

Key leaf traits indicative of photosynthetic plasticity in tropical tree species

**Letícia dosAnjos, Marco A. Oliva,
Kacilda N. Kuki, Marcelo S. Mielke,
Marília C. Ventrella, Mayra F. Galvão &
Luiz R. M. Pinto**

Trees

Structure and Function

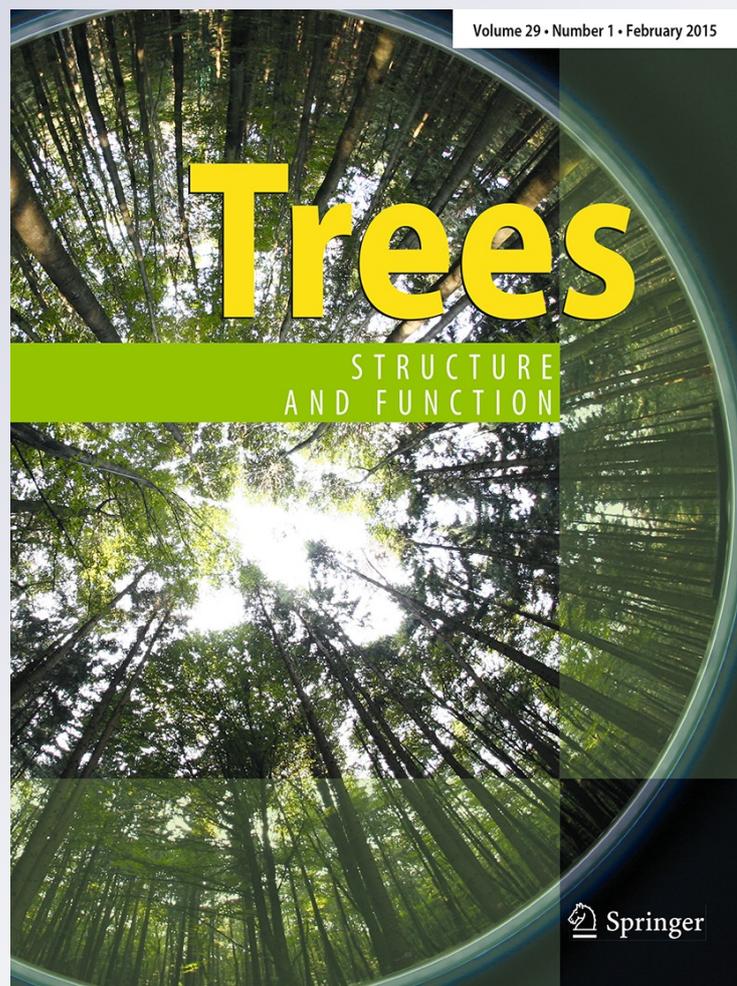
ISSN 0931-1890

Volume 29

Number 1

Trees (2015) 29:247-258

DOI 10.1007/s00468-014-1110-2



Your article is protected by copyright and all rights are held exclusively by Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Key leaf traits indicative of photosynthetic plasticity in tropical tree species

Letícia dosAnjos · Marco A. Oliva · Kacilda N. Kuki · Marcelo S. Mielke ·
Marília C. Ventrella · Mayra F. Galvão · Luiz R. M. Pinto

Received: 24 February 2014 / Revised: 16 September 2014 / Accepted: 9 October 2014 / Published online: 18 October 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract

Key message The main achievement of this work was an attempt to determine the best association between five tropical species and light environments, based on the physiological and morphological characters related to photosynthesis.

Abstract Information about light tolerance and photosynthetic plasticity of indigenous tropical tree species is still limited, particularly information of first years of plants' development. In this study, we evaluated the adjustments in response to different light environments of 25 leaf traits most commonly assessed in studies on light

acclimation of photosynthesis in current literature. This evaluation was used to investigate the photosynthetic plasticity on young plants of five tropical tree species belonged to different successional groups. All the species are commonly used in forest restoration programs in Brazil. Plants were grown for 6 months under different light conditions simulating environments that could exist due to variation in naturally occurring canopy openings of secondary tropical forests. The level of adjustment on leaf traits to environmental conditions was calculated via a plasticity index. The relation between leaf trait adjustments and species photosynthetic plasticity was investigated by multivariate Biplot analyses. We selected the seven most explicative leaf traits of the photosynthetic plasticity of the studied species in response to different light environments: dark respiration rate (R_d), Rubisco carboxylation capacity (V_{cmax}), total chlorophyll content (ChlT), contribution of spongy parenchyma (%SP), contribution of leaf collenchyma tissue (%C), chlorophyll parenchyma thickness (PP/SP) and specific leaf area (SLA). Based on the selected traits, we identified the traits most related to high plasticity (V_{cmax} , PP/SP, %SP, %C and SLA) and low plasticity (V_{cmax} , R_d and ChlT) and grouped species into three different patterns of photosynthetic plasticity. Our plasticity grouping was not correlated with species successional classification, indicating the importance of including physiological features related to light tolerance in species successional classifications. This work provides complementing information to traditional species successional groupings and to our current ability to select species for enrichment planting on restoration efforts.

Communicated by M. Buckeridge.

Electronic supplementary material The online version of this article (doi:10.1007/s00468-014-1110-2) contains supplementary material, which is available to authorized users.

L. dosAnjos · M. A. Oliva · K. N. Kuki ·
M. C. Ventrella · M. F. Galvão
Departamento de Biologia Vegetal, Universidade Federal de
Viçosa (DBV/UFV), Av. PH Rolfs, S/N,
PO box 36571-6 000, Viçosa, MG, Brazil

L. dosAnjos (✉) · M. S. Mielke
Departamento de Ciências Biológicas, Universidade Estadual de
Santa Cruz (DCB/UESC) Campus Soane Nazaré de Andrade,
Pav. Jorge Amado, BR 415, Km 16, PO box 45662-900, Ilhéus,
BA, Brazil
e-mail: leticiadosanjos@gmail.com

L. R. M. Pinto
Departamento de Ciências Exatas e Tecnológicas, Universidade
Estadual de Santa Cruz (DCET/UESC) Campus Soane Nazaré de
Andrade, Pav. Jorge Amado, BR 415, Km 16,
PO box 45662-900, Ilhéus, BA, Brazil

Keywords Forest restoration · Phenotypic plasticity ·
Light acclimation · Biplot analysis

Introduction

Enrichment planting is a common technique in tropical forest restoration (Lamb et al. 2005; Kuptz et al. 2010). This technique relies on planting a mixture of indigenous species of different successional groups simultaneously in forest gaps and in remnant fragments which have low resilience and are unable to regenerate naturally (Rodrigues et al. 2009; Hall et al. 2011). Mixed plantation with indigenous species reproduces the high biodiversity found in tropical forests and accelerates and guides the forest regeneration process (Carpanezzi 2005). This method is more effective when seedlings are produced under controlled conditions and selectively introduced in the field according to their light requirements (Kuptz et al. 2010; Kenzo et al. 2011). The light range tolerated by species across environments differs among plant ontogenetic stages and among successional groups (Rozendaal et al. 2006; Barros et al. 2012; dosAnjos et al. 2012; Houter and Pons 2012). Many successional classifications have been suggested in literature (Budowsky 1965; Denslow 1987; Bazzaz and Pickett 1980; Swaine and Whitmore 1988; Nascimento et al. 1999; Gandolfi 1991, 2003). Each successional classification relies on different expert knowledge of species functional traits. The majority of the available classifications rely on growth and reproductive traits (germination strategy, dispersal syndromes, life expectancy, growth biometric parameters and characteristics of wood). Interestingly, none of these classifications takes into account plant ecophysiological features, such as the photosynthetic plasticity for light acclimation. The photosynthetic machinery is not only the plant's source of energy and biomass accumulation, but also a site that is severely damaged during light stress conditions (Ruban 2009). Impairment of the photosynthetic apparatus makes plants more susceptible to other environmental stresses. As a consequence, plants have their growth and establishment impaired (Mittler 2006). Therefore the photosynthetic plasticity and the light tolerance are factors that should also be considered when grouping species into functional groups. The ability to acclimate to different light conditions should be particularly considered when selecting groups of species for enrichment planting in secondary forest restoration.

