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Changes in phenotypic variability of two tropical woody species due to short and long-term exposure to different irradiances

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ABSTRACT: Studies addressing the physiological and anatomical responses of plants under different light intensities normally are performed in short term. Thus, the present study includes a long term analysis. This study aims to verify whether the phenotypic variability off attributes in two woody tropical specieis, *Paquira aquatica* and *Sterculia foetida,* are compatible both under short term exposure to different light availability levels (full-light and half-light local irradiance) and long-term exposure in the same conditions. The study was developed in two phases: phase I (short term) and phase II (long term). The variables measured were referent to CO_2 assimilation responses to light intensity, chlorophyll *a* fluorescence, chlorophyll content, morpho-anatomical attributes and phenotypic variability. In phase I, *P. aquatica* had differences between treatments in A_{max} (maximum net photosynthesis) and LCP (light compensation point), without differences in these attributes in phase II. *S. foetida* plants had differences only in phase I in A_{max}. In anatomical attributes, *P. aquatica* had a higher palisade and spongy parenchyma and mesophyll thickness compared to *S. foetida* plants, without differences between light treatments. In *S. foetida*, the aforementioned cell layers were thicker in full-light treatment. Both species showed a higher phenotypic variability in the same physiological attributes in phase I and similar attributes for mesophyll thickness of the abaxial epidermis in phase II. The species showed different anatomical and physiological strategies, however with plastic responses in similar attributes, only observable after a longer period of exposure. The results indicated the importance of lengthy exposure to light, mainly in tropical species, wich are naturally exposed to elevated irradiance levels for an extended period of time in the field.

Key words: chlorophyll *a* fluorescence, gas exchange, plastic response, light curve.

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INTRODUCTION

Plants can alter physiological and anatomical attributes to fit the environment under different light conditions (Oguchi et al. 2003). The acclimation process in the leaf photosynthetic metabolism has a particular pattern under different light availability (Hallik et al. 2012). Plant species can adapt, both through natural selection or by modifying their physiology, morphology, and anatomy via phenotypic plasticity (Valladares 2008) in order to maintain stable growth. Furthermore, studies on phenotypic plasticity using variation coefficient are relevant to characterize species performance under environmental changes (Valladares et al. 2006).

Studies have shown the relationship between photosynthetic metabolism and light availability in herbaceous and shrubs (Oguchi et al. 2003) and tree species (Hallik et al. 2012; Kitao et al. 2016). However, most studies on light acclimation have been conducted over a short term period of exposure, not reflecting real field conditions, especially for tree species, which normally exhibit slower development.

In Brazilian Northeastern region, solar radiation is high from early morning onward (Frosi et al. 2013; Oliveira et al. 2014). In this region, most species are submitted to over double the radiation quantities needed to saturate photosynthetic machinery. In this study, we used two tropical woody species, *Pachira aquatica* and *Sterculia foetida*, which are natural to Brazilian's Northeast flora. Due to the high oil content in their seeds (Oliveira et al. 2000), the aforementioned species are important in recovery efforts in degraded areas by deforestation (Meli and Carrasco-Carballido, 2008; Santos et al. 2004). Furthermore, studies by Frosi et al. (2017) report that both species had drought tolerance and by Lustosa et al. (2017) also pointed that *S. foetida* possesses tolerance to NaCl contents on soil. Thus, the use of these species is feasible as economic and ecological alternative in the cultivation of semiarid regions.

In light of this conclusion, this study aims at verifying whether phenotypic variability attributes remained the same in both species when exposed to short (phase I) and long term (phase II) different light conditions. Moreover, this study attempts to identify whether both woody species show variations in the same attributes over time, due to belonging to the same family. We hypothesized that the attributes with high phenotypic variability of physiological and anatomical attributes are the more important to light acclimation. Furthermore, these attributes are the same among phases I and II and are similar in both species.

MATERIAL AND METHODS

Characterization of species

Pachira aquatica Aubl. and *Sterculia foetida* L. belong to the Malvaceae family. *P. aquatica* is native to the area from southern Mexico to northern South America, and is often found in flooded areas and riparian forests. It is an evergreen tree with a dense canopy, measuring 6-14 m in height and easily adapting to different edaphic conditions (Peixoto and Escudeiro 2002). *S. foetida* is native to the area from East Africa to North Australia and well adapted to tropical and sub-tropical climates. It is large, straight, and can grow up to 40 m in height (Vipunngeun and Palanuvej 2009).

