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Photoacclimation capacity in seedling and sapling of *Siparuna guianensis* (Siparunaeae): Response to irradiance gradient in tropical forest

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Abstract

Light is a limiting factor in plant establishment and growth in the understory of forests. In this paper, we assessed acclimation capacity of *Siparuna guianensis*, an early secondary successional species. We used seedlings and saplings in three regeneration areas with different irradiance regimes to determine the traits that confer photoplasticity. We examined whether these traits differ at different developmental stages. Anatomical characteristics, photochemical efficiency, photosynthetic capacity, and growth were analyzed. Multivariate component analysis revealed the formation of six clusters: three for seedlings (one for each regeneration area) and three for saplings (following the same pattern of seedlings, considering the area). Increased irradiance favored photosynthetic performance, independently of the developmental stage. The same trend was observed for most data on chlorophyll (Chl) *a* fluorescence and the ratios of net photosynthetic rate/intercellular CO_2 concentration (P_N/C_i) and P_N/PPFD. No parameter indicated photoinhibition stress. The CO_2- and light-response curve data indicated that seedlings were already acclimated to tolerate variation in irradiance. Anatomical adaptations, such as thickness of leaf blade and of adaxial cuticle, were observed in individuals growing in areas with higher irradiation. Thinning of spongy parenchyma and higher investment into a plant height were observed in seedlings, possibly due to the vertical stratification of CO_2 and light in the understory; because light is a more limiting resource than CO_2 in the lower stratum of the forest. Photoplasticity in *S. guianensis* is associated with a set of morphological, anatomical, photochemical, and biochemical traits, whereas biochemical performance is best acclimated to variation in irradiance. These traits differed in seedlings and saplings but they were modulated mainly by irradiance in both developmental stages.

Additional key words: chlorophyll; chlorophyll fluorescence; CO_2 uptake; growth; leaf anatomy; structural traits.

Introduction

Light dynamic in forest understories is influenced by canopy stratification and structure, mainly by the type of coverage (open or closed), which defines quality, periodicity, orientation, and distribution of PPFD (Chazdon *et al.* 1996, Montgomery and Chazdon 2002, Montgomery 2004). Multistratification of the understory also influences irradiance and the establishment of a light gradient by blocking direct radiation and increasing diffuse radiation

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Abbreviations: ab – abaxial surface; ad – adaxial surface; Car – carotenoids; CCU – closed canopy understory; Chl – chlorophyll; C_i – intercellular CO_2 concentration; ct – cuticle; DGL – diameter at ground level; DMSO – dimethylsulfoxide; ETR – electron transport rate; FV – final value; F_v/F_m – maximal quantum yield of PSII; g_s – stomatal conductance; GN – percentage gain H – height; ICU – intermediate canopy understory; IV – initial values; J_{max} – maximal electron transport rate; L_s – relative stomatal limitation to photosynthesis; NL – number of leaves; NPQ – nonphotochemical quenching; OCU – open canopy understory; PCA – principal component analysis; PC1 – first principal component; PC2 – second principal component; P_N – net photosynthetic rate; P_{Neff} – effective net photosynthetic rate; P_{NmaxC} – potential net photosynthetic capacity; P_{Nmax} – maximal net photosynthetic rate; P_{NmaxL} – maximal net photosynthetic capacity; pp – palisade parenchyma; LSP – light-saturation point; q_P – photochemical quenching; RH – relative humidity; SAC – shade adjustment coefficient; sp – spongy parenchyma; T – temperature; TLA – total leaf area; TPU – use of triose-phosphate; V_{cmax} – maximal carboxylation speed of Rubisco; VPD – vapor pressure deficit; ΔG – delta growth; $\Delta F/F_m'$ – effective quantum yield.

(Way and Pearcy 2012). The capacity to tolerate and respond to variations in light conditions is associated with phenotypic plasticity, the ability to promote genotype expression adjustments (Valladares *et al.* 2000). The extent to which plasticity affords the use of light in photosynthesis is a determining factor in the distribution of plant species in heterogeneous environments, such as forest understories (Ribeiro *et al.* 2005, Silvestrine *et al.* 2007, Souza *et al*. 2008, 2009; Miyashita *et al.* 2012, Rabelo *et al.* 2013).

The potential to acclimate to changes in light intensity varies among species and within species (Valladares *et al.* 2000). It has been hypothesized that this variation is linked to a set of morphological and physiological traits (Bazzaz 1979, Ribeiro *et al.* 2005, Gandolfi *et al.* 2009, dos Anjos *et al*. 2012, Nascimento and Marenco 2013, Rabelo *et al.* 2013), to growth and developmental conditions of individual plants (Kitajima *et al.* 1996, Pandey and Kushwaha 2005, Silvestrini *et al.* 2007), and to the ontogenetic stage of individuals (Sultan 2003, Ishida *et al.* 2005).

The Brazilian Atlantic Forest is one of the richest biomes in biodiversity (Myers *et al.* 2000), and is characterized by heterogeneous light availability that results from a multistratified understory (Lemos and Mendonça 2000). Seedlings represent an early and transitory stage in plant development. It has been reported that seedlings exhibit a high acclimation capacity to changes in the environment (Kitajima *et al.* 1996), a likely result of the constant development of new structures inherent to plant growth demands.

Siparuna guianensis Aublet is a shrub that grows in a variety of environments, from open areas to the forest understories. It is an early secondary species, native to the Atlantic Forest, and it is naturally distributed in primary and secondary forests, from Nicaragua across the whole of northern South America down to Paraguay (Renner and Hausner 2005). The wide distribution may be due to the ability to balance growth and the costs associated with acclimation to different environments (Kitajima 1994). This species is used as anti-inflammatory and analgesic drug, for the control of blood pressure, for the treatment of rheumatic diseases, and other applications (Valentini *et al.* 2010). In União Biological Reserve, Brazil, *S. guianensis* occurs in secondary forests and is the prevailing species in the understory of a native species regeneration forest in abandoned eucalyptus (*Corymbia citriodora*) plantations, which differ essentially in light availability (Evaristo *et al.* 2011, Lage-Pinto *et al.* 2012).

Two main questions were addressed in the present study: (*1*) What are the traits that confer photoplasticity to *S. guianensis*? (*2*) Do these traits differ during developmental stages? We assessed *S. guianensis* photoplasticity at two developmental stages based on the parameters of growth, leaf anatomy, photochemical efficiency, and photosynthetic capacity, under three different irradiance regimes. The underlying hypothesis was that photoplasticity of *S. guianensis* results from investment in different plastic traits (morphological, physiological, and/or anatomical) that vary with the developmental stage.