Unfortunately, information about light tolerance and photosynthetic performance of indigenous tropical tree species is still limited, particularly during the plants' first years of development. In this study, we evaluated the photosynthetic plasticity in response to different light conditions of five tropical tree species belonging to different successional groups. All the species are commonly used in recent forest restoration programs in Brazil (Barbosa et al. 2006). We grew young individuals for

6 months under different light conditions simulating environments that could exist due to variation in naturally occurring canopy openings of secondary tropical forests. The species photosynthetic plasticity was evaluated based on the adjustments of 25 leaf traits most commonly assessed in studies on light acclimation of photosynthesis in current literature. The level of adjustment was calculated via a plasticity index. Considering that photosynthetic plasticity is a multivariate phenomenon in which different metabolic processes act at the same time and interact with one another, the relation between leaf traits and species plasticity in response to light was investigated by multivariate Biplot analyses. We directed our efforts to address the following issues: (1) What are the main leaf traits that mostly influence the photosynthetic plasticity of the five studied species in response to light environments? (2) Do these species show different patterns of photosynthetic plasticity? Do these patterns correlate to their successional groupings? Based on the answers to these questions, this work intends to provide additional information for the discussion on the ecophysiology of tropical tree species and for the improvement of our current ability to select species for enrichment planting in restoration efforts of secondary highly biodiverse tropical rainforests.

Materials and methods

Study area, plant material and experimental design

The study was conducted in the Plant Biology Department of the Universidade Federal de Viçosa (20°45'25.64"S and 42°52'23.91"W), Brazil. Five tropical tree species of different successional groups from the Brazilian Atlantic Rain Forest (Table 1) were chosen based on their successful use in recent forest restoration programs (Barbosa et al. 2006).

Table 1 List of the five studied species with their scientific name, family and successional group

Species	Family	Successional group ^a
<i>Schinus terebinthifolius</i> Raddi.	Anacardiaceae	Early secondary
<i>Pseudobombax grandiflorum</i> (Cav.) A. Robyns	Malvaceae	Early secondary
<i>Joannesia princeps</i> Vell.	Euphorbiaceae	Early secondary
<i>Lecythis pisonis</i> Camb.	Lecythidaceae	Late secondary
<i>Hymenaea courbaril</i> L. var. <i>stilbocarpa</i> (Hayne) Y. T. Lee et Langenh	Leguminosae Caesalpinioideae	Late secondary

^a According to Bazzaz and Pickett (1980)

Table 2 Daily average of global light radiation (GLR, W m^{-2}), integrated photosynthetically active light radiation (PAR, $\text{mol m}^{-2} \text{ day}^{-1}$), air temperature (T , $^{\circ}\text{C}$) and relative humidity (RH, %), withminimum and maximum values, under three light conditions I_{100} (full sun), I_{50} (50 % of full sun) and I_{20} (20 % of full sun)

Treatments	GLR	PAR	T	RH
I_{100}	200.0 (161–228)	30.0 (24.6–40.3)	25.5 (24.4–26.3)	72.8 (71.0–75.5)
I_{50}	83.0 (49.5–100)	12.7 (11.1–17.2)	24.8 (23.8–25.3)	75.8 (73.7–76.9)
I_{20}	37.6 (27.4–42.4)	5.4 (4.3–8.0)	24.2 (23.1–24.8)	76.8 (72.9–79.0)

The species successional classification was based on the classification of Bazzaz and Pickett (1980), the advice of specialists and empirical experiences in the field.

Approximately 2-month-old seedlings were transplanted to plastic pots containing five liters of substrate, a mixture of regional red–yellow latosol, sand and humus (1:1:1). Soil acidity and fertility were corrected accordingly to Ribeiro (1999). Plants were subjected to three light treatments according to the total daily photosynthetically active radiation (PAR), simulating different light environments inside the fragments of secondary tropical forests (Piotto 2011; dos Santos et al. 2012). The light treatments consisted of full sun (I_{100}) (100 % of solar radiation, simulating a clearing area or a situation with more than 35 % of canopy openness) or artificial shade settings provided by shade cloths with 50 % (I_{50}) of incident solar radiation (simulating an understory light environment of 15–35 % of canopy openness) or 20 % of incident solar radiation (I_{20}) (simulating an understory light environment less than 15 % of canopy openness). The experimental design was completely randomized with 15 treatments (three light intensities versus five species) and with four replications per treatment. Incident photosynthetic radiation was monitored with a line quantum sensor (model LI-191, LI-COR Inc., Lincoln, Nebraska, USA). The environmental conditions under I_{100} , I_{50} and I_{20} are depicted in Table 2. Throughout the experiment, plants were kept well watered and fertilized.

A selection of 25 physiological and morphological leaf traits was made for the analysis of light acclimation of photosynthesis. The selection was based on a search in the most-cited studies on photosynthetic light acclimation. All measurements began only upon the emergence of at least a pair of young leaves, fully developed in the experimental light conditions. This took approximately 4 months of exposure of plant material to treatments settings. For each plant, measurements were made on the same leaves from the distal third of the plant, which were fully expanded, were not shaded by other leaves, had the most leaf typical angle of the plant and did not show herbivory or disease symptoms.

Fluorescence of chlorophyll *a*

The fluorescence of chlorophyll *a* was measured using a portable pulse amplitude modulated fluorometer

MINI-PAM (Walz, Effeltrich, Germany) equipped with a special leaf-clip holder (model 2030-B). Prior to measurements, leaves were kept in dark environment for 30 min. The minimum fluorescence (F_0) was obtained by exciting the sample area using a low intensity ($0.03 \mu\text{mol m}^{-2} \text{ s}^{-1}$) modulated red light, while the maximum fluorescence (F_m) was obtained by applying a saturating actinic light ($>6,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$) pulse of 0.8 s duration. The values of F_0 and F_m were then used for calculating the potential quantum yield of photosystem II (F_v/F_m), given by $F_v/F_m = (F_m - F_0)/F_m$ according to Genty et al. (1989).

Leaf gas exchanges

Leaf gas exchange was measured with a portable open gas-exchange system (LI-6400, LI-COR Biosciences Inc., Lincoln, Nebraska, USA) equipped with a blue/red light source (LI-6400-02B, LI-COR), under CO_2 concentration, temperature and H_2O vapor of the experimental ambient environment. Light response curves were then constructed for eight levels of irradiance (0, 50, 100, 250, 500, 1,000, 1,500 and 2,000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$), in decreasing order, with a 60-sec duration at each level. Data were fitted to the equation $A = \alpha\text{PAR} + A_{\text{max}} - [\sqrt{(\alpha + A_{\text{max}})^2 - (4\alpha\text{PAR}kA_{\text{max}})}] / (2k) - R_d$, as given by Prioul and Chartier (1977), where A is the net assimilation rate ($\mu\text{mol m}^{-2} \text{ s}^{-1}$), α is the apparent quantum yield of CO_2 (mol mol^{-1}), A_{max} is the maximum photosynthetic rate ($\mu\text{mol m}^{-2} \text{ s}^{-1}$), PAR is the light level ($\mu\text{mol m}^{-2} \text{ s}^{-1}$), k is the convexity coefficient ($0 < k < 1$), and R_d is the dark respiration rate in daytime ($\mu\text{mol m}^{-2} \text{ s}^{-1}$). The light compensation point (LCP) and light saturation point (LSP) were calculated from the light response curve using Photosyn Assistant Software (Version 1.1, Dundee Scientific, UK). The A/C_i curves, where C_i is internal CO_2 concentration, showing the relationship between the net assimilation and CO_2 partial pressure, were obtained with the aid of an injector device of the LI-COR 6400 (6400-01 CO_2 injector; LI-COR USA) using 12 g CO_2 cartridges under high pressure. The measurements were carried out for eight CO_2 levels (50, 100, 200, 400, 700, 1,000 and 1,500 $\mu\text{mol mol}^{-1}$) under controlled temperature (25°C) and light irradiance (PAR of 1,500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$). The maximum Rubisco carboxylation velocity (V_{cmax}) and

the electron transport rate associated with the regeneration of RuBP (j) were calculated according to Sharkey et al. (2007).

