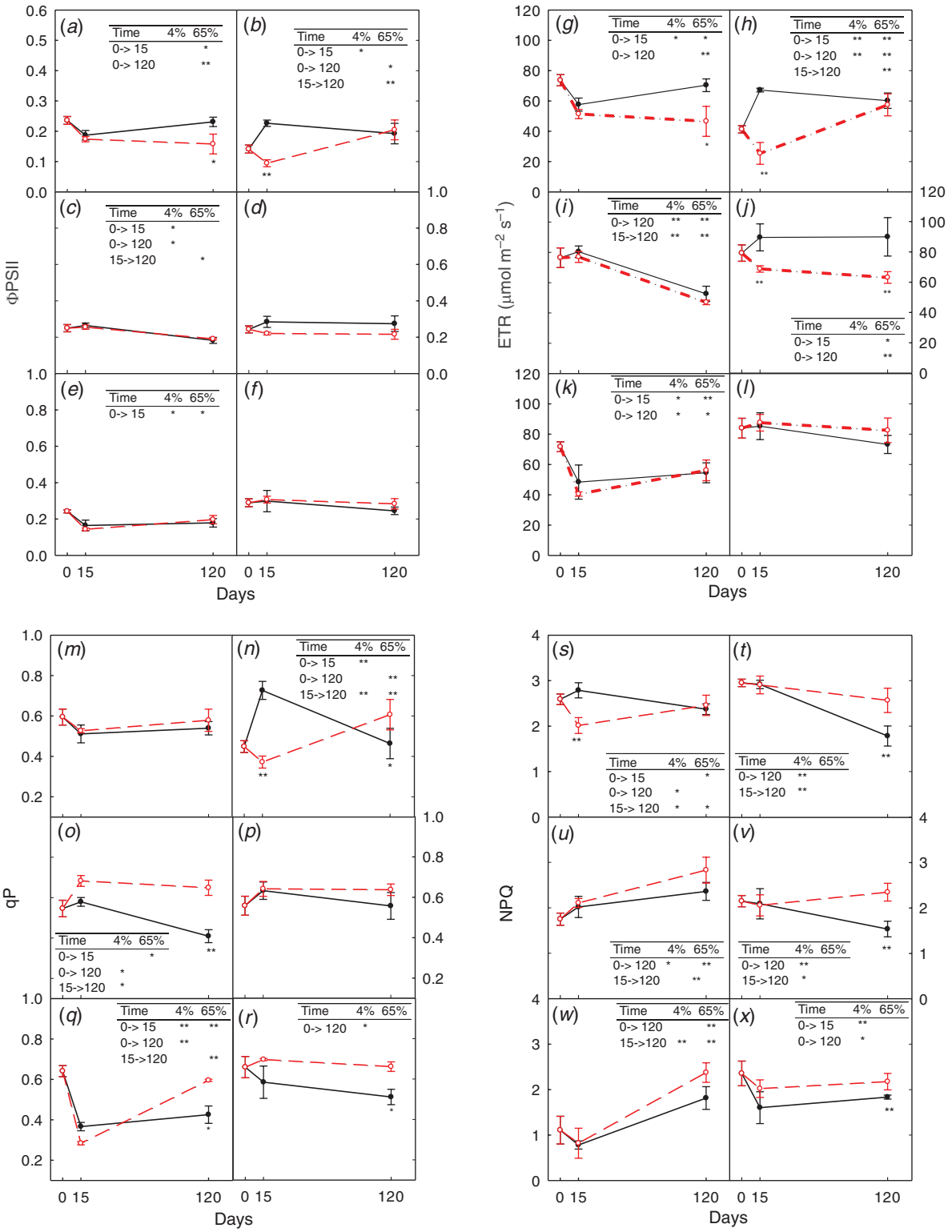

Fig. 3. Chlorophyll *a* fluorescence: (*a–f*) quantum yield of PSII (ΦPSII); (*g–l*) electron transport rate (ETR); (*m–r*) photochemical quenching coefficient (qP); (*s–x*) non photochemical quenching coefficient (NPQ). (*a*, *g*, *m*, *s*) *A. terniflora*; (*b*, *h*, *n*, *t*) *B. sulcata*; (*c*, *i*, *o*, *u*) *C. tachirensis*; (*d*, *j*, *p*, *v*) *M. karsteniana*; (*e*, *k*, *q*, *w*) *M. meridensis*; (*f*, *l*, *r*, *x*) *T. rubrivenium*. Solid lines, 4% photosynthetic photon flux (PPF); dashed lines 65% PPF. Asterisks in tables indicate significant changes in the response with time at each light level (**, $P < 0.01$; *, $P < 0.05$).