Materials and methods

Study area, plants, and sampling period: This study was carried out in the understories of three areas in União Biological Reserve (ReBio União), state of Rio de Janeiro, RJ, Brazil (22º27’30”S, 42º02’15”W). The vegetation is classified as dense ombrophilous forest, which occurs mainly in lowlands (IBGE 1992). The climate in the region is tropical wet (*Aw* in the Köppen 1948 climate classification). Annual mean temperature is 25ºC, and mean rainfall is 1,700 to 2,200 mm year^{-1}, with 85% of rains falling from October to March.

The areas studied were: (*1*) closed canopy understory (CCU), a secondary woodland edge strip (up to 50 m in a width) with native Atlantic Forest species, well-developed arboreal stratum, canopy coverage of 78.2%, and low irradiance at the forest understory; (*2*) intermediate canopy understory (ICU), an abandoned eucalyptus forest (*Corymbia citriodora*) near the native forest, with trees spaced at 2 m × 2 m, without forest management for 18 years, a well-developed understory, intermediate canopy coverage (57.7%); (*3*) open canopy understory (OCU), an abandoned eucalyptus forest 5 km from the native forest, with trees spaced at 2 m × 2 m, without forest management for 18 years, sparse understory vegetation, open canopy coverage (39%), and high irradiance (the highest irradiance of the three areas).

Vegetative *S. guianensis* individuals were at two developmental stages: seedlings (up to 50 cm tall and 2 cm diameter at ground level, with photosynthetically active leaves and without branches) and saplings (between 1.6 m and 2.0 m tall, up to 8 cm diameter at ground level and branched) were identified in the three areas and monitored throughout the study period (September to December 2012).

The study areas differed in canopy coverage, phytosociological characteristics (composition and distribution in the understory) and microclimate (PPFD, temperature, and vapor pressure deficit [VPD]) (Fig. 1). The forest coverage (on a scale of 0 to 100% coverage) was measured manually in 10 points (north, east, south, and west) in CCU, ICU, and OCU between 11:00 and 13:00 h in one sunny day with a spherical densiometer (*Model-A*, *Forestry Suppliers, Inc*., USA). The data are presented as means of 40 points. Temperature (T) and relative humidity (RH) were measured using a thermo-hygrometer (*910.15*, *CHH*, Alla, Brazil). PPFD was measured at the height of leaves (seedling and sapling) using a light meter radiometer (*Li-190* coupled to *Li-250 A*, *Li-Cor*, USA). The T, RH, and PPFD were measured manually for three

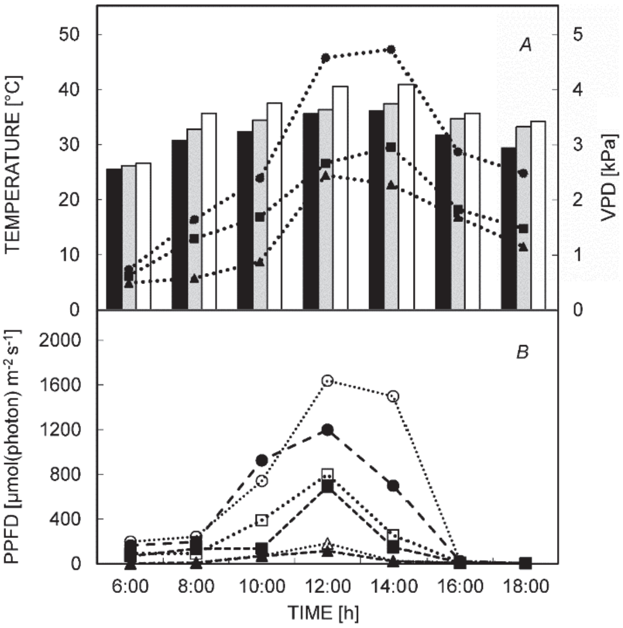

Fig. 1. Mean temperature (*bars*) and vapor pressure deficit (VPD, *dashed lines*) (*A*); and photosynthetic photon flux density (PPFD) (*B*) available for *Siparuna guianensis* at two developmental stages (seedling – *closed symbols and dashed lines*, and saplings – *open symbols and dotted lines*), in three areas of different irradiance regimes (closed canopy understory, *triangle and black bars*; intermediate canopy understory, *square and grey bars*; and open canopy understory, *circle and white bars*). Data were collected from 6:00 to 18:00 h in União Biological Reserve, Brazil ($n = 30$).

sunny days in ten points of CCU, ICU, and OCU at 6:00, 8:00, 10:00, 12:00, 14:00, 16:00, and 18:00 h. The data are presented as means of 30 points. VPD was calculated according to the formula:

$$\text{VPD} = 0.61137 \exp[17.502\ T/(240.97 + T)]\ (1 - \text{RH}/100)$$

and expressed in kPa (Unwin 1980).

Morphological and growth analyses: In each area surveyed, ten individuals (five at each developmental stage) were monitored monthly, for three months (September to December 2012). The parameters evaluated were: number of leaves (NL), height (H) from the ground to the apical bud (using a measuring tape), diameter at ground level (DGL, using a digital calipers), and total leaf area (TLA, calculated based on digital photographs analyzed using the software *Image J 1.46r*). For all parameters, the data presented were: (*1*) final value (FV), representing the last evaluation (December 2012); (*2*) delta growth (ΔG), which is the difference between the final and initial values (IV), $\Delta G = \text{Measurement}_{\text{(December 2012)}} - \text{Measurement}_{\text{(September 2012)}}$; and (*3*) percentage gain (GN), calculated as $\text{GN} = \Delta G \times 100/\text{IV}$.

Photosynthetic analysis: Eight individuals were analyzed (four of each developmental stage) in September and December 2012. For each plant, two healthy and completely developed leaves, always the third pair, were analyzed. A portable infrared gas analyzer (*CIRAS-2*, *PP Systems*, UK) was used to measure net photosynthetic rate [using leaves kept under controlled conditions in the sampling chamber of the analyzer at 25ºC, RH of 80%, and 380 μmol(CO_2) mol^{-1}]. Curves of photosynthetic responses to light and to CO_2 were constructed.

Net photosynthetic rate (*P_N*): Measurements were carried out at three times (8:00, 12:00, and 16:00 h) in September and December 2012. The parameters evaluated were maximal net photosynthetic rate (P_{Nmax}), measured under saturating artificial irradiance of 1,200 μmol m^{-2} s^{-1} (Lage-Pinto *et al.* 2012), and effective net photosynthetic rate (P_{Neff}), measured under natural light conditions in each environment (local irradiance, Fig. 1).

CO_2-response curve (*P_N/C_i*): Data used to construct the curves were obtained between 8:00 and 13:00 h in December 2012 using varying CO_2 concentrations (from 0 to 1,000 μmol mol^{-1}) in the chamber of the gas analyzer (Long and Bernacchi 2003). The curves were adjusted according to the equation developed by Monteiro and Prado (2006) based on P_N and C_i. Saturating irradiance was set at 1,200 μmol(photon) m^{-2} s^{-1} and RH was 80%. The variables calculated were: (*1*) maximal carboxylation speed of Rubisco (V_{cmax}); (*2*) maximal electron transport rate (J_{max}); (*3*) use of triose-phosphate (TPU); (*4*) potential net photosynthetic capacity ($P_{\text{N maxCO2}}$) based on the model proposed by Sharkey (1988) and Sharkey *et al.* (2007); and (*5*) relative stomatal limitation to photosynthesis (L_s), as described by Farquhar and Sharkey (1982). Stomatal conductance (g_s) data were obtained simultaneously.