Chlorophyll and carotenoid contents

The chlorophyll and carotenoid contents were obtained according to Hiscox and Israelstam (1979). Leaf disks of known area (0.785 cm^2) were collected, weighed using a precision scale, and immediately immersed in 5 mL of dimethyl sulfoxide (DMSO). The tubes were kept in darkness under room temperature for 48 h. The sample absorbances were measured at 480, 649 and 670 nm using a Hitachi U-2000 (Hitachi Instruments Inc., Danbury, CT) double beam spectrophotometer. The chlorophyll and carotenoid contents, expressed in mg per g of fresh weight, were estimated according to Wellburn (1994) from which the total chlorophyll (ChlT) content, and the chlorophyll a to chlorophyll b (Chl a/b) and the total chlorophyll to carotenoid (ChlT/Carot) ratios were calculated.

Nitrogen content

The nitrogen content was estimated according to Kjeldahl method. The leaf samples were mineralized, then digested and subsequently heated using sulfuric acid, sodium sulfate, copper sulfate and sodium selenite solutions. The nitrogen was distilled in an alkaline medium with boric acid as receptor and its content estimated by titration in 0.05 N hydrochloric acid. The total leaf nitrogen content (N) was then expressed per unit of leaf dry weight in g g^{-1} .

Leaf quantitative anatomy

Harvested leaves were fixed in FAA50 % for 48 h and later stored in 70 % alcohol according to Johansen (1940). From each leaf, a 25 mm^2 sample taken from the median region and comprising the midrib was enclosed in methacrylate (Leica Histo-resin). Cross section thicknesses ($5 \mu\text{m}$) of the plant material were obtained using an automatic advance rotary microtome RM-2155 (Leica Microsystems Inc., Deerfield, Illinois, USA). The sections were flushed with toluidine blue (O'Brien et al. 1964). To analyze the leaf surface, the technique of printing the epidermis with instant adhesive (methacrylate ester/Super-Bonder[®]) was used. The material was then photographed in a photomicroscope AX-70 TRF (Olympus Optical, Tokyo, Japan) equipped with a Photo system, and the digitized images analyzed with the use of the Image Pro-Plus 4,5 (Media Cybernetics, Inc., Rockville, Maryland, USA) software. Along the cross section of the interveinal region, we measured the thickness of the abaxial and adaxial epidermis, the thickness of the hypoderm when present, the thickness of the palisade and spongy parenchymas and the total leaf lamina thickness

(LT). The chlorophyll parenchyma thickness (PP/SP) and the contributions (in percentage) of the palisade (%PP) and spongy (%SP) parenchymas were then calculated for the total leaf blade thickness. Along the cross section of the midrib, were measured the total area of the midrib (MA) and calculated the contributions (in percentage) of the sclerenchyma (%S) and collenchyma (%C) tissues for the total midrib area. We measured and estimated the average area of the vessel lumen (AEV) and the average number and diameter of the xylem element vessels ($N^{\circ}\text{EV}$). On the leaf surface, we counted the number of stomata within ten fields of observation and calculated the stomatal density (SD) from the average number of stomata divided by the area of the field observed.

Leaf area, dry weight and specific leaf area

The leaf area (LA) was estimated using an electronic leaf area meter (MK2, Burwell, Cambridge, UK). To obtain the leaf dry weight (DW), leaves or leaflets were first dehydrated in an oven at $75 \text{ }^{\circ}\text{C}$ for 72 h and then weighed. The specific leaf area (SLA) was then calculated from the ratio between LA and DW.

Plasticity index

The quantitative estimation of the phenotypic change induced by light environment was assessed based on the phenotypic distances between individuals of a species, for a given leaf trait, exposed to the three different light environments, summarized in a relative distance plasticity index (RDPI) according to Valladares et al. (2006). The RDPI index ranges from 0 to 1 and it is obtained from the equation $\text{RDPI} = \Sigma (d_{ij} \rightarrow i'j' / (xi'j' + xij)) / n$, where i is the number of light treatments, j , the number of repetitions and n , the population size. $d_{ij} \rightarrow i'j'$ is the relative distance between treatments with repetition values taken in pairs with $i \neq i'$, and absolute value obtained as the difference $(xi'j' - xij)$. Hence, the relative distance, $d_{ij} \rightarrow i'j'$, is defined as $d_{ij} \rightarrow i'j' / (xi'j' + xij)$ for all pairs of treatment replications associated with different irradiance levels.

Statistical analysis

Since the photosynthetic plasticity is a result of multiple and correlated variables, we analyzed the phenomenon using a multivariate analytical method. We used the Principal Component Analysis (PCA) and Biplot graphics (Gabriel 1971; Kroonenberg 1997) by using “Biplot and Singular Value Decomposition Macros for Excel[®]” (Lipkovich and Smith 2002). Multicollinearity issue was overcome on the Biplot analysis by: (1) comparisons of the physiological meaning of each leaf trait; and (2)

Table 3 Average estimates of RDPI values for the parameters described from light to CO₂ response curves

Parameter	Light response curve					CO ₂ response curve	
	A_{\max}	R_d	α	LCP	LSP	V_{\max}	J
<i>S. terebinthifolius</i>	0.17	0.24	0.09	0.20	0.15	0.12	0.19
<i>P. grandiflorum</i>	0.11	0.17	0.09	0.11	0.11	0.12	0.08
<i>J. princeps</i>	0.10	0.44	0.11	0.48	0.16	0.20	0.08
<i>H. courbaril</i>	0.16	0.20	0.13	0.19	0.24	0.31	0.10
<i>L. pisonis</i>	0.15	0.48	0.23	0.16	0.09	0.31	0.19

A_{\max} maximum photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), R_d mitochondrial dark respiration rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), α apparent quantum yield of CO₂ (mol mol^{-1}), LCP light compensation point ($\mu\text{mol m}^{-2} \text{ s}^{-1}$), LSP light saturation Point ($\mu\text{mol m}^{-2} \text{ s}^{-1}$), V_{\max} maximum Rubisco carboxylation velocity, J electron transport rate associated with the regeneration of RuBP

verification of eigenvalues close to zero in the PCA (Carrascal et al. 2009). The PCA analysis was calculated by means of a matrix with five species and the plasticity indexes (RDPIs). The relevance of variables was judged according to the direction, orientation and magnitude of their vectors. For a better comprehension of Biplot graphics, a review can be found in Kroonenberg (1997). The data matrix was applied to test the relationships between (1) leaf traits; (2) species; (3) species and leaf traits, and to select the most important traits. First, we selected the leaf traits that were significant for species vs. light level interaction, using ANOVA by GLM Procedure/SAS. Several Biplot analyses were performed to select the most explanatory and relevant leaf traits to obtain a better picture of species plasticity in response to light.

Results

The RDPIs calculated for the five species and the 25 leaf traits are presented in supplementary material. To have a parsimonious analysis, we previously divided all RDPIs in two matrices: a first matrix containing the RDPI values from the leaf traits derived from light to CO₂ regression curves (Table 3); and a second matrix containing the RDPI values from other leaf traits. First, we selected seven leaf traits (ChIT, SLA, SD, %PP, %SP, PP/SP, %C) that were significant for the species vs. light condition interaction by using ANOVA (Table 4). Subsequently, we performed a series (rounds) of Biplot analyses for the first matrix containing the RDPIs of the five species and the seven parameters derived from the light and CO₂ model curves. At this point, the aim was to evaluate the correlations among variables (parameters derived from the light and CO₂ model curves), among species and among variables and species.

Since double-centering transformation is useful for constructing Biplots for multiplicative interaction diagnostics in two-way tables, we corrected the effects of species and variables to zero. The first two principal components (PC) of the analysis explained 89.2 % of the multivariate variability of this group of variables. Four leaf traits explained 90.5 % of the variability associated with the first two PC: R_d (26.9 %), LCP (38.5 %), LSP (10.8 %) and V_{\max} (14.3 %), [other variables (9.5 %)]. Next, a second round of Biplot analyses was performed for a matrix containing the four leaf traits selected from light to CO₂ regression curves and the seven leaf traits selected by ANOVA (11 variables in total). In this analysis, the first two PC explained 81.6 % of total multivariate variability. Seven variables explained 91.2 % of that variability: R_d (25.5 %), V_{\max} (9.6 %), ChIT (8.3 %), SLA (12.2 %), %SP (8.3 %), PP/SP (16.7 %) and %C (10.6 %), [other variables (8.8 %)].