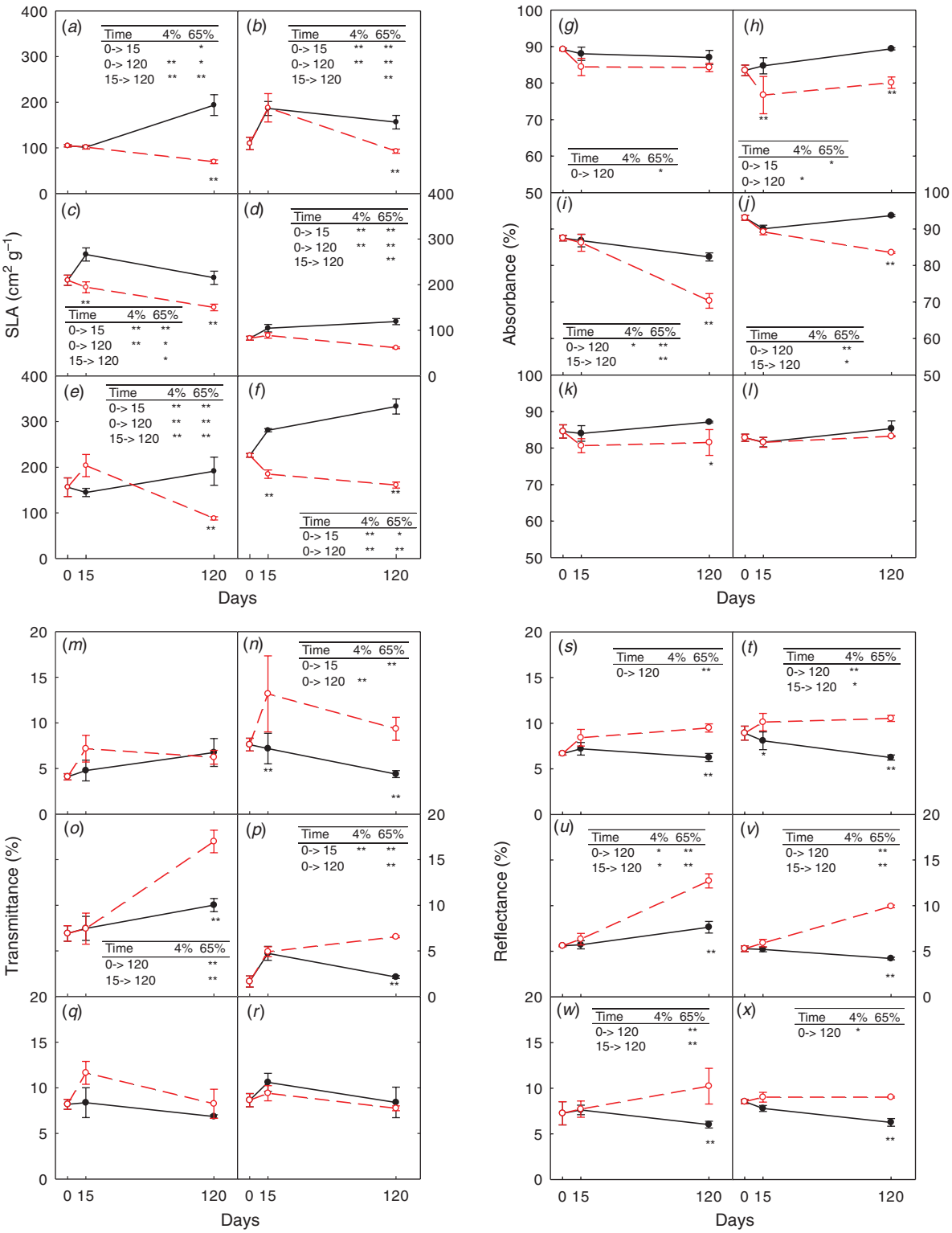

Fig. 4. (*a–f*) Specific leaf area; (*g–l*) absorbance; (*m–r*) transmittance; (*s–x*) reflectance. (*a*, *g*, *m*, *s*) *A. terniflora*; (*b*, *h*, *n*, *t*) *B. sulcata*; (*c*, *i*, *o*, *u*) *C. tachirensis*; (*d*, *j*, *p*, *v*) *M. karsteniana*; (*e*, *k*, *q*, *w*) *M. meridensis*; (*f*, *l*, *r*, *x*) *T. rubrivenium*. Values on Day 0 correspond to the 20% photosynthetic photon flux (PPF) treatment. Solid lines, 4% PPF; dashed lines 65% PPF. Asterisks indicate significant changes in the response with time at each light level (**, $P < 0.01$; *, $P < 0.05$).