Light-response curve (*P_N*/PPFD): Curves were constructed using PPFD between zero and 2,000 μmol m^{-2} s^{-1}. Measurements were carried out between 8:00 and 13:00 h in December 2012. Curves were adjusted according to the model described by Prado and Moraes (1997). CO_2 concentration was set at 380 μmol mol^{-1} and RH was 80%. The derivative variables were maximal net photosynthetic capacity (P_{NmaxL}), and light-saturation point (LSP).

Chl *a* fluorescence: Analyses were carried out in September and December 2012 with the same individuals and leaves used in the photosynthetic analysis. Measurements were made between 12:00 and 14:00 h using a modulated light portable fluorometer (*FMS2*, *Hansatech*, UK). Leaves were previously adapted to the dark for 30 min using leaf clips, and then exposed to the weak, modulated beam light (approximately 6 μmol m^{-2} s^{-1} at 660 nm), followed by exposure (for 0.8 s) to high-intensity (10,000 μmol m^{-2} s^{-1}) actinic white light, according to Genty *et al.* (1989). The variables determined were: (*1*) maximal quantum yield of PSII (F_v/F_m); (*2*) photochemical quenching (q_P); (*3*) nonphotochemical quenching (NPQ); and (*4*) effective quantum yield ($\Delta F/F_m'$), which was obtained under saturating light (1,200 µmol m^{-2} s^{-1}) simultaneously with (P_{Nmax}). The electron transport rate (ETR) was calculated according to Bilger *et al.* (1995). The data are presented as means of September and December data.

Photosynthetic pigments: Two 0.502 cm² disks were punched from leaves of four individuals (seedlings and saplings) in December 2012 in the CCU, ICU, and OCU areas. The leaves used were the same as the ones used in the photosynthetic and Chl *a* fluorescence analysis. The disks were kept in plastic tubes containing 5 mL of organic solvent (dimethylsulfoxide, DMSO) protected from light for 5 d. After this time, the organic solvents were analyzed in a spectrophotometer (*Model TCC-240A, Shimadzu,* Japan) at 480, 649, and 665 nm. All the laboratory procedures were carried out under a low-light environment. Chl *a*, Chl *b*, carotenoids (Car), total Chl (*a*+*b*), and the Chl *a*/*b* ratio were calculated according to Wellburn (1994).

Anatomical measurements: Leaf fragments of the same leaves used in previous analyses were immediately fixed in a solution of 2.5% glutaraldehyde and 4% formaldehyde in 0.05 M sodium cacodylate buffer at pH 7.2 (Klein *et al.* 2004). The samples were washed three times with buffer for 30 min and then fixed with 1.0% osmium tetroxide in the same buffer for 2 h at room temperature. The fixed samples were dehydrated using an acetone series and embedded in epoxy resin (*Epon*®). Microtome sections (1.0 mm) were cut with an ultramicrotome (*Reicheit Ultra-Cut S*, *Leica*, Germany) and stained with 0.05% aqueous solution toluidine blue (O'Brien *et al.* 1964). The slides were sealed with *Entellan*® (*Merck)* and examined using an optical microscope (*Axioplan Fluotescence, Zeiss,* Germany). Images were obtained with a *PowerShot A640* camera (*Canon*, USA) and the *Axiovision* software (*Zeiss*, Germany). Measurements were taken following leaf anatomy parameters in the epidermis, mesophyll, and vascular bundle. Anatomical description and measurements were performed with the aid of the image analysis software, *AnalySIS*®, in the *Axioplan Zeiss* microscope. The following leaf blade parameters were analyzed or measured (*Analysis Sis Link/Oxford – Zeiss*): leaf blade thickness, palisade parenchyma, spongy parenchyma, adaxial epidermis face, abaxial epidermis face, and adaxial cuticle.

Shade adjustment coefficient (SAC): The shade adjustment coefficient was calculated as an indicator of phenotypic plasticity to irradiance variations in thicker understories, CCU and ICU, in comparison with the OCU (Laisk *et al.* 2005). This coefficient was calculated as SAC = 1 – (shade/sun), in which "shade" represents the attributes measured in the areas with lower irradiance, and "sun" represents the same attributes, measured in the areas with higher irradiance. Thus, SAC was calculated for CCU as SAC = 1 – (CCU/OCU), while for ICU the coefficient was calculated as SAC = 1 – (ICU/OCU).

Statistical analyses: All data were evaluated using the analysis of variance (two-way *ANOVA*), and means were compared using the *Tukey*'s test ($p \leq 0.05$). A principal component analysis (PCA) was carried out to confirm the clustering of samples based on the parameters evaluated (Souza *et al.* 2008) using the software *PC-ORD version 4* (*MJM Software Design*, Gleneden Beach, OR, USA).

Results

Morphological and growth analyses: All growth parameters increased with rising irradiance (Table 1). The ΔG of all parameters was higher in the saplings than in seedlings, similarly to the GN [%] for the parameters such as the number of leaves (NL) and total leaf area (TLA). The GN in height (H) was greater in the seedlings than in saplings. The GN in a diameter at the ground level (DGL) was the parameter that varied the least with the developmental stage and with the area (between 26.7 to 34 %, except in saplings from ICU).

Maximal and effective carbon gain: P_{Nmax} and P_{Neff} differed significantly among the areas. Higher P_N were observed in plants growing in areas with greater irradiance (ICU and OCU). P_{Neff} did not differ significantly between the seedlings and saplings only in plants growing in OCU (Fig. 2*B*,*D*,*F*). P_{Nmax} and P_{Neff} observed at 8:00 and 12:00 h were higher than those at 16:00 h in most measurements.

Photosynthetic pigments: Contents of Chl *a* and Chl *b*, Chl *a/b* ratio, and total Chl did not vary significantly with irradiance or developmental stage (Table 2). Yet, Car contents were significantly higher in the seedlings and saplings growing in OCU (45% and 52% higher, respectively; Table 2).

Photochemical efficiency: The F_v/F_m ratio did not differ significantly between plants at different developmental stages and under irradiance gradient (Table 2). However, other parameters used to assess photochemical efficiency varied with irradiance. q_P was significantly higher in the saplings growing in OCU than in CCU and ICU (Table 2). Saplings growing in ICU and OCU had higher $\Delta F/F_m'$ and ETR than those in CCU.