Plant material and growth conditions

The experiment was conducted in a greenhouse (8°08'58"S, 34°56'55"W) in the northeast region of Brazil. The design for the experiment was completely randomized in a factorial arrangement of 2 light levels [full-light and half-light] × 2 species [*Pachira aquatica* and *Sterculia foetida*], totaling 4 treatments: *P. aquatica* under full and half light; *S. foetida* under full and half light, with three plants for each treatment. Exclusively for density epidermal cell and epidermal thickness, we also were considering the leaf surface (abaxial and adaxial) as factor.

Seeds from two woody species were disinfected with 1% (v/v) sodium hypochlorite for 5 min and germinated, one plant per pot, in pots with 10 L of a mixture of soils from an organic horizon, a red clay horizon and washed sand in a ratio of 3:1:1, respectively. *S. foetida* and *P. aquatica* plants with 60 and 90 days of development, respectively, were subjected to two different light intensities: full-light and half-light local irradiance. The half-light irradiance was obtained with the use of a screen structure over the plants, which reduced the light radiation by 50%. Throughout the experiment, the plants were kept at pot hydration capacity (500 mL) every day. The all experiment lasted 417 days. The phase I (short term) refer to approximately three months of exposure (153 days), where the measurements were performed →

among 132 to 153 days. After these measurements, the plants continued under these conditions (full and half-light) until 417 days of exposure, characterizing phase II (long term). In phase II, measurements were performed among 390 to 417 days. These intervals were determined to verify whether changes in physiological attributes remain the same over time, whereas most studies have only analyzed short time periods in order to verify acclimation strategies.

The vapor pressure deficit (VPD) was calculated according to Campbell and Norman (1998). It was obtained using temperature and relative air humidity, measured with a digital thermal hygrometer (Termo-Higro SH 122, J Prolab., São José dos Pinhais, BR) in each light condition. For full-light and half-light irradiance, the VPD average was 2.4 kPa.

Plant water status

The leaf water potential (Ψ_l) was measured at the end of phases I and II in 5 plants per treatment, at predawn, using a pressure chamber (model 3035, Soil Moisture Equipment Corp., Santa Barbara, CA, USA).

Gas exchange and chlorophyll fluorescence

Gas exchange and chlorophyll fluorescence measurements were performed in 5 replicates in one third of each expanded leaf. The measurements were recorded from 9 h to 10 h 30 min with an infrared gas analyser (IRGA, LI-COR, model LI – 6400XT, Lincoln, NE, US) in a leaf chamber fluorometer (6400–40) during phase I and phase II for each treatment. The chamber had an area of 2 cm^2 and a gas flow of 400 $mmol \cdot s^{-1}$. The PPFD of 2,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was used for the full-light treatment and 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the half-light treatment. The VPD leaf average under full-light and half-light irradiance was 4 and 3 kPa, respectively. Net photosynthetic rate (A), stomatal conductance (g_s), and transpiration rate (E), were measured.

For the chlorophyll *a* fluorescence variable, the leaves were adapted to the dark for 30 min to determine minimal fluorescence from a dark-adapted leaf (F_o). Maximum fluorescence from a dark-adapted leaf (F_m) was calculated after a saturation pulse of ~7000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Fluorescence emission from a light-adapted leaf (F') and maximum fluorescence from a light-adapted leaf (F_m') were determined for light-adapted leaves undergoing stable photosynthesis. The data was used to calculate the maximum quantum efficiency of PSII photochemistry (F_v/F_m), electron transport rate (ETR), PSII operating efficiency (F_q'/F_m'), photochemical quenching (qP), and nonphotochemical quenching (NPQ) (Baker, 2008).

CO_2 assimilation responses to light intensity (*A*-PPFD)

The *A*-PPFD curves were obtained by varying PPFD values from 20 to 2,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with an infrared gas analyzer (IRGA, LI–COR, model LI–6400XT, Lincoln, NE, US) in 3 plants from each species per treatment. The curves were designed in a controlled environment (temperature: 25°C; relative humidity: 68%) where the plants were exposed at 153 (phase I) and 410 days (phase II) of light availability.