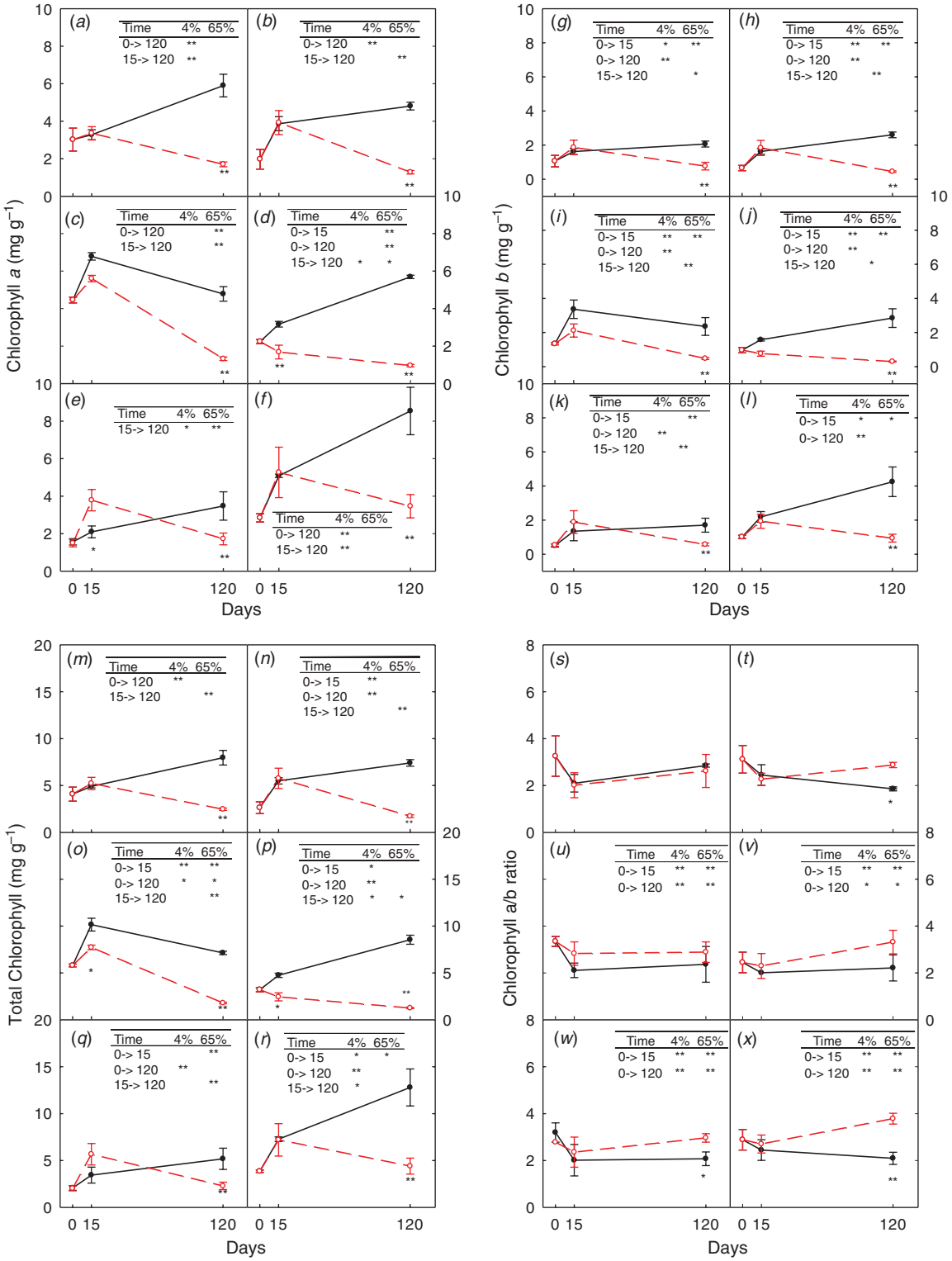

Fig. 5. (*a–f*) Chlorophyll *a*; (*g–l*) chl *b*; (*m–r*) total chlorophyll; (*s–x*) chl *a*/*b* ratio. (*a*, *g*, *m*, *s*) *A. terniflora*; (*b*, *h*, *n*, *t*) *B. sulcata*; (*c*, *i*, *o*, *u*) *C. tachirensis*; (*d*, *j*, *p*, *v*) *M. karsteniana*; (*e*, *k*, *q*, *w*) *M. meridensis*; (*f*, *l*, *r*, *x*) *T. rubrivenium*. Values on Day 0 correspond to the 20% light treatment. Solid lines, 4% photosynthetic photon flux (PPF); dashed lines, 65% PPF. Asterisks indicate significant changes in the response with time at each light level (**, $P < 0.01$; *, $P < 0.05$).

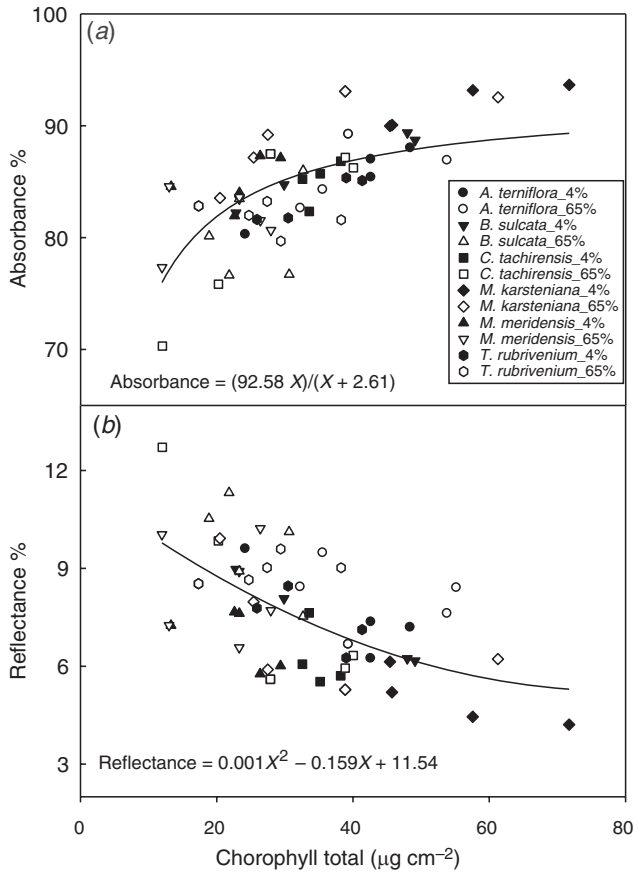

Fig. 6. Relationship between (*a*) leaf absorbance and (*b*) reflectance with total chlorophyll content (*X*) for six species grown under photosynthetic photon flux 4% and 65%. All coefficients were significant ($P < 0.001$).

reached at a very low PAR because of the low radiation environment in which these species have grown (dense fog all year round). The changes in LCP influence greater plasticity for the adaptation of the species to different light conditions, which results in a smaller separation of niches by light among species (Sterck *et al*. 2013). In addition to low R_D, shade-tolerant plants usually exhibited lower light compensation and saturation points to maintain net C gains (Chen *et al*. 2011). We found that *A. terniflora* and *M. karsteniana* showed traits of shade-tolerant plants and had a low ability to acclimate to HPPF. On the other hand, *C. tachirensis* and *B. sulcata* were more flexible in their responses, showing adjustments under both low and high PPF. Finally, *T. rubrivenium* and *M. meridensis* increased their R_D and LCP, although only *T. rubrivenium* was able to enhance A_{max} and light saturation points after 120 days under HPPF. Thus *T. rubrivenium* could respond favourably to sudden increases in PPF, whereas *M. meridensis* was less responsive in terms of C assimilation rates. Higher photosynthetic plasticity is a trait shown by shade-intolerant and pioneer species (Zhang *et al*. 2015).