A positive correlation was observed between Car contents and NPQ in plants growing in areas or the strata with greater irradiance (upper stratum in ICU and OCU, Table 3). In ICU, this correlation was observed in the saplings, while in OCU it was exhibited by both saplings and seedlings. A negative correlation between these

Table 1. Mean growth values of *Sipurana guianensis* seedlings and saplings between September 2012 and December 2012 in three areas of different irradiance regimes (closed canopy understory, CCU; intermediate canopy understory, ICU; and open canopy understory, OCU) in União Biological Reserve, Brazil. ΔG – delta growth; DGL – diameter at ground level; FV – final value; GN – percentage gain; H – height; NL – number of leaves; TLA – total leaf area. *Capital letters* compare areas considering one developmental stage. *Lowercase letters* compare developmental stage considering one same area ($p \leq 0.05$; $n = 5$).

	CCU						ICU						OCU					
	Seedling			Sapling			Seedling			Sapling			Seedling			Sapling		
Parameter	FV	ΔG	GN	FV	ΔG	GN	FV	ΔG	GN	FV	ΔG	GN	FV	ΔG	GN	FV	ΔG	GN
NL	8.6^{Bb}	1.89^{Cb}	28.17^{Bb}	72^{Aa}	23.09^{Ba}	47.21^{Ca}	18^{Ab}	4.51^{Bb}	33.43^{Bb}	80.2^{Aa}	34.97^{Aa}	77.32^{Ba}	12.8^{Ab}	5.7^{Ab}	80.28^{Ab}	66.6^{Ba}	34.9^{Aa}	110.09^{Aa}
H [m]	0.27^{Bb}	0.03^{Bb}	17.39^{Ba}	1.95^{Aa}	0.18^{Ba}	10.17^{Bb}	0.31^{Ab}	0.04^{Bb}	14.81^{Ba}	2.01^{Aa}	0.21^{Ba}	11.67^{Ba}	0.34^{Ab}	0.07^{Ab}	25.93^{Aa}	1.92^{Aa}	0.29^{Aa}	17.79^{Ab}
DGL [mm]	3.24^{Bb}	0.71^{Bb}	$28,26^{\mathrm{ABa}}$	12.84^{Ba}	2.98^{Ba}	30.22^{Aa}	4.8^{Ab}	1.01^{Ab}	26.65^{Ba}	16.3^{Aa}	2.17^{Ca}	15.36^{Bb}	4.1^{Ab}	1.04^{Ab}	33.99^{Aa}	17.5^{Aa}	4.3^{Aa}	32.58^{Aa}
TLA [m^2]	2.0^{Bb}	0.33^{Cb}	$19,76^{\mathrm{Bb}}$	34.3^{Ca}	10.1^{Ca}	41.74^{Aa}	5.1^{Ab}	0.95^{Bb}	22.89^{Bb}	59.9^{Aa}	20.6^{Aa}	52.42^{Aa}	5.2^{Ab}	1.65^{Ab}	46.48^{Ab}	46.1^{Ba}	16.3^{Ba}	54.70^{Aa}

parameters was observed in areas or strata with lower irradiance (CCU and bottom stratum in ICU, Table 3).

P_N/C_i and P_N/PPFD curves: Higher irradiance increased photochemical efficiency. Similarly, irradiance increased all parameters representing the stomatal conductance and biochemical stage of photosynthesis (P_{NmaxL}, LSP, P_{NmaxC}, V_{cmax}, J_{max}, TPU, and L_s) (Table 2). These parameters did not vary with the developmental stage. An opposite pattern was exhibited by g_s, with low values in areas exposed to higher irradiance (OCU and ICU), compared with the area exposed to lower irradiance (CCU). Seedlings had higher g_s values than saplings in all environments.

Anatomical parameters: Adaxial cuticle and palisade parenchyma thickness values were significantly higher in plants growing in high-irradiance areas (Table 4). Besides elongation of palisade parenchyma cells, two strata were also observed in this tissue in plants growing in the areas with higher irradiance (Fig. 3*C-F*). Leaf blade thickness and spongy parenchyma thickness were significantly higher in saplings, independently of irradiance (Table 4, Fig. 3). Seedlings in OCU presented more convex, outer periclinal wall of adaxial epidermis face cells, compared with saplings (Fig 3*A*, *arrow*).

Shade adjustment coefficient (SAC): Concerning physiological traits (Table 2), the highest SAC values were observed mainly for the parameters that represent stomatal conductance and biochemical efficiency of photosynthesis in plants under CCU, independently of developmental stage. Except for some parameters, such as Car contents, lower phenotypic plasticity was observed in photosynthetic pigments than in biochemical traits. Considering the anatomical traits assessed (Table 4), the highest SAC values were observed for adaxial cuticle thickness among plants growing in the environments with lower irradiance and those growing in OCU, in both developmental stages.

Principal component analysis (PCA) was carried out using all parameters assessed. It revealed that two principal components explained 64.8% of the total variance in the dataset, 42.0% in the first principal component (PC1), and 22.8% in the second (PC2) (Fig. 4). Six clusters were formed, indicating the existence of a response pattern of plants at different development stages to variation in irradiance conditions. In PC1, the groups distributed along an environmental gradient (from OCU to CCU) were influenced mainly by the variables linked with photoprotection (Car and adaxial cuticle) and photosynthetic performance (q_P, P_{Nmax}, P_{Neff}, P_{NmaxL}, P_{NmaxC}). Differentiation considering the developmental stage was underlined by differences in the most significant parameters in PC2, especially anatomical traits and Chl contents, and biochemical efficiency (V_{cmax}, P_{NmaxC}, and P_{Neff}) (Fig. 4).

Discussion

Low irradiance may reduce growth rates, as it was observed in the present study for number of leaves, diameter at ground level, and total leaf area, even though development may remain constant (Chazdon *et al.* 1996, Strauss-Debenedetti and Bazzaz 1996, Marenco and Vieira 2005, Silvestrine *et al.* 2007, Zhou *et al.* 2010, Azevedo and Marenco 2012).

The high values of P_{Nmax}, P_{NmaxL}, and P_{NmaxC} recorded for seedlings and saplings growing under higher irradiance demonstrated the adaptability of the photosynthetic apparatus to growing irradiance gradients, already at the seedling stage, and pointed to irradiance as a limiting factor (Poorter 1999, Poorter and Pothmann 1992, Ronquim *et al.* 2003, Montgomery 2004).