The *A*-PPFD curve was obtained using the Prado and Moraes (1997) as illustrated by Eq. 1:

$$A = A_{max} (1 - e^{-k(PPFD - LCP)}) \quad (1)$$

where A = net photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$), A_{max} = maximum net photosynthesis ($\mu mol \cdot m^{-2} \cdot s^{-1}$), e = Euler constant, k = constant of proportionality, PPFD = Photosynthetic photon flux density ($\mu mol \cdot m^{-2} \cdot s^{-1}$), and LCP = light compensation point ($\mu mol \cdot m^{-2} \cdot s^{-1}$). From the curves, the maximum net photosynthesis (A_{max}), light compensation point (LCP), light saturation point (LSP), and dark respiration rate (R_D) of the plants were calculated. R_D was calculated by attributing a zero value for PPFD. LSP was found at 90% of A_{max} in the adjusted PPFD curve by using Eq. 1. The rate of CO_2 production by photorespiration (P_R) can then be calculated as described in Franco and Lüttge (2002) on Eq. 2:

$$P_R = [\alpha_L (ETR_{sat}) - 4(A_{sat} + R_D)]/12 \quad (2)$$

where αL = light absorbance of leaves of each species [however, typical values are in the range of 0.8 (Long et al. 1993), and this value was used in this study], ETR = electron transport rate at light saturation point, A_{sat} = net photosynthesis at light saturation point, and R_D = dark respiration. The data were processed using the OriginPro 8 SRO software version 8.0724 (OriginLab Corporation, Northampton, USA).

Chlorophyll content

Chlorophyll (Chl) content and leaf anatomical analyses were performed in 3 replicates from each treatment with the same leaves used in the gas exchange and chlorophyll fluorescence measures. Chl *a* and *b* contents were analyzed in phase I and phase II of each treatment in both species by macerating 80 mg of leaf tissue in 2 mL of acetone (80%) with $CaCO_3$ to prevent chlorophyllase activity, according to Lichtenthaler and Buschmann (2001). The absorbance was measured at 470.0, 646.8, 663.2, and 710 nm using a double beam spectrophotometer (Genesys 10S UV-Vis, Thermo Scientific, Madison, Wisconsin, USA).

Leaf anatomical analyses

The anatomical attributes were evaluated by the end of phase II. Leaf samples exposed to different light availabilities were collected from 3 replicates and fixed in FAA 50 for 48 h and stored in 70% ethanol. The samples were dehydrated in an ethanol-butanol series, embedded in paraffin, and transversely sectioned using a 10 μm Zeiss rotary microtome (model HYRAX M55). The sections were stained using 1% Alcian blue and 1% safranin and placed on semi-permanent slides using Canada balsam (Purvis et al. 1964). The dissociation of the epidermis was performed in *S. foetida* with a hydrogen peroxide and acetic acid 1:1 solution (Franklin 1945), and in *P. aquatica* with a 50% sodium hypochlorite solution, washed in distilled water and stained with safranin (1%). The semi-permanent slides were mounted in 50% glycerin (Purvis et al. 1964), and the structures were recorded using a Leica digital imaging system (LAS EZ Version 2.0.0 ICC 50) attached to a Leica photomicroscope (model DM500). The images were analyzed using the ImageJ software version 1.47r. Stomatal and epidermal cell densities, epidermal thickness, and leaf palisade and spongy parenchyma thickness were measured in 20 randomly selected microscopic fields obtained from three leaves per plant, and the stomatal index was calculated according to Salisbury (1928).

Phenotypic variability measures

A general coefficient of variation for physiological (gas exchange and chlorophyll *a* fluorescence) and anatomic attributes was calculated using data from both phases (phase I and II) for each species (Valladares 2006) as described in Eq. 3:

$$\%CV = SD/TotalAv \times 100 \quad (3)$$

where SD is the standard deviation of TotalAv, and TotalAv is the average of each characteristic in each phase for each species.

The confidence interval was calculated using the Bootstrap confidence interval tool in the R program and using 999 randomizations (Version 0.99.486 – 2009-2015 RStudio, Inc.).

