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Structural and ecophysiological adaptations to forest gaps

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Abstract To survive new microclimatic conditions of a forest gap environment, plant species must physiologically and structurally adjust. A morpho-anatomical, ultrastructural and ecophysiological study was performed at three different times in a forest gap that was created by illegal selective logging. The study followed the early successional *Actinostemon verticillatus* and the late-successional *Metrodorea brevifolia,* to elucidate the adaptive strategies of acclimation to gaps. Additionally, *Schinus terebinthifolius* was included in the study in order to test the plasticity of a pioneer species that grows on forest edges, where this species had higher values of leaf thickness, leaf mass area and succulence. *M. brevifolia* had succulent leaves, high leaf area and a thin cuticle. *A. verticillatus* presented the densest leaves and was the only species to show leaf morpho-anatomical plasticity. Ultrastructural and physiological differences were observed only in *A. verticillatus* and *M. brevifolia* leaves from the gap: increase in the stroma volume, oil droplets, plastoglobuli, photochemical and non-photochemical quenching. Photosynthetic efficiency showed that the early stages of gap formation are the most critical. Acclimation strategies of *A. verticillatus* suggest this species invests in the efficiency of photosynthesis by increasing its leaf thickness, leaf mass area and in water content maintenance by increasing the density of its leaves, at the expense of gas exchange, was compensated by a high density of stomata. *M. brevifolia* compensates for the higher cost of leaves and lower leaf plasticity with ultrastructural changes that are used to adjust the photosynthetic process, which promotes a shorter leaf payback time.

Keywords Gaps · Acclimation strategies · Leaf morpho-anatomy · Chloroplasts · Photosynthesis · Successional groups

In Memoriam: Ricardo A. Cruz.

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Introduction

The early stage of a forest gap represents a great challenge and/or opportunity for tree seedlings and other plants that remain after its formation. New microclimatic conditions that develop suddenly in a forest, such as during gap formation, can have high irradiance and temperatures, and low levels of moisture (Denslow 1987; Brown 1993) to which surviving species need to quickly adjust both physiologically and structurally. Thus, the species that will adapt and develop to the new conditions constitutes the paradigm of species diversity in tropical ecosystems (Denslow 1987).

In a recent review, Schliemann and Bockheimb (2011) list numerous works that emphasize the impacts of gaps on forest structure, nutrient cycling, microclimates, and forest management. Other studies have focused on photosynthetic light acclimation (Naidu and Delucia 1998; Yamashita

et al. 2000) and the anatomical leaf changes that are involved during photosynthetic adjustments (Oguchi et al. 2003, 2006).

Leaf physiology and structural changes are one of the primary means for plants to respond to microclimatic changes in a gap. However, plants differ in their capacity of change phenotypically (phenotypic plasticity) in response to the environment (Via et al. 1995). Species of different successional groups demand different levels of light, temperature, and nutrients (Strauss-Debenedetti and Bazzaz 1996). According to Bazzaz and Pickett (1980), the survival strategies used to separate tree species into two successional groups (early and late) are supported by growth features and shade adaptation. The early successional group includes pioneer species, which are light demanding and their germination is inhibited by far-red light (shade avoidance syndrome) that dominates the understory. Species in the late-successional group are shade tolerant and exhibit a lower light acclimation potential (Yamashita et al. 2000).

Highly diverse environments, such as a tropical ecosystem, imply high levels of ecological redundancy (Peterson et al. 1998). Thus, in a tropical ecosystem, it is appropriate to use successional groups, where botanical identity is substituted for functionality instead of the species, which is based on intrinsic assumptions of the taxonomic characteristics (Quesada et al. 2009). Additionally, studying the phenotypic plasticity of different successional groups that are acclimating to gaps can serve as a good model to predict the behavior of forests to climate change. Thus, how to choose an appropriate method that can be used to predict the acclimation process integrating ecological and phenotypic plasticity studies?

Sultan (2004) enumerates three complex aspects of plasticity that have substantial ecological importance. The first is *cross-generational plasticity,* where the adaptive legacy is transmitted to the offspring in response to environmental conditions. The second is *dynamic plasticity,* which relates to the variation in ontogenetic trajectories and the rates of phenotypic response, namely, as the plastic response may depend on the timing of its expression. The third is the *plasticity of anatomical and architectural traits,* where the adaptive strategies are analyzed at the tissue and cellular level.