P_{Neff} values characterize CO_2 assimilation capacity, which reflects the actual carbon gain under natural irradiance conditions. Natural irradiance at 16:00 h was low, but still high enough to promote photosynthetic activity. The obtained results showed that saplings had higher carbon gain values and that photosynthesis was favored by higher irradiance. The differences in P_{Neff} and growth between seedlings and saplings were attributed to the vertical stratification of light in the understory, reducing quantity, quality, and duration of irradiance in understories (Chazdon *et al.* 1996, Miyashita *et al.* 2012, Way and Pearcy 2012), and reducing carbon gain, mostly in seedlings, especially in CCU and ICU. The fact that height was the only growth parameter with higher GN in seedlings suggests that photoassimilates are translocated preferentially to grant these plants better access to this limiting resource (Smith 1982, Poorter 1999, Huang *et al.* 2011). While the saplings showed better P_{Neff} in CCU and ICU, P_{Neff} in the seedlings verged on the P_{Nmax} assimilation in OCU, indicating that effective photosynthetic performance of the seedlings increased with irradiance (Fig. 2). P_{Nmax} values characterize CO_2 assimilation capacity under saturated light conditions. They were lower at 16:00 h in all areas, due to stomatal closure (data not shown). The results of the present study suggest that all biochemical parameters increased with irradiance. The biochemical

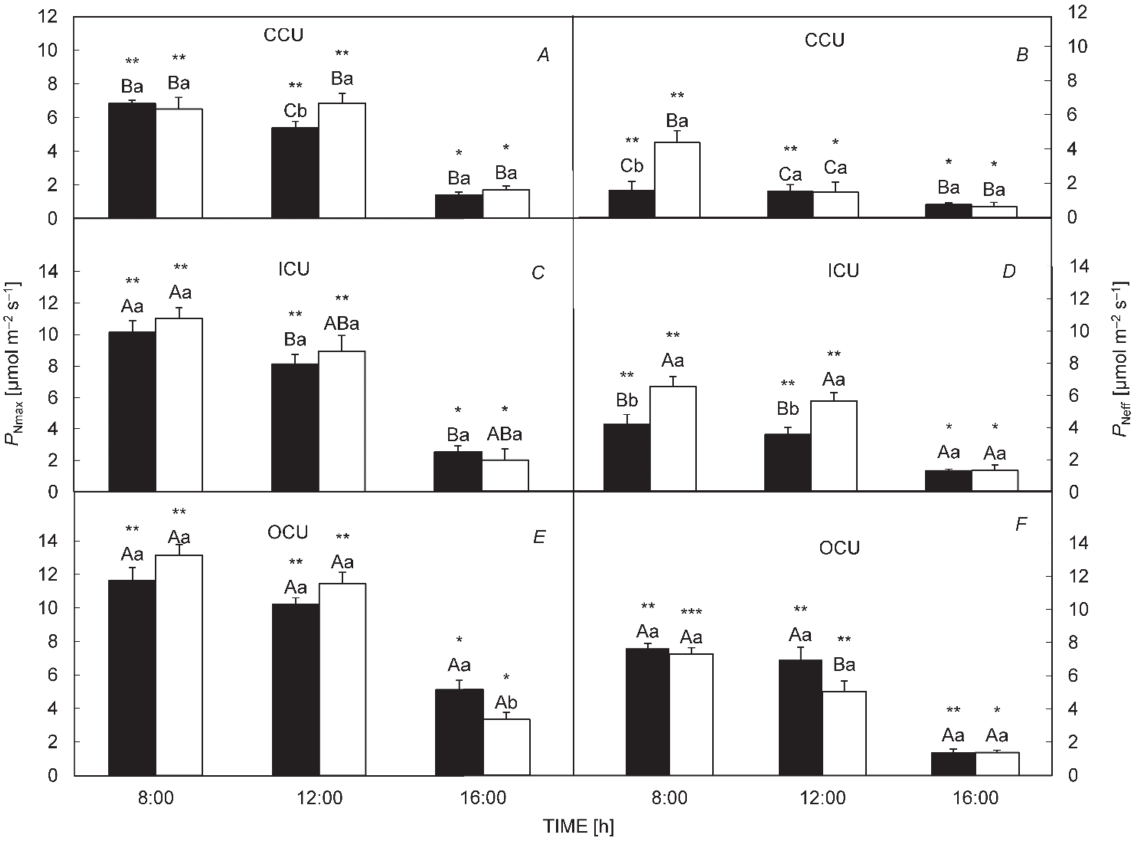

Fig. 2. Maximal net photosynthetic rate (P_{Nmax} – *A*,*C*,*E*), and effective net photosynthetic rate (P_{Neff} – *B*,*D*,*F*) in *Sipurana guianensis* at 8:00 , 12:00 , and 16:00 h in seedlings (*black bars*) and saplings (*white bars*) in three areas of different irradiance regimes (closed canopy understory, CCU; intermediate canopy understory, ICU, and open canopy understory, OCU) in União Biological Reserve, Brazil. *Capital letters* compare areas considering one developmental stage. *Lowercase letters* compare developmental stage considering one same area. * Compares hours considering one developmental stage (8:00, 12:00, and 16:00 h) ($p \leq 0.05$; $n = 4$).

Table 2. Various parameters and shadow adjustment coefficient (SAC) of *Sipurana guianensis* in seedlings and saplings in three areas of different irradiance regimes (closed canopy understory, CCU; intermediate canopy understory, ICU; and open canopy understory, OCU) in União Biological Reserve, Brazil. Mean chlorophyll (Chl) *a*; Chl *b*; carotenoids (Car); chlorophyll *a*/*b* ratio (Chl *a*/*b*), total chlorophyll; maximal quantum yield of PSII (F_v/F_m): photochemical quenching (q_P); nonphotochemical quenching (NPQ); effective quantum yield ($\Delta F/F_m'$); electron transport rate (ETR); maximal photosynthetic capacity (P_{NmaxL}); light saturation point (LSP); potential photosynthetic capacity (P_{NmaxC}); maximal carboxylation speed of Rubisco (V_{cmax}); maximal electron transport rate (J_{max}); use of triose-phosphate (TPU); relative stomatal limitation to photosynthesis (L_s); and stomatal conductance (g_s). *Capital letters* compare areas considering one developmental stage. *Lowercase letters* compare developmental stage considering one same area ($p \leq 0.05$; $n = 4$).