Therefore and for practical reasons, we generated another Biplot graph with those seven selected and most relevant variables (R_d , V_{\max} , ChIT, SLA, %SP, PP/SP and %C), with no loss of relevant information (supplementary material). To evaluate the correlations among variables, we developed Biplots for multiplicative interaction diagnostics and corrected the effects of species and variables to zero. The first two components of the analysis explained 93.7 % of the total multivariate variability. In this analysis, the variables SLA, %SP and PP/SP showed high and positive correlations among them (≥ 0.89), as well as high and negative correlation with R_d (≤ -0.57). The variables V_{\max} and PP/SP were negatively correlated (-0.22). Finally, to evaluate the importance of each selected variable on the plasticity of each species in response to light environments, a last Biplot analysis was performed (Fig. 1). In this analysis only the effects of variables were corrected to zero. The system explained 83.6 % of total variability and provided a clear picture of the effects of variables over species and precise information for species grouping. In this Biplot graph, the origin represents the average response of all species. The larger the magnitude (length) of the vector of a species towards the origin the greater the influence of that species on the average performance of all species, due to the species effect plus the interaction effect between traits and species. The angle between vectors of the species provides information about the degree of similarity of responses among the species. Therefore, *Schinus terebinthifolius* and *Lycythis pisonis* presented the largest distances from the origin (larger vectors), indicating that they were the species that mostly contributed to the variability of the system and presented the most different physiological behaviors. The species *S. terebinthifolius* and *L. pisonis* showed vectors in opposite directions and orientation, which means they had opposite physiological behavior. The variables V_{\max} and

Table 4 ANOVA with the effect of species ($n = 5$), light condition ($n = 3$; $I_{100} = 100\%$ of solar radiation, $I_{50} = 50\%$ of incident solar radiation, $I_{20} = 20\%$ of solar radiation) and interactions ($n = 15$)

Parameter	Species				Light				Interaction				R^2 Total	Model		SST
	F	P	R^2	SS	F	P	R^2	SS	F	P	R^2	SS		SS	R^2	
F_v/F_m	19.23	***	0.77	0.06	3.45	*	0.07	0.01	1.99	ns	0.16	0.01	0.00	0.08	0.69	0.11
ChlT ^a	17.49	***	0.62	0.61	6.45	**	0.11	0.11	3.67	**	0.26	0.26	0.01	0.98	0.71	1.37
Chla/b	8.02	***	0.55	0.01	8.09	***	0.27	0.01	1.33	ns	0.18	0.00	0.00	0.03	0.57	0.05
ChlT/Carot	3.59	*	0.42	0.16	6.35	*	0.37	0.14	0.88	ns	0.21	0.08	0.01	0.38	0.43	0.89
N	4.91	**	0.59	0.29	2.46	ns	0.15	0.07	1.10	ns	0.26	0.13	0.01	0.49	0.43	1.16
LA	5.48	***	0.40	0.30	8.37	***	0.31	0.23	1.94	ns	0.29	0.21	0.01	0.75	0.55	1.37
DW	3.93	**	0.50	0.39	1.50	ns	0.10	0.07	1.58	ns	0.40	0.32	0.03	0.78	0.41	1.91
SLA ^a	11.03	***	0.48	0.49	6.59	**	0.07	0.07	4.27	***	0.05	0.05	0.01	1.02	0.67	1.52
SD ^a	7.83	***	0.09	0.03	10.80	***	0.12	0.04	4.57	***	0.05	0.02	0.00	0.37	0.67	0.55
LT	3.92	**	0.60	0.07	1.80	ns	0.14	0.02	0.83	ns	0.26	0.03	0.00	0.11	0.37	0.31
%PP ^a	5.21	***	0.39	0.08	7.70	***	0.29	0.06	2.13	*	0.32	0.07	0.00	0.20	0.54	0.37
%SP ^a	26.29	***	0.51	0.33	11.64	***	0.11	0.07	9.57	***	0.37	0.24	0.00	0.65	0.82	0.79
PP/SP ^a	10.88	***	0.45	0.52	8.11	***	0.17	0.20	4.71	***	0.39	0.45	0.01	1.17	0.68	1.71
MA	1.53	ns	0.41	0.10	0.31	ns	0.04	0.01	1.03	ns	0.55	0.13	0.02	0.24	0.25	0.98
%C ^a	10.51	***	0.61	0.75	2.79	ns	0.08	0.10	2.65	*	0.31	0.38	0.02	1.23	0.60	2.04
%S	18.05	***	0.82	0.82	2.81	ns	0.06	0.06	1.29	ns	0.12	0.12	0.01	1.00	0.66	1.51
$N^{\circ}EV$	2.05	ns	0.57	0.13	1.58	ns	0.22	0.05	0.36	ns	0.20	0.05	0.02	0.23	0.24	0.94
AEV	2.44	ns	0.44	0.14	0.92	*	0.08	0.03	1.30	ns	0.47	0.15	0.01	0.31	0.33	0.94

ns Variable not relevant, F_v/F_m potential quantum yield of photosystem II, ChlT total chlorophyll content ($mg\ g^{-1}$), Chla/b chlorophyll a to chlorophyll b ratio, ChlT/Carot total chlorophyll to carotenoid ratio, N ($mg\ g^{-1}$) nitrogen content ($mg\ g^{-1}$), LA leaf surface (cm^2), DW leaf dry weight (g), SLA specific leaf area ($cm^2\ g^{-1}$), SD stomatal density ($n^{\circ}\ mm^2$), LT leaf lamina thickness (μm), %PP percentage of palisade parenchyma, %SP percentage of spongy parenchyma, PP/SP palisade to spongy parenchyma ratio, MA midrib area (μm^2), %C percentage of collenchyma, %S percentage of sclerenchyma, $N^{\circ}EV$ number of xylem element vessels ($n^{\circ}\ mm^2$), AEV average area of the xylem element vessels (μm^2)

F values, Sum of Square (SS), level of significance (P) and R^2 are indicated for species, light, interaction and for the model (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

^a Variables selected by ANOVA and used on the Biplot analysis

PP/SP were most responsible for the divergent plasticity on those species. The photosynthetic plasticity response of *S. terebinthifolius* was mostly explained by the highest influence of the traits V_{cmax} , PP/SP, %C, %SP and SLA. The plasticity of *L. pisonis* was strongly and positively influenced by R_d , ChlT and V_{cmax} , as well as negatively influenced by PP/SP. The positive influence of PP/SP, %SP and %C on *S. terebinthifolius* and the negative influence of PP/SP on *L. pisonis* were visually confirmed on the microscopy images of leaf and midrib cross sections (Fig. 2). The species *Pseudobombax grandiflorum*, *Hymenaea courbaril* and *Joannesia princeps* in particular were the species closest to the origin. It means they were the species closest to the average response of all species and had moderate plasticity, indicating an intermediate response group.

Hence, we conclude that the first principal component of the analysis was important to set the plasticity contrast

and clearly separate *S. terebinthifolius* (high plasticity) and *L. pisonis* (low plasticity) from the other species. The second principal component of the analysis set the medium–low plasticity contrast and was especially important to separate *J. princeps* and *H. courbaril* (medium–low plasticity) from *P. grandiflorum* (medium plasticity). Since *J. princeps*, *H. courbaril* and *P. grandiflorum* were too similar to be separated, we established three patterns or groups of photosynthetic plasticity: (1) high plasticity—composed of the early secondary species *S. terebinthifolius*; (2) medium plasticity—composed of the early secondary species *P. grandiflorum* and *J. princeps* and of the late secondary species *H. courbaril*; and (3) low plasticity—composed of the late secondary species *L. pisonis*. Species belonging to the same successional position were clearly clustered into different plasticity groups and, thus, species successional groups were not correlated with plasticity grouping.