According to Maxwell and Johnson (2000), plants subjected to stress by high light exposure should show lower F_v/F_m than nonstressed plants. In our study, for all species, the sudden increase in light exposure (0–15 days) reduced F_v/F_m, indicating excessive excitation energy. Griffin *et al*. (2004) found similar responses in tropical trees and shrubs species of *Illcium* subject to high light conditions. Although the shade-intolerant species (*M. meridensis* and *T. rubrivenium*) showed photoinhibition when changed to HPPF, by Day 120, however, they had recovered their former values of F_v/F_m, indicating acclimation to HPPF. When considering the HPPF level, *A. terniflora* and *M. karsteniana* reduced their initial F_v/F_m and did not recover by Day 120. In the case of *A. terniflora*, this response was also associated with lower ΦPSII and ETR, which did not recover with time. When plants are not able to dissipate excess energy, the emitted fluorescence increases, indicating that the efficiency in electron transfer is lower or that excess light limits the photochemical process (Takahashi and Badger 2011). The partially shade-tolerant *C. tachirensis* showed moderate recovery in F_v/F_m under HPPF from Day 15 to Day 120 and increased NPQ values, indicating an effective mechanism for dissipation of excess excitation energy, allowing acclimation to increased irradiance. However, although *B. sulcata* kept a low F_v/F_m and did not increase NPQ, it showed a significant recovery in ΦPSII, ETR and qP. By Day 120, all species except *B. sulcata* showed increases in NPQ values, suggesting increased thermic dissipation of excess energy, which is an efficient way of avoiding PSII photodamage and acclimating to HPPF. Therefore, the six species were susceptible to photoinhibition; however, the partially shade-tolerant and shade-intolerant species showed a dynamic photoinhibition response and recovered their F_v/F_m after 4 months under HPPF, whereas the shade-tolerant species showed chronic photoinhibition.

In addition to biochemical responses, the leaves of the studied species underwent changes in other traits to acclimate to changes in PPF. All species showed significant SLA reductions under HPPF and increases under LPPF after 120 days. García-Núñez *et al*. (1995) reported similar findings for juveniles of *Alchornea triplinervia* (Spreng.) Müll.Arg. and *Decussocarpus rospigliosii* (Pilg.) de Laub. in the same forest. Evans and Poorter (2001) pointed out that when light is a limiting resource, leaves increase their light capture efficiency by increasing SLA.

As expected, all species grown under LPPF showed lower stomatal density and larger stomatal size. Conversely, under HPPF, stomatal density increased for all species, being significantly larger on newly formed leaves on Day 120, indicating an acclimation mechanism to high irradiance. Increased stomatal density has been reported in acclimation experiments for plants grown under high light exposure (Boardman 1977) and is correlated with increasing gas exchange rates mediated by higher stomatal conductance (Oberbauer and Strain 1986).

The six species showed larger absorbance when cultivated under LPPF than under HPPF; however, this difference was mostly caused by a reduction in the absorbance of leaves of plants grown under HPPF. Under LPPF, absorbance increased slightly or remained stable (Fig. 4g–*l*). Likewise, for plants grown in HPPF, larger transmittance and reflectance were observed with time as a photoprotective strategy. These results agree with the hypothesis proposing that species acclimated to high irradiance can reduce heat on their leaves by increasing the reflectance and reducing absorbance (Poorter *et al*. 2000). The observed increase