Parameters	CCU Seedling	CCU Sapling	SAC - CCU Seedling	SAC - CCU Sapling	ICU Seedling	ICU Sapling	SAC - ICU Seedling	SAC - ICU Sapling	OCU Seedling	OCU Sapling
Chl *a* [μmol m^{-2}]	307.0^{Aa}	369.7^{Aa}	0.09	0.11	291.3^{Aa}	369.1^{Aa}	0.14	0.11	338.1^{Aa}	416.0^{Aa}
Chl *b* [μmol m^{-2}]	103.8^{Aa}	118.1^{Aa}	0,03	0.04	97.1^{Ab}	124.3^{Aa}	0.09	–0.01	106.9^{Aa}	123.6^{Aa}
Car [μmol m^{-2}]	75.8^{Ba}	85.7^{Ba}	0.31	0.33	73.5^{Ba}	89.9^{Ba}	0.33	0.28	110.1^{Aa}	125.7^{Aa}
Chl *a*/*b*	29.5^{Aa}	31.3^{Aa}	0.07	0.07	30.1^{Aa}	30.1^{Aa}	0.05	0.11	31.6^{Aa}	33.5^{Aa}
Total Chl [μmol m^{-2}]	410.9^{Aa}	487.7^{Aa}	0.08	0.10	388.4^{Ab}	493.4^{Aa}	0.13	0.09	445.1^{Aa}	539.6^{Aa}
F_v/F_m	0.82^{Aa}	0.82^{Aa}	–0.01	0.08	0.81^{Aa}	0.82^{Aa}	0.00	0.08	0.81^{Aa}	0.89^{Aa}
q_P	0.81^{Aa}	0.85^{Ba}	0,05	0.04	0.85^{Aa}	0.85^{Ba}	0.00	0.04	0.85^{Ab}	0.89^{Aa}
NPQ	0.61^{Aa}	0.44^{Bb}	–0.17	–0.76	0.57^{Aa}	0.59^{Aa}	–0.10	–1.36	0.52^{Aa}	0.25^{Cb}
$\Delta F/F_m'$	0.12^{Aa}	0.11^{Ba}	0.08	0.35	0.15^{Aa}	0.17^{Aa}	–0.15	0.00	0.13^{Ab}	0.17^{Aa}
ETR [μmol m^{-2} s^{-1}]	56.19^{Ba}	57.67^{Ba}	0.08	0.27	76.27^{Ab}	84.1^{Aa}	–0.24	-0.08	61.31^{Ab}	78.63^{Aa}
P_{Nmax} [μmol(CO$_2$) m^{-2} s^{-1}]	8.97^{Ca}	9.23^{Ca}	0.41	0.37	12.72^{Ba}	13.18^{Ba}	0.16	0.10	15.16^{Aa}	14.66^{Aa}
LSP [μmol(photon) m^{-2} s^{-1}]	527.91^{Ca}	456.25^{Ba}	0.50	0.40	714.59^{Ba}	712.36^{Aa}	0.32	0.06	1,047.17^{Aa}	757.18^{Ab}
P_{NmaxC} [μmol(CO$_2$) m^{-2} s^{-1}]	16.59^{Ca}	14.46^{Ba}	0.32	0.38	19.01^{Ba}	20.68^{Aa}	0.22	0.11	24.45^{Aa}	23.33^{Aa}
V_{cmax} [μmol m^{-2} s^{-1}]	45.50^{Ba}	51.3 ^{Ba}	0.48	0.45	105.67^{Aa}	93.21^{Aa}	0.20	0.00	87.67^{Aa}	93.67^{Aa}
J_{max} [μmol m^{-2} s^{-1}]	58.23^{Ba}	57.32^{Ba}	0.28	0.28	78.27^{Aa}	81.33^{Aa}	–0.03	0.01	81.09^{Aa}	80.33^{Aa}
TPU [μmol m^{-2} s^{-1}]	5.27^{Ba}	4.57^{Ba}	0.23	0.33	5.93^{ABa}	6.42^{Aa}	0.13	–0.07	6.83^{Aa}	6.87^{Aa}
L_s	15.04^{Bb}	22.78^{Aa}	0.47	0.15	26.30^{Aa}	24.63^{Aa}	0.08	0.08	28.51^{Aa}	26.82^{Aa}
g_s [mol(H$_2$O) m^{-2} s^{-1}]	0.12^{Aa}	0.10^{Ab}	–0.49	–0.38	0.10^{Ba}	0.09^{Bb}	–0.22	–0.26	0.08^{Ca}	0.07^{Cb}

Table 3. Pearson correlation *r* values between nonphotochemical quenching (NPQ) and carotenoid contents in *Sipurana guianensis* seedlings and saplings in three areas of different irradiance regimes (closed canopy understory, CCU; intermediate canopy understory, ICU; and open canopy understory, OCU) in União Biological Reserve, Brazil ($p \leq 0.05$; $n = 4$). *Bold items* – positive correlation, *italic* – negative correlation.

	Correlation carotenoid × NPQ CCU	ICU	OCU
Seedling	-0.0079	*-0.998*	**0.996**
Sapling	*-0.0753*	**0.739**	**0.714**

performance of plants varies with PPFD (de Pury and Farqhuar 1997), and the uniform distribution of PPFD promotes the balance between Rubisco and Rubisco activase. Studies have shown that plants exposed to low or fluctuating PPFD show higher ability and speed of photosynthetic induction, which are associated with higher levels of Rubisco activase, among other factors (Yanhong *et al.* 1994, Küppers *et al.* 1996, Valladares *et al.* 1997, Montgomery and Givnish 2008, Santiago and Dawson 2014).

The greater irradiance in ICU and OCU, the higher resulting drop in RH, and higher VPD values increased L_s in both saplings and seedlings, and reduced g_s. L_s represents the limitation to CO$_2$ entry, while g_s is associated with the restriction to the release of water vapor. Mott and Woodrow (1993) evaluated stomatal opening in photosynthetic induction, and suggested that Rubisco activation is a function of intercellular CO$_2$ concentration. The performance of plants in OCU indicated that the higher PPFD available might favor an adaptation of biochemical characteristics (SAC values), since higher L_s did not reduce CO$_2$ assimilation, which might be associated with Rubisco activity (higher V_{cmax} values). The higher irradiance favors V_{cmax} (Pearcy *et al.* 1994) and generally is followed by an increase in J_{max} (Sassenrath-Cole and Pearcy 1992) and in the capacity to use chloroplast products (saccharose and starch) (Sharkey *et al.* 2007).

In the present study, increases in V_{cmax} and J_{max} were observed in the areas with higher irradiance, suggesting an increase in the capacity to use chloroplast products. However, this parameter was not measured and therefore this hypothesis could not be confirmed.

Studies on leaf pigment contents suggest that the photosynthetic apparatus adapts to changes in irradiance (Portes *et al.* 2010, Silva *et al.* 2010, Vieira *et al.* 2011, Lage-Pinto *et al.* 2012) throughout leaf development (Rosevear *et al.* 2001). Chls also undergo photooxidation under high irradiance (Hendry and Price 1993, Araújo and Deminicis 2009). The results of the present study showed that concentration of Car varied with irradiance (SAC values), thus, the constant Chl contents and the F_v/F_m

Table 4. Mean values of leaf blade thickness, adaxial cuticle, adaxial epidermis face, palisade parenchyma, spongy parenchyma, and abaxial epidermis face in *Sipurana guianensis* seedlings and saplings in three areas of different irradiance regimes (closed canopy understory, CCU; intermediate canopy understory, ICU; and open canopy understory, OCU) in União Biological Reserve, Brazil. SAC: shadow adjustment coefficient. *Capital letters* compare areas considering one developmental stage. *Lowercase letters* compare developmental stage considering one same area ($p \leq 0.05$; $n = 4$).