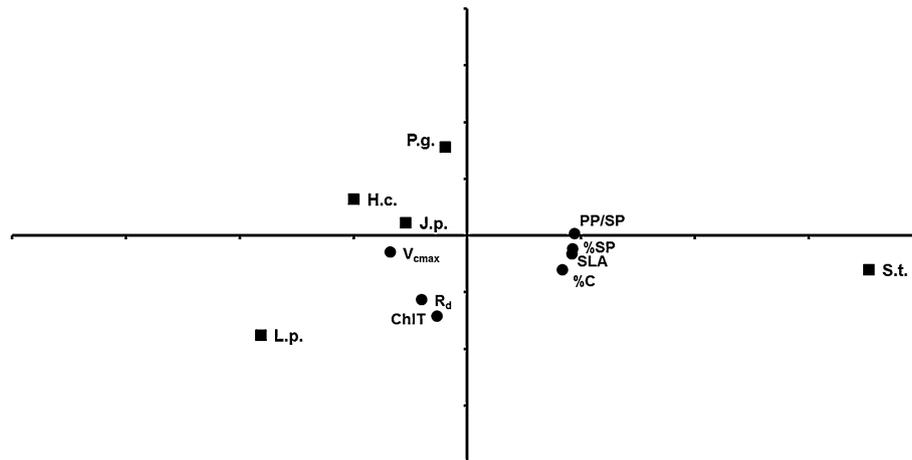


Fig. 1 Biplot analysis with the seven most relevant and non-collinear variables selected for the five indigenous tree species of the Brazilian Atlantic Rain Forest. Only effects of variables were corrected to zero. (solid square) species; (solid circle) leaf traits. R_d mitochondrial dark respiration rate, V_{cmax} maximum Rubisco carboxylation velocity,

$ChLT$ total chlorophyll content, PP/SP palisade to spongy parenchyma ratio, $%SP$ percentage of spongy parenchyma, $%C$ percentage of collenchyma, SLA specific leaf area, $S.t.$ *Schinus terebinthifolius*, $P.g.$ *Pseudobombax grandiflorum*, $J.p.$ *Joannesia princeps*, $H.c.$ *Hymenaea courbaril*; $L.p.$ *Lecythis pisonis*

Discussion

Leaf traits explicative of species photosynthetic plasticity in response to different light environments

In this study, we analyzed the adjustment in response to different light environments of 25 most commonly evaluated leaf traits in studies on light acclimation of photosynthesis in current literature. This group of traits included (1) physiological characters evaluated via infrared gas exchange under different light intensities and different CO_2 concentrations and via fluorescence of chlorophyll *a*; (2) biochemical characters assessed via quantification of photosynthetic pigments and leaf nitrogen; (3) morphological characters assessed via growth variables; and (4) anatomical characters evaluated via quantitative optical microscopy. We understand photosynthetic plasticity as a response resulted from many correlated variables and, thus, a multivariate response. Therefore, we analyzed the variability on photosynthetic plasticity in response to different light environments among five species as a multivariate phenomenon and concluded that this multivariate variability could be explained by seven key non-collinear leaf traits: R_d , V_{cmax} , $ChlT$, $%SP$, PP/SP , $%C$ and SLA . That means in this particular case study, we can explain the differences on photosynthetic plasticity among the five studied species only by measuring those seven leaf traits. Unfortunately, we did not find any other similar work to compare our finding and further studies are required to test if those same leaf traits would be sufficient to differentiate photosynthetic plasticity patterns among other groups of species. We believe that the identification of key leaf traits can facilitate the description of photosynthetic plasticity

and help the comparison of responses among different species and the meta-analyses of published data. Therefore, the method proposed in this study is a guideline for future studies to provide more precise and scientific information in predicting species' light tolerance and to support the choice of species most suitable for forest enrichment plantings.

The leaf trait selection we made in this work corroborates other studies on photosynthetic light acclimation. The importance of V_{cmax} for photosynthetic plasticity, for example, has been verified by other authors (Niinemet et al. 2007; Cavanagh and Kubien 2014). According to Farquhar et al. (1980), the capacity for carboxylation of RuBP by Rubisco (V_{cmax}) is one of the main processes that model the CO_2 assimilation in C3 plants. Saturating light intensities and increasing temperatures decrease the ratio CO_2/O_2 in solution, for a given pCO_2/pO_2 . This environmental condition causes stomata closure and further reduction on CO_2 levels at the site of Rubisco. Under this situation, Rubisco's kinetic phenotype has to be adjusted to improve plant photosynthetic performance. Studies have shown that the relative specificity of Rubisco for CO_2 vs. O_2 (Galmes et al. 2005), the Rubisco's K_c (von Caemmerer and Quick 2000) and the regulation of Rubisco expression (Yamori et al. 2006) varies with growth environment and life habit. Therefore, the capacity of adjustments in V_{cmax} is an important trait for predicting plant photosynthetic ability to light acclimate. Nonetheless, the competence of a plant to keep a high photosynthetic performance depends not only on the carbon gain capacity but also on the respiratory costs for maintaining a light compensation point in accordance to the availability of light in the environment, particularly under low light intensities (Givnish 1988). The importance

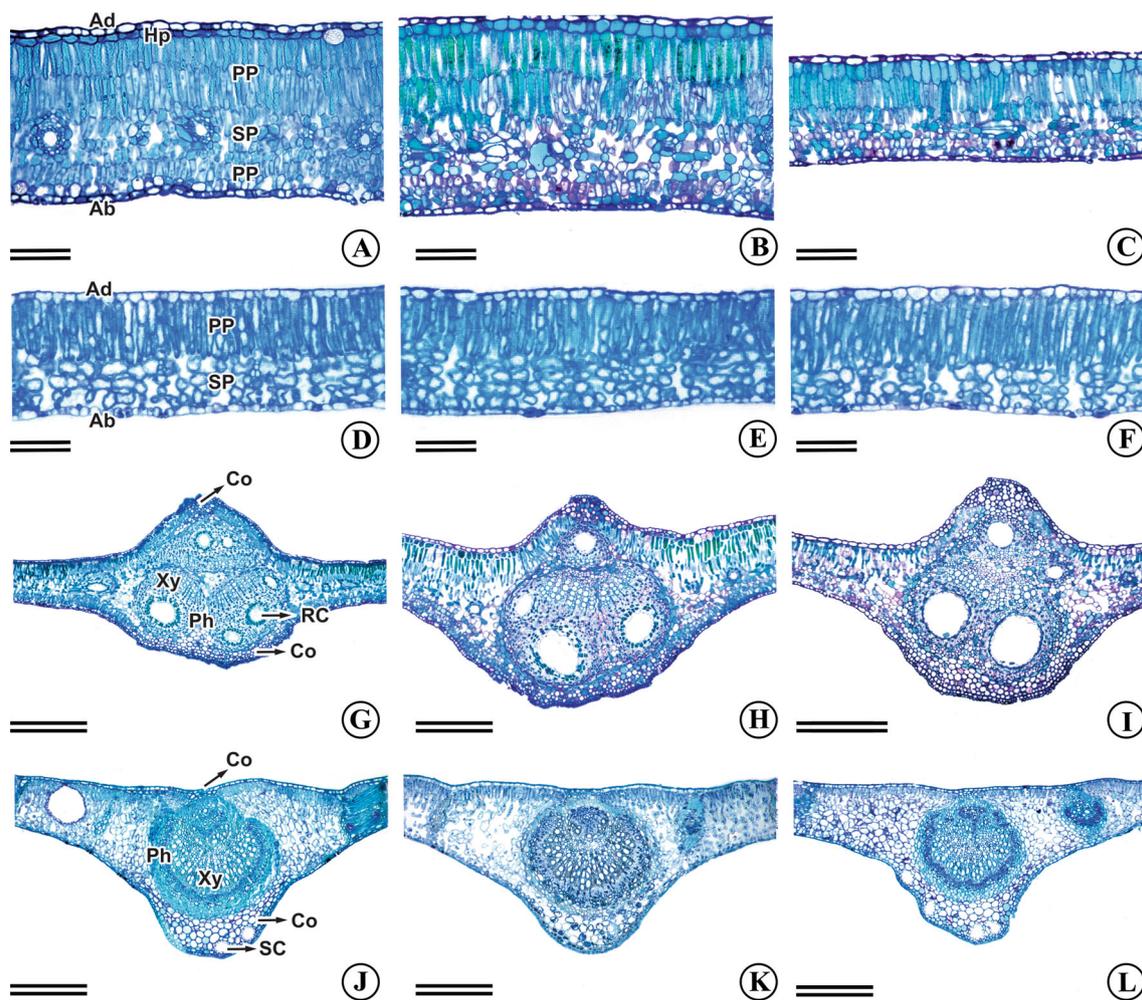


Fig. 2 Cross sections of leaf blade (a–f) and of leaf midrib (g–l) of indigenous tree species of the Brazilian Atlantic Rain Forest, under three light conditions, I_{100} (full sun), I_{50} (50 % of full sun) and I_{20} (20 % of full sun). a–c *S. terebinthifolius*, d–f *L. pisonis*, g–i *S. terebinthifolius*, j–l *H. courbaril*, a, d, g, j, m I_{100} . b, e, h, k, n I_{50} . c,