Statistical analyses

The data for gas exchange, chlorophyll *a* fluorescence, stomatal density, stomatal index mesophyll thickness and the palisade and the spongy parenchyma were submitted to the factorial ANOVA, comparing light availability conditions (full-light and half-light) and species (*P. aquatica* and *S. foetida*).

Density epidermal cells and epidermal thickness (anatomical attributes) were subjected to a factorial ANOVA, comparing light availability conditions (full-light and half-light), leaf surface (abaxial and adaxial) and species (*P. aquatica* and *S. foetida*). For these attributes, the leaf surface is the important factor, mainly in different light conditions, since the leaves are very plastic. When significant differences were observed, the means were compared using the Student Newman Keuls test at a 5% probability. In analyses, all the prerequisites (normality and homogeneity) were respected. The data was analyzed using the Statistica 7.0 software (StatSoft. Inc., Tulsa, OK, USA).

RESULTS

Plant water status

The plant water status (Ψ_l) at predawn did not differ in phases I and II among treatments for both species. The Ψ_l at predawn was –0.27 MPa for *P. aquatica* and –0.34 MPa for *S. foetida* (data not shown).

CO_2 assimilation responses to light intensity (*A*-PPFD)

A_{max} and LCP showed differences between light treatments and species in phase I. *P. aquatica* plants had lower A_{max} →

under half-light irradiance compared to full-light treated plants, while *S. foetida* plants exhibited opposite behaviour, with reduction above 30% (Figure1 and Table 1). LCP did not differ in *S. foetida*; however, it was 60% lower in *P. aquatica* plants under full-light irradiance compared to the half-light treatment.

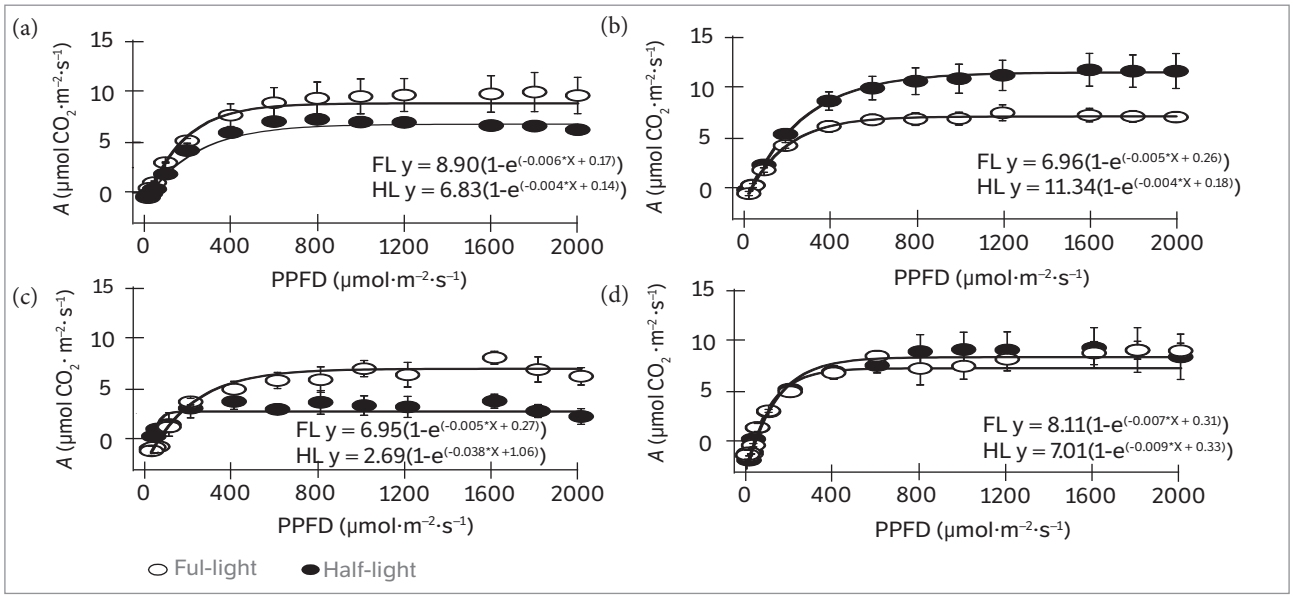

Figure 1. Light response curves of net photosynthesis (*A*) in function of photosynthetic photon flux density (PPFD) of *Pachira aquatica* (a,c) and *Sterculia foetida* (b,d) plants at 153 (phase I – a,b) and 410 days (phase II – c,d) under controlled conditions (constant temperature at 25°C and relative humidity at 68%) exposed to 1,000 (half-light - HL) and 2,000 µmol·m^{-2}·s^{-1} (full-light - FL) irradiance (n = 3 ± SE)