The present study focused on the plasticity of morpho-anatomical traits, adding insight into ultrastructural and ecophysiological characteristics and how phenotypic adjustments occur in the leaves of plants. This study used a forest gap created by illegal selective logging of commercial timber species (mostly *Paratecoma peroba* (Record) Kuhlm. and *Myrocarpus frondosus* Fr. All.) and followed the response of fully expanded leaves of understory saplings. The morpho-anatomical, ultrastructural and ecophysiological responses of leaves after gap formation were compared with leaves from a closed forest to clarify the adaptive strategies of acclimating to the gap. In addition, to test the acclimation capacity of a pioneer plant, we included a woody species that occurs on the edge of the forest.

Materials and methods

Study site

The study was carried out at the Guaxindiba Ecological Station, located in the city of São Francisco do Itabapoana (21º24′S, 41º04′W) in northern Rio de Janeiro, Brazil. This is the largest fragment (ca. 1200 ha) of lowland forest on tertiary sediments in Brazil, which is also known as tabuleiro Atlantic forest (RadamBrasil 1983), and classified as seasonal semi deciduous lowland forest based on its phytogeographic features (Veloso et al. 1991). According to Köppen (1948), the climate of this region is classified as Aw, and has an intense dry season from May to August (RadamBrasil 1983). Mean annual rainfall is approximately 1,000 mm; the wettest month is December and the driest is August (Villela et al. 2006). The mean annual temperature is 23 °C (RadamBrasil 1983) and the soils are ultisols (USDA classification) that have a low capacity of water retention and are poor in nutrients (Villela et al. 2006).

The forest fragment consists mainly of secondary semi deciduous forest and has a history of disturbance mainly from logging for commercial timber species, charcoal production, and plantation farming (Villela et al. 2006); consequently, the fragment has artificial canopy gaps of different sizes and ages.

Microclimatic measurements (Table 1) were made a closed area of forest (Forest) and in the gap studied, 2 months after it formed (Gap2m), at four cardinal points of each of five chosen individuals. Solar irradiance ($\mu mol\ m^{-2}\ s^{-1}$) was measured with a LI-250A light meter (Li-Cor Inc.), and temperature (°C) and humidity (%) were measured with a therm-hygrometer HT-300 (Instrutherm). Statistical differences were determined by the Mann–Whitney U rank sum test Statistica 7, StatSoft, USA (Statsoft Inc. 1998).

Plant material and sampling

The sampling was carried out from February to April 2008, at the end of the wet season. Three woody species of different successional status were studied: a pioneer species, *Schinus terebinthifolius* Raddi (Anacardiaceae); an early successional species, *Actinostemon verticillatus* (Kl.) Baill. (Euphorbiaceae); and a late-successional species, *Metrodorea brevifolia* var. *nigra* Engl. (Rutaceae). Three

Table 1 Microclimatic differences between the forest and a forest gap, 2 months (Gap2m) after gap formation in the Guaxindiba ecological station, Rio de Janeiro State, Brazil

Sites	Forest	Gap2m
Temperature (°C)*	32	39
Humidity (%)*	66.5	48
Irradiance (μmol m^{-2} s^{-1})*	50.24	546.4

Values representing the median evaluated from February to April, 2008

* Statistically different (determined by the Mann–Whitney *U rank sum test*)

individuals of each species were measured in each site (forest and forest gap); individuals were about 1–2.5 m tall.

All species responses were measured under field conditions and the forest gap used was created by illegal selective logging. The plant species were studied in a single forest gap (around 10 m in diameter) at three different times relative to when the gap was created: 1-day-old gap (Gap1d); 7-day-old gap (Gap7d); 2-month-old gap (Gap2m). As a control, all data obtained were compared to the closed forest (Forest), which lacked signs of logging. In addition, the pioneer *S. terebinthifolius,* which occurs exclusively along forest edges, was studied. In this part of the study, for comparison, leaves fully exposed to sun were classified as gap leaves and those exposed the least to sun were considered forest leaves.

For the general leaf measurements, anatomical measurements, and chloroplast analysis, fully expanded leaves from the third node were collected from the higher branches of three individuals in the forest and Gap2m sites. It is emphasized that in the Gap2m, only leaves that had been formed after the gap was created were collected. For the chlorophyll *a* fluorescence we choose fully expanded leaves, from the third node of the higher branches, and these same leaves were analyzed in the Gap1d and Gap7d.