f, i, l I_{20} . a–f Scale Bar = 150 μ m. Ad Adaxial epiderme, Ab Abaxial epiderme, Hp Hypodermis, PP Palisade parenchyma, SP Spongy parenchyma, SSc Sclerenchyma, SC Secretory cell. G–L: Scale Bar = 400 μ m. Xy Xylem, Ph Phloem, Co Collenchyma, RC Resin canal, SSc Sclerenchyma, SC Secretory cell, LC Laticifer cell, SCa Secretory cavity

of adjustments in R_d has been verified by Walter and Reich (2000), and Baltzer and Thomas (2007) studying seedlings of species of different light tolerances, and by Gianoli et al. (2012) studying the phenotypic plasticity in ecophysiological traits of tropical wood climbing plants across light environments. Under low light leaves may also present the ability to increase the efficiency of light capture through high chlorophyll content in combination with a high specific leaf area (SLA) (Evans and Poorter 2001; Rozeendal et al. 2006; Shao et al. 2014).

Morphological adjustments play great impact on photosynthetic activity (Hanba et al. 2002; Ivancich et al. 2014). Since the mesophyll is the main site of photosynthesis, adjustments on %SP and on the ratio PP/SP are associated with regulation on the surface for diffusing light and CO_2 and on adjustments of the number and volume of cells performing photosynthetic activity, as well as on the

content of chloroplasts and proteins involved in the photosynthetic process (Terashima et al. 2001; Oguchi et al. 2006). The association between PP/SP and the photosynthetic performance in response to different light environments was also observed by Niinemets (2001) on different trees and shrubs, and by other authors (Wyka et al. 2007; Pereira et al. 2009; Mattos et al. 2009). In *Nothofagus pumilio* Patagonian seedlings, palisade parenchyma was the most sensitive tissue to irradiance and it was associated with greater foliar photosynthetic rates per unit area at medium and high irradiance levels (Ivancich et al. 2014). In this study PP/SP showed a negative correlation with V_{cmax} . We suggest that, for the studied species in this work, the velocity of reaction of Rubisco might compensate for a higher content of enzymes contained in a higher number/volume of palisade cells. Also, PP/SP showed a positive correlation with %C. This result suggests that plants which

are capable to enhance the carboxylation rate in response to an increase in environmental light irradiance may also be capable of developing strategies to overcome water deficit, by investing on collenchyma tissue to increase structural support and water storage. Leaf collenchyma tissue is a dynamic mechanical tissue and in terms of %C it plays an important role by helping on the maintenance of water balance in situations of high transpiration rates, such as that undergone in high light and higher temperatures (Villar-Salvador et al. 1997; Leroux 2012).

Species photosynthetic plasticity grouping based on selected leaf traits

Based on our analysis of the relationship among leaf traits adjustments and species' photosynthetic performances, we could distinguish three different groups (or patterns) of photosynthetic plasticity. The most plastic of all species in our study was *S. terebinthifolius* (High-plasticity group). That means this species was the most capable of functional adjustments for stress tolerance and carbon acquisition to cope with changes in light environment. This capacity was mainly attributed to the greater ability for anatomical adjustments on the chlorophyll parenchyma thickness, a feature also verified by Sabbi et al. (2010), and on the percentage of leaf collenchyma. Other leaf traits that did not appear to be important in this study, but can also help to confirm the high photosynthetic plasticity in *S. terebinthifolius* are the high light compensation and saturating points and the higher capacity to regulate xylem hydraulics (number and size of element vessels). These results provide a basis to justify high photosynthetic plasticity as an adaptive edge for *S. terebinthifolius* that confers to this species the capacity to colonize areas of high environmental instability, as it is evident by its frequent occurrence in Brazilian early secondary forests (Ewe and Sternberg 2003) and its competitive and aggressive behavior as invasive species (Ewe and Sternberg 2005; Stratton and Goldstein 2001). Similarly, high photosynthetic capacity was also attributed to the success of other invasive species, such as the *Bischofia javanica* in Bonin Islands (Yamashita et al. 2000) and the *Sphaeropteris cooperi* in Hawaii (Durand et al. 2001).

In contrast to *S. terebinthifolius*, *L. pisonis* was the least plastic species (Low-plasticity group), i.e., the light environment that favors one species disfavors another. Although the level of adjustments showed by *L. pisonis* was the second highest of all species (*S. terebinthifolius* and *L. pisonis* explained 82.4 % of total multivariate variability), those adjustments must not be mistaken for an adaptive advantage, as for *S. terebinthifolius*. Instead, those adjustments should be interpreted as a reflection of lack of adaptation. The great variation in V_{cmax} and in ChIT

presented by *L. pisonis* reflects, first and foremost, the occurrence of chronic photooxidative damages in its carbon-fixing enzymatic complex and its pigments content, other than the ability to adjust these features as a function of changes in light environment. Another leaf trait that was not considered important in our analysis, but can be used to confirm the hypothesis of photoinhibition in *L. pisonis* is the decrease in the fluorescence parameter F_v/F_m , a phenomenon also observed by dosAnjos et al. (2012) and in other late secondary species such as *Fagus sylvatica* (Einhorn et al. 2004), *Cariniana legalis* (Ribeiro et al. 2005) and *Chrysophyllum sanguinolentum* (Santos et al. 2006). *L. pisonis* was the most shade-tolerant. Under low irradiance, this species showed higher plasticity for reducing the respiration rate and for increasing the efficiency of carboxylation and the chlorophyll content of light-harvesting antennas. Based on the strong association of *L. pisonis* with V_{cmax} , R_d and on the association of *S. terebinthifolius* with V_{cmax} , PP/SP, %SP, %C and SLA, we could conclude that the indicator leaf traits of low species plasticity in response to light environmental changes in this study are V_{cmax} , R_d and ChIT and the indicators leaf traits of high species plasticity are V_{cmax} , PP/SP, %C, %SP and SLA. This result supports the hypothesis of genetic restrictions in morphological plasticity for late secondary species (Valladares et al. 2000; Rozendaal et al. 2006), which states that shade-tolerant species would exhibit less morphological plasticity due to the longer leaf longevity influencing the speed of tracking environmental changes.

The late secondary species *H. courbaril* and the early secondary species *P. grandiflorum* and *J. princeps* were clustered in a Medium-plasticity group. These species showed decreases in V_{cmax} and ChIT under high irradiances. Nonetheless, the oxidative stress suffered by these species was not associated with long-term photoinhibition, as it was in *L. pisonis*, and they were all capable to overcome the light stress and maintain high photosynthetic capacity under high irradiance. Finally, species belonging to the same successional position were clearly clustered into different plasticity patterns. Thus, we conclude that species successional classification was not correlated with our photosynthetic plasticity grouping. This result shows the importance of including in successional classifications ecophysiological information of species' light tolerance before making decisions about each species and each place they should be planted in enrichment planting efforts.