Table 1. Maximum net photosynthesis (A_{max}), light compensation point (LCP), light saturation point (LSP), dark respiration (R_D), photorespiration (P_R) and chlorophyll (Chl) in the leaves of young plants from *Pachira aquatica* and *Sterculia foetida* grown under different light availability (full-light and half-light of irradiance) after 150 (phase I) and 365 (phase II) days of exposure in treatments. Values represent the mean values of experimental replicates (n = 3 ± SE).

	Pachira aquatica		*Sterculia foetida*	
	Full-light	**Half-light**	**Full-light**	**Half-light**
		Phase I		
A_{max}	9.83 ± 1.88^{A}	6.88 ± 0.41^{B}	6.99 ± 0.62^{B}	11.37 ± 1.69^{A}
LCP	22.85 ± 0.78^{C}	56.58 ± 0.05^{A}	48.33 ± 4.80^{AB}	41.41 ± 3.96^{B}
LSP	565.91 ±113.07^{NS}	485.92 ± 45.58	489.52 ± 23.71	598.52 ± 23.71
R_D	–0.50 ± 0.25^{NS}	–1.25 ± 0.40	–1.34 ± 0.20	–1.35 ± 0.20
P_R	2.78 ± 0.52^{NS}	2.46 ± 0.12	2.31 ± 0.14	3.42 ± 0.34
Chl *a*	2.15 ± 0.02^{C}	1.88 ± 0.10^{C}	4.36 ± 0.15^{B}	4.75 ± 0.15^{A}
Chl *b*	0.70 ± 0.01^{C}	0.57 ± 0.02^{C}	1.25 ± 0.05^{B}	1.44 ± 0.06^{A}
		Phase II		
A_{max}	6.60 ± 1.10^{AB}	3.31 ± 0.79^{B}	8.10 ± 1.18^{A}	6.72 ± 0.08^{AB}
LCP	71.81 ± 3.93^{A}	54.39 ± 6.75^{A}	34.40 ± 8.59^{B}	45.76 ± 3.20^{B}
LSP	603.27 ± 77.96^{A}	186.83 ± 15.36^{B}	313.08 ±131.92^{AB}	323.96 ± 2.36^{AB}
R_D	–1.06 ± 0.12^{NS}	–2.39 ± 0.11	–1.93 ± 0.52	–1.65 ± 0.67
P_R	4.57 ± 0.38^{A}	1.26 ± 0.25^{B}	2.05 ± 0.33^{B}	2.34 ± 0.26^{B}
Chl *a*	1.76 ± 0.22^{A}	1.75 ± 0.27^{A}	0.96 ± 0.14^{B}	1.12 ± 0.21^{B}
Chl *b*	0.44 ± 0.02^{A}	0.43 ± 0.06^{A}	0.22 ± 0.01^{B}	0.36 ± 0.04^{B}

*Different letters in lines denote significant differences between treatments (full and half-light) and species (*P. aquatica* and *S. foetida*) by Newman Keuls test ($P < 0.05$) in each phases (phase I and II).

A_{max} did not differ in phase II for both species. *P. aquatica* showed higher LCP than *S. foetida*. *P. aquatica* showed higher LSP under full-light compared to the half-light treatment. *P. aquatica* plants showed higher *P*r under full-light compared to half-light irradiance and *S. foetida* plants (Figure1 and Table 1).

Chlorophyll content

Chlorophyll content differed between treatments and species in phase I. *P. aquatica* plants had lower concentration of Chl *a* and Chl *b* than *S. foetida*. However, *S. foetida* plants had higher chlorophyll concentration under half-light irradiance when compared to full-light treatment (Table 1).

In phase II, *P. aquatica* plants showed higher concentrations of Chl *a* and Chl *b* than *S. foetida* (Table 1).