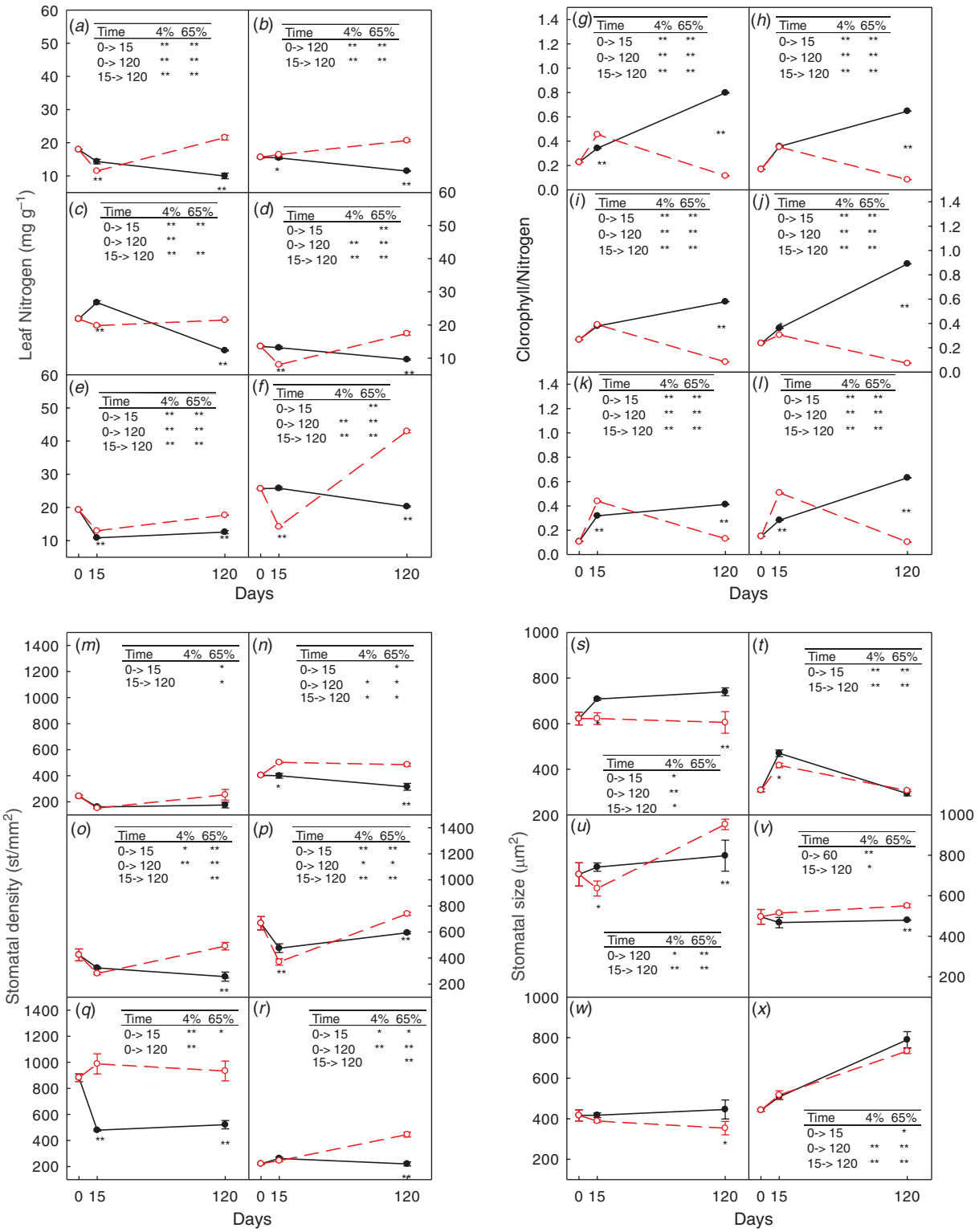

Fig. 7. (*a–f*) Leaf N; (*g–l*) chl : N; (*m–r*) stomatal density; (*s–x*) stomatal size. (*a*, *g*, *m*, *s*) *A. terniflora*; (*b*, *h*, *n*, *t*) *B. sulcata*; (*c*, *i*, *o*, *u*) *C. tachirensis*; (*d*, *j*, *p*, *v*) *M. karsteniana*; (*e*, *k*, *q*, *w*) *M. meridensis*; (*f*, *l*, *r*, *x*) *T. rubrivenium*. Values on Day 0 correspond to the 20% light treatment. Solid lines, 4% photosynthetic photon flux (PPF); dashed lines, 65% PPF. Asterisks indicate a significant response in time at each light level (**, $P < 0.01$; *, $P < 0.05$).

in leaf absorbance in all species under LPPF supports the hypothesis that leaves developed in shaded environments are capable of absorbing a larger proportion of incident irradiance as a response to light limitations (Givnish 1988).