Recommendations for enrichment plantings based on species photosynthetic plasticity

Our results based on the photosynthetic plasticity suggest that young plants of the five species analyzed in this study should not be planted all together in the same conditions, as

it is usually done in restoration practices. *S. terebinthifolius*, for example, should be suitable for planting in large canopy gaps of secondary forests, over 20 % of sunlight. Otherwise, seedlings would be under suboptimal conditions to achieve high photosynthetic rates and their survival could be reduced on the first years after transplantations, as it happened to seedlings of the Brazilian native golden trumpet tree *Tabebuia chryso-tricha* planted under more than 50 % shading (Endres et al. 2010) and to the Brazilian native palm *Euterpe edulis* under more than 80 % shading (dos Santos et al. 2012). *L. pisonis* is suitable for planting in low light conditions, i.e., small canopy gaps of less than 20 % of full sunlight. If not, seedling would undergo photoinhibition and have their early growth compromised as verified on seedlings of the Brazilian native rubber tree *Hevea brasiliensis* planted under full sunlight (Senevirathna et al. 2003). *H. courbaril*, *J. princeps* and *P. grandiflorum* could be recommended for planting under different light conditions, avoiding high light intensities such as large gaps and degraded areas with no vegetation cover. We emphasize, however, that these recommendations are speculative and that care should be taken in predicting success to maturity of those species, as our investigation was limited to light in artificial conditions, to a small group of species and to the early phase of establishment of plants. In short, all the species studied in this work have potential to be used in enrichment planting for forest restoration, since they are planted in suitable light conditions according to their light tolerance. This work represents an attempt to select key physiological traits for species grouping according to their light tolerance. We believe this method complements the current knowledge on species successional groups and will help guiding species selection for enrichment plantings in tropical secondary forests restoration.

Conclusions

In this study, we analyzed the association of the adjustments on 25 leaf traits and the photosynthetic plasticity of five species of different successional classes. We found that seven leaf traits can be used in this study to distinguish species photosynthetic plasticity patterns and predict species light tolerance. The results of this study show that phenotypic plasticity indices of leaf ecophysiological traits provide different and complementing information to traditional successional grouping and that this information can help to improve our current ability to select species for enrichment planting.

Author contribution L. dosAnjos and M.A. Oliva conceived and designed the experiments. L.dosAnjos and

M.F. Galvão performed the experiments. L.R.M. Pinto performed statistical analyses. L. dosAnjos, M.S. Mielke, L.R.M. Pinto, K.N. Kuki, and M.C. Ventrella analyzed the data. L.dosAnjos wrote the manuscript.

Acknowledgments The authors thank the following institutions: CAPES (Brazilian Higher Education Council), CNPq (Brazilian National Council for Scientific and Technological Development) and FAPEMIG (Minas Gerais State Research Support Foundation) for their invaluable financial support. Our sincere thanks also go to Vale do Rio Doce Company for providing the seedlings for this work.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Baltzer JL, Thomas SC (2007) Determinants of whole-plant light requirements in Bornean rain forest tree saplings. *J Ecol* 95:208–221
- Barbosa LM (2006) Recuperação florestal de áreas degradadas no Estado de São Paulo: histórico, situação atual e projetos. In: Barbosa LM (Coord) Manual para Recuperação de Áreas Degradadas do Estado de São Paulo – Matas Ciliares do Interior Paulista. Prefeitura de Guaratinguetá, CATI Guaratinguetá: FAPESP, SMA/SP, GEF, IBT/SP e Governo de São Paulo, pp 4–25
- Barros FV, Goulart MF, Sá Telles SB, Lovato MB, Valladares F, de Lemos-Filho J (2012) Phenotypic plasticity to light of two congeneric trees from contrasting habitats: Brazilian Atlantic Forest versus Cerrado (savanna). *Plant Biol* 14:208–215
- Bazzaz FA, Pickett STA (1980) Physiological ecology of tropical succession: a comparative review. *Ann Rev Ecol Syst* 11:287–310
- Budowsky G (1965) Distribution of tropical American rain forest species in the light of succession process. *Turrialba* 15:40–42
- Carpanezzi AA (2005) Fundamentos para a reabilitação de ecossistemas florestais. In: Galvão APM, Porfirio-da-Silva V (eds) Restauração Florestal—Fundamentos e Estudos de Caso. Embrapa, Colombo, pp 27–46
- Carrascal L, Galván MI, Gordo O (2009) Partial least squares regression as an alternative to current regression methods used in ecology. *Oikos* 118:681–690
- Cavanagh AP, Kubien DS (2014) Can phenotypic plasticity in Rubisco performance contribute to photosynthetic acclimation? *Photosynth Res* 119:203–214
- Denslow JS (1987) Tropical rainforest gaps and tree species diversity. *Ann Rev Ecol Syst* 18:431–451
- dos Santos MLS, França S, Gomes FP, do Nascimento JL, dos Anjos L and Mielke MS (2012) Low light availability affects leaf gas exchange, growth and survival of *Euterpe edulis* seedlings transplanted into the understory of an anthropic tropical rainforest. *South Forests* 74:167–174
- dosAnjos L, Oliva MA, Kuki KN (2012) Fluorescence imaging of light acclimation of Brazilian Atlantic Forest tree species. *Photosynthetica* 50:95–108
- Durand LZ, Glodstein G (2001) Photosynthesis, photoinhibition and nitrogen use efficiency in native and invasive tree ferns in Hawaii. *Oecologia* 126:345–354
- Einhorn KS, Rosenqvist E, Leverenz JW (2004) Photoinhibition in seedlings of *Fraxinus* and *Fagus* under natural light conditions: implications for forest regeneration? *Oecologia* 140:241–251