Leaf anatomical attributes

Both species had stomata only on abaxial side. *Pachira aquatica* showed lower stomatal index under half-light irradiance when compared to samples under full-light irradiance. *S. foetida* showed no difference of stomatal index between treatments. However, *P. aquatica* showed the highest index values (Table 2).

Density of epidermal cells was higher on adaxial face of *P. aquatica*, while *S. foetida* showed higher density on abaxial face under full-light treatment for both species (Table 2). Epidermal thickness differed between species and sides, being thicker in *P. aquatica* plants than *S. foetida* plants. The highest values were obtained on adaxial faces of both species under both light treatments (Table 2).

P. aquatica plants showed thicker palisade and spongy parenchyma and mesophyll than *S. foetida*. These attributes differed in *S. foetida* plants between treatments, with higher values under full-light treatment than half-light irradiances (Table 2).

Phenotypic variability

S. foetida and *P. aquatica* showed similar phenotypic variability for all physiological attributes in phase I (Table 3), with greater variability in F_q'/F_m', g_s, NPQ and qP. In phase II, *P. aquatica* showed greater variability in g_s, NPQ, *A*, *E* →

Table 2. Anatomical attributes in the leaves of young plants from *Pachira Aquatica* and *Sterculia foetida* under different light availability (full-light and half-light of irradiance) after 365 days of exposure (phase II). Values represent the mean values of experimental replicates (n = 3 ± SE)

	Pachira aquatic			
	Full-light		**Half-light**	
	Adaxial	**Abaxial**	**Adaxial**	**Abaxial**
Stomatal density (0.02 mm²)	-	21.90 ± 0.72^{A}	-	15.20 ± 0.73^{B}
Density epidermal cells (0.02 mm²)	89.80 ± 0.94^{C}	52.70 ± 1.76^{E}	83.10±1.9^{D}	51.30 ± 1.7^{E}
Stomatal index (%)	-	29.30 ± 1.23^{A}	-	22.90 ± 1.0^{B}
Epidermal thickness (µm)	21.30 ± 0.61^{A}	11.30 ± 0.36^{B}	19.50±0.9^{A}	10.30 ± 0.48^{B}
Palisade parenchyma (µm)	48.80 ± 1.11^{A}		49.90 ± 1.79^{A}	
Spongy parenchyma (µm)	87.60 ± 2.94^{A}		93.17 ± 3.78^{A}	
Mesophyll thickness (µm)	136.45 ± 2.97^{A}		143.14 ± 5.25^{A}	
	Sterculia foetida			
Stomatal density (0.02 mm²)	-	22.25 ± 0.89^{A}	-	20.15 ± 0.82^{A}
Density epidermal cells (0.02 mm²)	83.10 ± 1.26^{D}	129.60 ± 2.6^{A}	81.30 ± 0.76^{D}	109.70 ± 2.16^{B}
Stomatal index (%)	-	14.68 ± 0.55^{C}	-	15.50 ± 0.52^{C}
Epidermal thickness (µm)	9.12 ± 0.57^{C}	6.17 ± 0.35^{D}	8.42 ± 0.44^{C}	6.36 ± 0.45^{D}
Palisade parenchyma (µm)	27.86 ± 1.12^{B}		23.46 ± 1.12^{C}	
Spongy parenchyma (µm)	30.38 ± 0.73^{B}		25.85 ± 0.55^{C}	
Mesophyll thickness (µm)	58.24 ± 1.51^{B}		49.30 ± 0.95^{C}	

*Different letters in lines denote significant differences between species (*P. aquatica* and *S. foetida*), treatments (full-light and half-light of irradiance) and faces (adaxial and abaxial) for stomatal density; density epidermal cells; stomatal index and epidermal thickness, and denote significant differences between species (*P. aquatica* and *S. foetida*) and treatments (full-light and half-light of irradiance) for palisade and spongy parenchyma and mesophyll thickness by Newman-Keul test ($P < 0.05$).

Table 3. Coefficient of variation (CV) and confidence intervals (CI) of biological attributes of *Pachira aquatica* and *Sterculia foetida* plants under different light availability (full-light and half-light of irradiance) after 150 (phase I) and 365 (phase II) days of exposure.