Larger absorbances under LPPF than under HPPF were related to chlorophyll content (Fig. 6*a*). In this sense, our results agree with Rozendaal *et al*. (2006), who found that the leaves of plants grown under LPPF had higher chlorophyll concentrations per unit of DW compared with those grown under HPPF. The varying proportions found between chlorophyll types constitute an adjustment of leaves to the incident light. Chlorophyll *a* is relatively more abundant in the light chemical reaction centres, whereas, chl *b* is more abundant in the capture antenna complexes, where light is absorbed. As expected, the chl *a*/*b* ratio was higher in sun leaves than in shade leaves. Poorter *et al*. (2000) compared sun and shade leaves in adult *T. rubrivenium* trees in a Venezuelan cloud forest and found a significantly higher chl *a*/*b* ratio in sun leaves than in shade leaves, similar to what we found for the juveniles of this species. The larger chl *a*/*b* ratio in sun leaves reflected the larger PSII reaction complex centres and the lower number of light-harvesting complexes that contain mainly chl *b* (Evans and Poorter 2001; Walters 2005).

The leaves of all species increased foliar N concentration under HPPF and lower chl : N with respect to LPPF on Day 120, in agreement with other studies carried out in tropical forests. For example, Poorter *et al*. (2000) reported that foliar N in sun leaves was 60% higher than in the shade leaves for of *T. rubrivenium* adult trees. This response is regarded as characteristic of shade-tolerant plants, indicating that a larger foliar N proportion was invested in chlorophyll to increase light capture at the expense of N investment in Rubisco for C fixation (Poorter *et al*. 2000; Rozendaal *et al*. 2006).

For *T. rubrivenium*, it seems that in spite of the similar ΦPSII seen under both light treatments, there were more open reaction centres under the high light treatment (higher qP). This could partially explain other changes in the biochemical machinery (higher N content, lower chl : N ratio) and morphoanatomical characteristics (lower SLA, higher stomatal density), the capacity of this species to increase the assimilation rate at higher PPF.

Overall, our results agree with the notion that at least in the seedling–sapling stage, many tree species comprise a broad functional group of shade-tolerant generalists, characterised by a limited capacity of photosynthetic acclimation to high radiation conditions. Meanwhile, species with preferences for more illuminated environments comprise a group of specialists able to acclimate to a sudden increase in radiation exposure such as that occurring in treefall gaps (Chazdon *et al*. 1996; Barker *et al*. 1997; Krause *et al*. 2001; Goldstein *et al*. 2016). This model helps us to elucidate an array of functional traits related to photosynthetic performance that influence tree species' distribution along the gradient of light availability in the understorey of our study site, characterised by deep shaded conditions extended in a narrow range of canopy openness (Quevedo *et al*. 2015, 2016). Generally, morphological (SLA) and anatomical (stomatal density) traits were more plastic than photosynthetic traits (e.g. A_{max}, F_v/F_m) to changes in the light environment. Our results clearly show that chronic photoinhibition and the consequent inability of shade-tolerant plants to increase the C assimilation rates under high irradiance is a key factor contributing to explaining their absence in the upper end of the light gradient (above 12.6% of canopy openness). On the other hand, we could distinguish subtle variations in the studied photosynthetic traits, allowing us to separate the species that behaved like partially shade-tolerant plants that were able to recover from the photoinhibited state (*B. sulcata*, *C. tachirensis*) from the shade-tolerant functional group.

The long-term persistence of tree species in the understorey may depend on other traits that are not directly related to photosynthetic capacity, such as patterns of C allocation or defence against herbivores (Kitajima 1994). Nonetheless, our results support that photoinhibition is an effective mechanism of acclimation to sudden changes in light availability in the seedling–sapling stage (Mulkey and Pearcy 1992; Yamashita *et al*. 2000). This issue is a key factor in explaining tree species' responses to disturbances and hence to understand successional patterns in forest ecosystems.

Conflicts of interest

The authors declare no conflicts of interest.

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