- Endres L, Câmara CA, Ferreira VM, Silva JV (2010) Morphological and photosynthetic alterations in the yellow-ipe, *Tabebuia chrysostricha* (Mart. Ex DC.) Standl., under nursery shading and gas exchange after being transferred to full sunlight. *Agrofor Syst* 78:287–298
- Evans JR, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell Environ* 24:755–767
- Ewe SML, Sternberg LSL (2003) Seasonal gas exchange characteristics of *Schinus terebinthifolius* in a native and disturbed upland community in Everglades National Park, Florida. *Forest Ecol Manage* 179:27–36
- Ewe SML, Sternberg LSL (2005) Growth and gas exchange responses of Brazilian pepper (*Schinus terebinthifolius*) and native South Florida species to salinity. *Trees* 19:119–128
- Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C3 species. *Planta* 149:78–90
- Gabriel KR (1971) The biplots graphic display of matrices with application to principal component analysis. *Biometrika* 58:453–467
- Galmes J, Flexas J, Keys AJ, Cifre J, Mitchell R, Madgwick P, Haslem R, Medrano H, Parry MaJ (2005) Rubisco specificity factor tends to be larger in plant species from drier habitats and in species with persistent leaves. *Plant Cell Environ* 28:571–579
- Gandolfi S. (1991). Estudo florístico e fitossociológico de uma floresta residual na área do Aeroporto Internacional de São Paulo, município de Guarulhos, SP. Campinas: UNICAMP, 1991. 232 p. Dissertação Mestrado (Ciências Biológicas)—Universidade Estadual de Campinas
- Gandolfi S. (2003). Regimes de luz em florestas estacionais semidecíduais e suas possíveis conseqüências. In: Claudino-Sales, V. *Ecosistemas Brasileiros: Manejo e Conservação*. Expressão Gráfica e Editora. Fortaleza, Ceará. 392 p
- Genty B, Briantais J, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* 990:87–92
- Gianoli E, Saldaña A, Jiménez-Castillo M (2012) Ecophysiological traits may explain the abundance of climbing plant species across the light gradient in a temperate rainforest. *PLoS One* 7(6):e38831
- Givnish TJ (1988) Adaptation to sun and shade: a whole-plant perspective. *Aust J Plant Physiol* 15:63–92
- Hall JS, Ashton MS, Garen EJ, Jose S (2011) The ecology and ecosystem services of native trees: implications for reforestation and land restoration in Mesoamerica. *Forest Ecol Manage* 261:1553–1557
- Hanba YT, Kogami H, Terashima I (2002) The effect of growth irradiance on leaf anatomy and photosynthesis in *Acer* species differing in light demand. *Plant Cell Environ* 25:1021–1030
- Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. *Can J Botany* 57:1332–1334
- Houter NC, Pons TL (2012) Ontogenetic changes in leaf traits of tropical rainforest trees differing in juvenile light requirement. *Oecologia* 169:33–45
- Ivancich HS, Lencinas MV, Pastur GJM, Esteban RMS, Hernández L, Lindstrom I (2014) Foliar anatomical and morphological variation in *Nothofagus pumilio* seedlings under controlled irradiance and soil moisture levels. *Tree Physiol* 32:554–564
- Johansen DA (1940) *Plant microtechnique*. McGraw-Hill Book Co. Inc., New York
- Kenzo T, Yoneda R, Matsumoto Y, Mohamad Azani A, Nik Majid N (2011) Growth and photosynthetic response of four Malaysian indigenous tree species under different light conditions. *J Trop For Sci* 23:271–281
- Kroonenberg PM (1997) *Introduction to Biplots for GxE tables*. Research Report 51, The University of Queensland, Brisbane, Australia
- Kuptz D, Grams TEE, Günter S (2010) Light acclimation of four native tree species in felling gaps within a tropical mountain rainforest. *Trees* 24:117–127
- Lamb D, Erskine PD, Parrotta JA (2005) Restoration of degraded tropical forest landscapes. *Science* 310:1628–1632
- Leroux O (2012) Collenchyma: a versatile mechanical tissue with dynamic cell walls. *Ann Bot* 110(6):1083–1098
- Lipkovich I, Smith EP (2002) Biplot and singular value decomposition macros for Excel[®]. *J Stat Softw* 7:1–15
- Mattos FS, Wolfgramm R, Gonçalves FV, Cavatte PC, Ventrella MC, DaMatta FM (2009) Phenotypic plasticity in response to light in the coffee tree. *Environ Exp Bot* 67:421–427
- Mittler R (2006) Abiotic stress, the field environment and stress combination. *Trends Plant Sci* 11:15–19
- Nascimento HEM, Dias AS, Tanabez AAJ, Viana VM (1999) Estrutura e dinâmica de populações arbóreas de um fragmento de Floresta Estacional Semidecidual na região de Piracicaba, São Paulo. *Braz J Bot* 59:329–342
- Niinemets Ü (2001) Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology* 82:453–469
- Niinemets Ü, Lukjanova A, Turnbull MH, Sparrow AD (2007) Plasticity in mesophyll volume fraction modulates light-acclimation in needle photosynthesis in two pines. *Tree Physiol* 27:1137–1151
- O'Brien TP, Feder N, McCully ME (1964) Polychromatic staining of plant cell walls by toluidine blue. *Protoplasm* 59:368–373
- Oguchi R, Hikosaka K, Hiura T, Hirose T (2006) Leaf anatomy and light acclimation in woody seedlings after gap formation in a cool-temperate deciduous forest. *Oecologia* 149:571–582
- Pereira DC, Barros CF, Scarano FR (2009) In situ variation in leaf anatomy and morphology of *Andira legalis* (Leguminosae) in two neighbouring but contrasting light environments in a Brazilian sandy coastal plain. *Acta Bot Bras* 23:267–273
- Piotto D (2011) Spatial dynamics of forest recovery after swidden cultivation in the Atlantic forest of southern Bahia, Brazil. Dissertation, Faculty of the Graduate School of Yale University
- Prioul JL, Chartier P (1977) Partitioning of transfer and carboxylation components of intracellular resistance to photosynthetic CO₂ fixation: a critical analysis of the methods used. *Ann Bot* 41:789–800
- Ribeiro AC (1999) Recomendação de calagem e adubação de substratos para mudas, covas e canteiros. In: Ribeiro AC, Guimarães PTG, Álvares VH (eds) *Comissão de Fertilidade do Solo do Estado de Minas Gerais—Recomendações para o uso de corretivos e fertilizantes em Minas Gerais—5ª Aproximação*. MG, Departamento de Solos da Universidade Federal de Viçosa, Viçosa, p 237
- Ribeiro RV, Souza GM, Oliveira RF, Machado EC (2005) Photosynthetic responses of tropical tree species from different successional groups under contrasting irradiance conditions. *Braz J Bot* 28:149–161
- Rodrigues RR, Lima RAF, Gandolfi S, Nave AG (2009) On the restoration of high diversity forests: 30 years of experience in the Brazilian Atlantic Forest. *Biol Conserv* 142:1242–1251
- Rozendaal DMA, Hurtado VH, Pooter L (2006) Plasticity in leaf traits of 38 tropical tree species in response to light: relationships with demand and adult stature. *Funct Ecol* 20:207–216
- Ruban AV (2009) Plants in light. *Commun Integr Biol* 2(1):50–55
- Sabbi CLB, Ângelo AC, Boeger MR (2010) Influência da luminosidade nos aspectos morfoanatômicos fisiológicos de folhas de

- Schinus terebinthifolius* Raddi (Anacardiaceae) implantadas em duas áreas com diferentes graus de sucessão, nas margens do Reservatório Iraí, Paraná, Brasil. *Iheringia* 65:171–181
- Santos UMJr, Gonçalves JFC, Feldpausch TR (2006) Growth, leaf nutrient concentration and photosynthetic nutrient use efficiency in tropical tree species planted in degraded areas in central Amazonia. *Forest Ecol Manage* 226:299–309
- Senevirathna AMWK, Stirling CM, Rodrigo VHL (2003) Growth, photosynthetic performance and shade adaptation of rubber (*Hevea brasiliensis*) grown in natural shade. *Tree Physiol* 23:705–712
- Shao Q, Wang H, Guo H, Zhou A, Huang Y, Sun Y, Li M (2014) Effects of Shade Treatments on Photosynthetic Characteristics, Chloroplast Ultrastructure, and Physiology of *Anoectochilus roxburghii*. *PlosOne* 9:1–10
- Sharkey TS, Bernacchi CJ, Farquhar GD, Singsaas EL (2007) Fitting photosynthetic carbon dioxide curves for C3 leaves. *Plant Cell Environ* 30:1035–1040
- Stratton LC, Goldstein G (2001) Carbon uptake, growth and resource-use efficiency in one invasive and six native Hawaiian dry forest tree species. *Tree Physiol* 21:1327–1334
- Swaine MD, Whitmore TC (1988) On the definition of ecological species groups in tropical rain forests. *Vegetatio* 75:81–86
- Terashima I, Miyazawa SI, Hanba YT (2001) Why are sun leaves thicker than shade leaves? Consideration based on analyses of CO₂ diffusion in the leaf. *J Plant Res* 114:93–105
- Valladares F, Wright SJ, Lasso E, Kitajima K, Pearcy RW (2000) Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. *Ecology* 81:1925–1936
- Valladares F, Sanchez-Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *J Ecol* 94:1103–1116
- Villar-Salvador P, Castro-Díez P, Pérez-Rontomé C, Montserrat-Martí G (1997) Stem xylem features in three *Quercus* (Fagaceae) species along a climatic gradient in NE Spain. *Trees* 12:90–96
- von Caemmerer S, Quick WP (2000) Rubisco: physiology in vivo. In: Leegood RC, Sharkey TD, von Caemmerer S (eds) *Photosynthesis: advances in photosynthesis and respiration*, vol 9. Springer, Dordrecht, pp 85–113
- Walters MB, Reich PB (2000) Trade-offs in low-light CO₂ exchange: a component of variation in shade tolerance among cold temperate tree seedlings. *Funct Ecol* 14:155–165
- Wellburn AR (1994) The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J Plant Physiol* 144:307–313
- Wyka T, Robakowski P, Zytkowski R (2007) Acclimation of leaves to contrasting irradiance in juvenile trees differing in shade tolerance. *Tree Physiol* 27:1293–1306
- Yamashita N, Ishida A, Kushima H, Tanaka N (2000) Acclimation to sudden increase in light favoring an invasive over native trees in subtropical islands, Japan. *Oecologia* 125:412–419
- Yamori W, Suzuki K, Noguchi K, Nakai M, Terashima I (2006) Effects of Rubisco kinetics and Rubisco activation state on the temperature dependence of the photosynthetic rate in spinach leaves from contrasting growth temperatures. *Plant Cell Environ* 29:1659–1670