Biological Attributes	Phase I		Phase II	
	P. aquatica CV (CI)	*S. foetida* CV (CI)	*P. aquatica* CV (CI)	*S.foetida* CV (CI)
	Physiological			
A	25.34 (18.58-33.62)	33.63 (26.28-42.42)	59.51 (46.09-76.44)	33.64 (22.03-47.34)
g_s	41.25(34.06-50.95)	49.07 (36.75-63.68)	66.40 (50.43-85.34)	49.78 (37.14-65.79)
E	30.53 (23.73-38.92)	32.18 (24.05-41.63)	58.22 (45.43-73.94)	37.75 (24.91-52.95)
F_q'/F_m'	42.69 (36.57-50.27)	56.53 (46.89-69.51)	34.90(27.91-43.71)	37.80 (31.58-45.56)
q_p	36.48 (29.67-44.47)	45.46 (37.94-56.05)	29.96 (21.69-39.73)	36.26 (29.80-44.40)
F_o	20.97 (14.76-28.65)	27.06 (17.69-39.02)	45.18 (30.21-63.68)	30.51 (17.16-45.00)
F_m	11.98 (9.46-15.35)	23.84 (13.85-37.20)	10.27 (7.98-13.12)	10.52 (8.27-13.34)
F_v/F_m	7.88 (5.67-10.54)	9.77 (6.94-13.23)	12.21 (8.28-16.86)	7.14 (5.17-9.60)
NPQ	38.48 (28.32-50.92)	53.35 (40.51-68.15)	65.15 (50.71-84.40)	36.25 (28.11-46.39)
ETR	22.11 (14.70-30.91)	33.65 (28.27-41.28)	24.86 (19.06-32.15)	17.29 (12.69-22.87)
	Anatomic			
Mesophyll thickness	---	---	13.69 (10.29-18.02)	13.36 (11.06-16.15)
Abaxial epidermal thickness	---	---	17.85 (14.57-21.60)	28.48 (23.05-34.59)
Adaxial epidermal thickness	---	---	17.50 (13.95-21.71)	26.02 (20.88-32.41)
Palisade parenchyma thickness	---	---	16.20 (12.32-20.59)	21.13 (17.89-24.88)
Spongy parenchyma thickness	---	---	16.84 (13.14-21.22)	13.04 (10.29-16.30)
Adaxial epidermal cell density	---	---	7.17 (5.82-8.77)	5.70 (4.75-6.86)
Abaxial epidermal cell density	---	---	14.86 (11.09-19.26)	12.22 (9.88-14.90)
Stomatal density			25.17 (21.23-29.82)	18.57 (15.69-22.01)
Stomatal index			22.90 (18.46-28.23)	15.92 (13.59-18.71)

and F_o, while *S. foetida* presented greater variability in g_s, F_q'/F_m', *E*, qP, NPQ and *A*. Furthermore, *P. aquatica* showed greater variability only in NPQ compared to *S. foetida*. In phase II, *P. aquatica* had greater variability in *A*, *E*, and F_o, while *S. foetida* showed decreased variability of F_q'/F_m', $F_{m,}$ and ETR, compared to phase I.

In anatomical attributes, *P. aquatica* showed the greatest variability of stomatal density, stomatal index, and abaxial and adaxial epidermal thickness. Abaxial and adaxial epidermis and palisade parenchyma were thicker in *S. foetida*. Furthermore, *S. foetida* showed greater variability of abaxial epidermis thickness than *P. aquatica*.

DISCUSSION

Net photosynthetic rate changed during experimental period in *P. aquatica* and *S. foetida,* demonstrating the acclimation capacity of these woody species. In phase I, *P. aquatica* and *S. foetida* had differences in A_{max}, while *P. aquatica* showed differences in LSP and P_r in phase II, which were higher under full-light treatment. Results suggest that fully expanded leaves of trees could change photosynthetic activity when more mature, showing a dynamic pattern (Hallik et al. 2012).