	CCU		SAC-CCU		ICU		SAC-ICU		OCU	
Thickness [µm]	Seedling	Sapling	Seedling	Sapling	Seedling	Sapling	Seedling	Sapling	Seedling	Sapling
Leaf blade thickness	181.6^{Ab}	201.5^{Aa}	0.05	0.05	171.1^{Ab}	210.4^{Aa}	0.10	0.01	191.0^{Ab}	211.7^{Aa}
Adaxial cuticle	1.0^{Ca}	0.82^{Ca}	0.47	0.63	1.3^{Ba}	1.4^{Ba}	0.32	0.38	1.9^{Ab}	2.24^{Aa}
Adaxial epidermis face	31.2^{Aa}	36.4^{Aa}	-0.03	-0.22	32.8^{Aa}	33.2^{Aa}	-0,09	-0.11	30.2^{Aa}	29.9^{Aa}
Palisade parenchyma	36.9^{Ba}	41.9^{Ba}	0.22	0.19	38.6^{Bb}	50.1^{Aa}	0.19	0.03	47.4^{Aa}	51.8^{Aa}
Spongy parenchyma	83.4^{ABb}	102.3^{Aa}	0.07	0.03	78.8^{Bb}	100.0^{Aa}	0.12	0.05	89.6^{Ab}	105.7^{Aa}
Abaxial epidermis face	31.9^{Aa}	31.1^{Aa}	-0.23	-0.11	31.3^{Aa}	25.9^{Aa}	-0.20	0.08	26.0^{Aa}	28.1^{Aa}

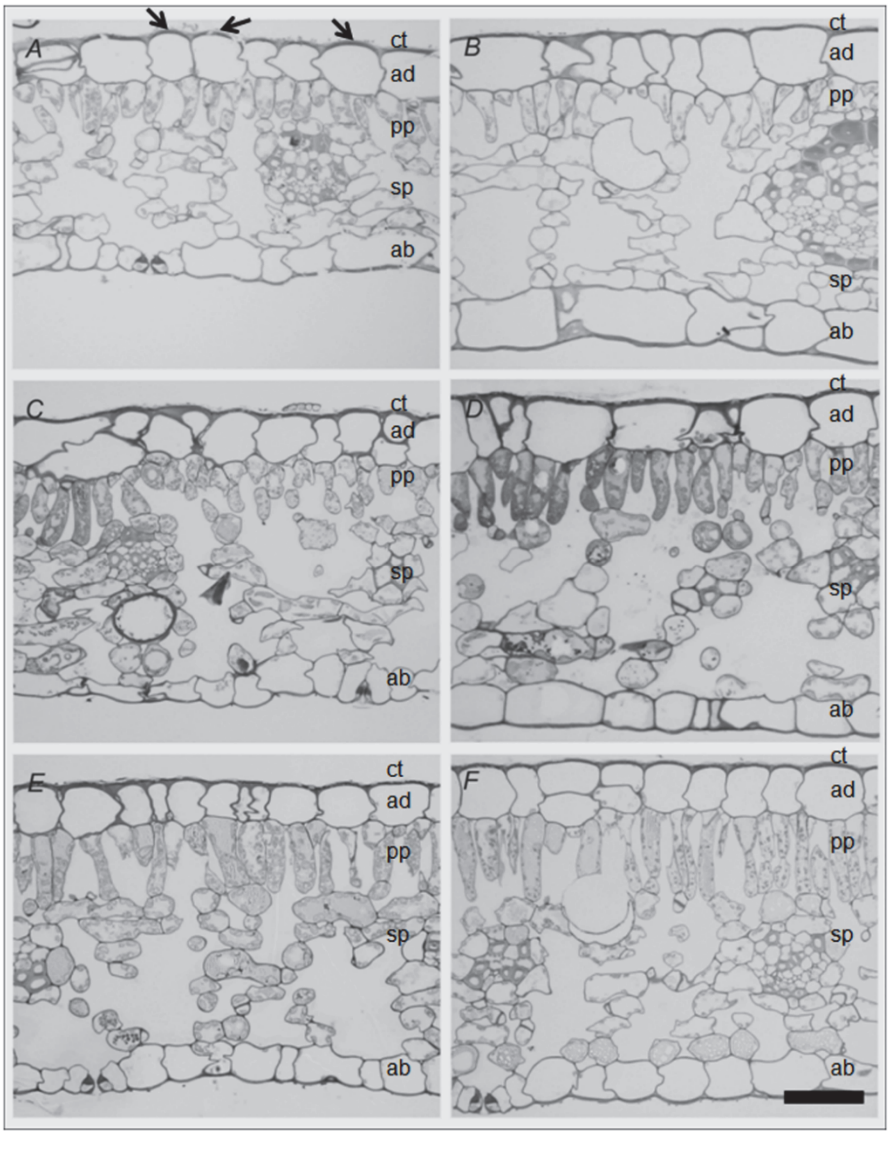

Fig. 3. Cross-sections of leaves of seedlings (*A*,*C*,*E*) and saplings (*B*,*D*,*F*) of *Siparuna guianensis* in three areas of different irradiance regimes (closed canopy understory, CCU, *A*,*B*; intermediate canopy understory, ICU, *C*,*D*; and open canopy understory, OCU, *E*,*F*) in União Biological Reserve, Brazil). Bar: 50 µ; magnification 40 ×. ad – adaxial surface; ab – abaxial surface; pp – palisade parenchyma; sp – spongy parenchyma; ct – cuticle; outer periclinal wall (*arrow*).

values might indicate photoprotection mediated by Car and NPQ (Demmig-Adams *et al.* 1990, Demmig-Adams and Adams 1996, Merzlyak and Solovchenko 2002). The positive correlation between Car and NPQ in seedlings and saplings growing in OCU and in saplings in ICU suggests that the photoprotection pathway was not determined by developmental stage and that it was modulated mainly by irradiance. Additionally, this correlation may be associated with the production of Car in response to increased irradiance (Demmig-Adams *et al.* 1990). These results suggest this species possesses capacity to acclimate independently of the environment or developmental stage, since all F_v/F_v values were within the 0.75−0.85 range. This indicates that PSII was not affected, and that energy conversion efficiency was preserved (Bolhàr-Nordenkampf *et al.* 1989). Another piece of evidence of the acclimation capacity of *S. guianensis* was the high q_P value (over 0.8). However, in spite of the little variation among environments, significant differences were observed between OCU and CCU, only in saplings.

In the present study, LSP and ETR increased with irradiance in the three environments analyzed. It is known that plants growing in shaded environments present low LSP (Bazzaz 1979, Bazzaz and Pickett 1980). The results obtained in the present study showed that the capacity to use irradiance (LSP) was higher than the irradiance available in all areas studied. This, taken together with the ETR data, indicates that light is a limiting factor in the photosynthetic activity of *S. guianensis* in these environments, and that photochemical and biochemical reactions improve with increasing irradiance. However, when *S. guianensis* in the understory was exposed to sun due to abrupt forest gaps, photoinhibition and photooxidation were observed (Lage-Pinto *et al.* 2012). This suggests that, when the leaves of this species develop under low irradiance, the plant is still susceptible to the effects of higher irradiance levels, in spite of its plasticity.