P_R may represent strong electron drain in C_3 plants related to protection mechanisms (Silva et al. 2015), which could be stimulated by excess light energy, as observed in *P. aquatica* under full-light irradiance during phase II. Allocation of high excitation energy to P_R could mitigate deleterious effects of excessive irradiance, such photoinhibition (Guan and Gu 2009; Osmond and Grace 1995). This alteration of P_R in *P. aquatica* plants was followed by greater variability of NPQ, compared to *S. foetida* between phase I and II. NPQ is non-radiative energy dissipation by thermal processes, being a mechanism of photoprotection, reducing probability →

of photodamage and photooxidative stress in chloroplasts (Bonifacio et al. 2011; Silva et al. 2010). Furthermore, *P. aquatica* plants had higher concentration of Chl *a* and *b* in phase II compared to *S. foetida*. Chlorophyll also could act as alternative electron drain promoting photoprotection of photosynthetic machinery combined with P_R and NPQ. Light adaptation involves both structural and functional differences, as adaptation of pigment levels (Lichtenthaler 2007; Urban et al. 2007). In most cases, these traits promote advantages to light interceptions and would support photosynthetic machinery activity (Lichtenthaler et al. 2013).

Foliar anatomical aspects also revealed strategies used by species, which may have influenced gas exchange directly (Yang et al. 2014). *P. aquatica* plants showed the highest values in palisade and spongy parenchyma and mesophyll thickness in both irradiances, followed by *S. foetida* under full-light irradiance. Furthermore, full-light irradiance stimulated higher epidermal cell density on the adaxial and abaxial surface of *P. aquatica* and *S. foetida* plants, respectively. Parenchyma and mesophyll thickness are crucial for providing more space to chloroplasts position (Terashima et al. 2001). Thus, mesophyll thickness is very important to achieve high *A* values under different light availability (Oguchi et al. 2003).

Other traits with influence on gas exchange are stomatal density and stomatal index which varied between light treatments, being higher under full-light treatment in *P. aquatica* plants. Stomatal development can be affected by several environmental variables; stomatal index of dicot plants increased with light intensity (Lake et al. 2001; Schoch et al. 1980). Indeed, stomatal densities are higher in plants grown under full sunlight or high light intensities than those in plants grown under low light irradiance (Willmer and Fricker 1996). We observed such a behavior only in *P. aquatica* plants. Thus, leaf morphological traits, including stomatal density and distribution, may affect gas exchange and relationships with environmental factors, such as light intensity (Nilson and Assmann 2007).

Coefficient of variation (CV) analysis can be a useful tool for exploring phenotypic variability and alternative to estimate phenotypic plasticity (Valladares et al. 2006). Higher CV values correspond to greater trait variability, allowing a practical measure of relative phenotypic variation as an indicator of plasticity. Phenotypic variability showed that both species changed all physiological and anatomical attributes. In this study, physiological attributes showed greater variation compared to anatomic attributes (Table 3). *P. aquatica* plants increased variability in the same physiological attributes in both phases of light exposure, such as photosynthesis, while *S. foetida* had reduced variability in phase II, mainly in photochemical attributes. This distinct behaviour shows that degrees of response could modify over a long period of exposure to different irradiances in this species. Increased variability of *P. aquatica* at phase II reduced differences between treatments, especially photosynthesis, indicating this species acclimatization. Reduced variability of photochemical attributes in *S. foetida* suggests this species already acclimated in terms of photosynthetic machinery to different light conditions over short exposure time to different irradiances. Results indicated that both species had different plastic responses under a long time of exposure to different light conditions.

Therefore, our initial hypothesis was partly corroborated. Some attributes, more variable in phase I, occurred in both species in phase II, such as g_s and NPQ in *P. aquatica* and g_s, F_q'/F_m', qP and NPQ in *S. foetida*. Furthermore, both species exhibited plastic responses in the same attributes in both phases. F_q'/F_m', g_s, NPQ and qP varied in both species in phase I, while F_q'/F_m', qP and F_o, and stomatal density as stomatal index were observed in phase II (Table 3). Furthermore, the high phenotypic variability was important to initial acclimation to light conditions. However, after some time, a reduction of this variability may occur, indicating acclimatization, as observed in *S. foetida* in some attributes in phase II.

CONCLUSION

Both species showed different anatomical and physiological strategies leading to acclimation under different irradiances, including excess radiation. Plastic responses occurred in similar attributes. The adequate measurement and documentation of this behaviour was only possible due to long-term analyses. This study suggests that extensive time is necessary for acclimatization of woody species under different light intensities. In this work, we used two species that are potential economic alternative to cultivation on semiarid regions. Studies on this profile, reflecting the light environmental condition and the time of exposure in the field, could be interesting to optimize tolerance, development and production of these species in the future.

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