In this study, the convex cell shape on the adaxial epidermis face of the seedlings growing in CCU revealed that these individuals were better acclimated to harvest diffuse light and sunflecks, which are typical of closed canopy forests (Vogelmann *et al.* 1996, Brodersen and Vogelmann 2007, Silva *et al.* 2010). This result is corroborated by research that shows the association between the curvature of the external periclinal wall and the capacity of chloroplasts to harvest and use light in the photosynthetic process (Haberlandt 1914, Vogelmann and Martin 1993, Vogelmann *et al.* 1996, Silva *et al.* 2010).

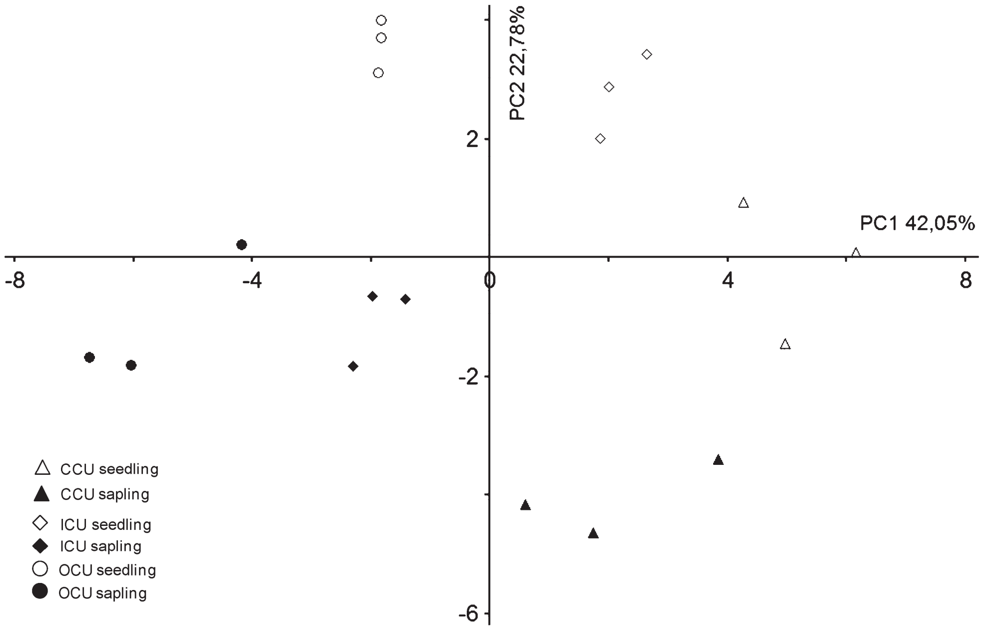

Fig. 4. Principal component analysis generation plot considering all growth parameters, the P_N/C_i (net photosynthetic rate/intercellular CO_2 concentration) and the P_N/PPFD (net photosynthetic rate/photosynthetic photon flux density) curves, chlorophyll *a* fluorescence, photosynthetic pigment contents, and anatomical parameters of *Siparuna guianensis* in three areas of different irradiance regimes (closed canopy understory, CCU; intermediate canopy understory, ICU; and open canopy understory, OCU) in União Biological Reserve, Brazil).

Previous studies have shown that the increase in leaf blade thickness in response to greater irradiance is a result of the elongation of palisade parenchyma cells and of a higher number or a larger space between cells of the spongy parenchyma (Rozema *et al.* 1997, Pandey and Kushwaha 2005, Johnson *et al.* 2005, Silva *et al.* 2010, Rabelo *et al.* 2012). Greater photosynthetic parenchyma thickness promotes light harvesting and CO_2 fixation by chloroplasts, increasing photosynthetic activity (Vogelmann and Martin 1993). Chloroplasts tend to change orientation in the cytoplasm, in order to maximize or minimize light absorption, under low- and high-irradiance conditions, respectively (Brugnoli and Björkman 1992). This movement may be favored by the elongation of the photosynthetic parenchyma. The acclimation of chloroplasts according to irradiance may maximize photosynthetic capacity and reduce photodamage (DeLucia *et al.* 1996). The elongation of photosynthetic parenchyma may be considered a beneficial change in the structural photosynthetic mechanism, since it reduces resistance to CO_2 diffusion in the intracellular space and facilitates CO_2 permeability through the cell wall, plasmatic membrane, cytosol, chloroplast, and stroma towards Rubisco (Evans 1999, Terashima *et al.* 2001).

The lower compaction of the spongy parenchyma, more pronounced in the seedlings than in saplings, did not depend on irradiance. The phenomenon might be interpreted as an acclimation strategy involving lower investment in carbon absorption by photosynthetic tissues due to the availability of higher concentrations of CO_2, as observed near the soil, where microbiological respiration activity produces higher amounts of the gas (Kao *et al.* 2000). Another explanation for the phenomenon in seedlings is the lower irradiance in the lower forest stratum, which may determine greater investment in light harvesting, compared to CO_2 assimilation. In the present study, the consistent height gain and the undersized spongy parenchyma of seedlings highlighted the notion that the limiting factor under those conditions was indeed light harvesting capacity, not CO_2 concentration.

S. guianensis individuals growing in CCU had thinner adaxial cuticles than those in OCU. Thicker cuticles may be associated with increased reflection and result in low sunlight absorption (Holloway 1982). In the present study, the thinner adaxial epidermis surface observed might be also considered as an adaptive strategy, together with thicker spongy parenchyma. Variations in structure and position of elements of the leaf blade in response to changes in irradiance lend more plasticity to species (Strauss-Debenedetti and Berlyn 1994, Oguchi *et al.* 2003, Silva *et al.* 2010). The anatomical adaptations observed might be linked not only to differences in irradiance between the areas analyzed, but also to vertical light stratification of the understories.

The ordination plot (PCA) revealed that distinct clusters formed in and between the environments, proportionally to irradiance. The differentiation of clusters characterizes the different response of plants concerning photosynthetic capacity and carbon gain throughout development, considering the areas plants originally grow in. However, clustering cannot be attributed to one single set of plastic attributes.

According to hypothesis, *S. guianensis* acclimation involves morphological, anatomical, photochemical, and biochemical traits. These traits were different for seedlings and saplings. However, SAC values indicated that biochemical performance of these plants was the attribute most easily adaptable to variation in irradiance. Both irradiance and developmental stage are important factors for direct response to acclimation, though the irradiance played a more important role in the activation of these responses, compared with the developmental stage. Even though this species is widely distributed in Central and South America, it should be taken into account that the data obtained in the present study refer to one species only, and that more species should be investigated to validate these plastic attributes as reference for the developmental stages of other plants.

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