Electron microscopy

Leaf fragments of all species studied were collected in the Forest and Gap2m sites, fixed for 2 h in a solution of 2.5 % glutaraldehyde and 4.0 % formaldehyde, buffered with 0.05 M sodium cacodylate, to pH 7.2, at room temperature. After being rinsed with the same buffer, the samples were post-fixed with 1.0 % osmium tetroxide in a 0.05 M sodium cacodylate buffer, at pH 7.2, for 1 h. Subsequently, the samples were dehydrated in an ascending series of acetone solutions. The material was infiltrated and embedded in epoxy resin (Polybed). Ultrathin sections (70 nm) were collected in copper grids (300 mesh), stained with 1.0 % uranyl acetate, followed by 5.0 % lead citrate. Sections were observed at 80 kV using a ZEISS TEM 900 transmission

electron microscope. For scanning electron microscopy (SEM), the samples were fixed, post-fixed, dehydrated (as described previously) and rinsed in the same buffer. Afterwards, the samples were CO_2 critical-point-dried (CPD 030 Baltec). Dried samples were adhered to stubs with carbon adhesive tape (3 M) and sputter coated with 20 nm of gold (SCD 050 Baltec). The material was observed using a ZEISS DSM962 microscope operating at 25 kV.

Morphological leaf measurements

The leaf area (LA) (cm^2) was measured using an electronic leaf area meter (LiCor mod. LI-3100). Leaf discs were taken from fifteen leaves from each of the three individuals of *S. terebinthifolius, A. verticillatus* and *M. brevifolia* and hydrated, placing the leaf disks in distilled water for 24 h. Following this, the saturated mass of the discs was weighed on a digital balance (0,001 g) and their thickness (THIC) was measured using a digital caliper rule (0.001 ± mm). The discs were then dried at 55 °C for 72 h and their mass was measured. From this data, the succulence (SUC g m^{-2}) was calculated based on the difference between the saturated mass and dry mass, divided by leaf disc area. The leaf mass area (LMA g m^{-2}) was calculated by dividing leaf dry mass by disc area. Leaf density (DEN mg mm^{-3}) was calculated by dividing LMA by thickness.

Anatomical leaf measurements

The leaf anatomy variables were made from the following: cuticle, adaxial surface, palisade parenchyma, spongy parenchyma, leaf blade, and stomata density with the aid of an Axioplan Zeiss (Oberkochen, Germany) light microscope coupled with a Hamamatsu C3077 digital camera and the software Analysis®-LINK/ISIS/ZEISS (Oxford, UK).

Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence parameters were determined at 9:00 a.m., 12:00 and 15:00 p.m. using a portable modulated pulse fluorimeter (MINI PAM, Walz, Germany). Three intact, totally expanded and healthy leaves from each of the three individuals of the species from the four sites (Forest, Gap1d, Gap2m and Gap7d) were kept in the dark for 30 min using clamps and exposed to a weak, modulated light beam (approximately 6 μmol m^{-2} s^{-1} at 660 nm), followed by exposure for 0.8 s to highly intense (10,000 μmol m^{-2} s^{-1}) actinic white light (adapted from Genty et al. 1989; Van Kooten and Snel 1990). The chlorophyll *a* fluorescence emission parameters were recorded as minimal fluorescence (F_0), maximum fluorescence (F_m), maximum quantum efficiency (F_v/F_m), maximum primary efficiency (F_v/F_0) and the fluorescence quencher

coefficients qP (photochemical quenching), qN and NPQ (non-photochemical quenching).

Photosynthetic pigments

Analyses of the content of chlorophyll *a,* chlorophyll *b* and carotenoids were carried out using an organic solvent extraction method with a dimethylsulfoxide (DMSO) reagent. Three leaf discs were taken at 12:00 p.m. from each of the three individuals. The discs were sliced and placed in Falcon[TM] tubes containing 5 ml of DMSO and were kept in the dark. The extracts were analyzed in a spectrophotometer at the wavelengths 480, 649 and 665 nm and quantified using the equations from Wellburn (1994) with values expressed in µmol cm^{-2}.

Statistical analysis

The leaf and anatomical comparisons among the three species and the sites were made using Notched Box Plot (McGill et al. 1978). The analyses were done with the software Systat 12.02.00 (Systat 1992). Statistical analyses of chlorophyll *a* fluorescence parameters and pigments comparisons were performed using a one-way ANOVA followed by Tukey's test ($p < 0.05$) (Zar 1999). The principal component analysis (PCA) was based on a comparison between the quantitative leaf characters of the species in the forest and Gap2m (Ludwig and Reynolds 1988); a statistical software package was used for the analysis (Statistica 7, StatSoft, USA Statsoft Inc. 1998).

The index of the phenotypic plasticity (Valladares et al. 2000) was calculated for the three species as the difference between the minimum and maximum mean value among the forest and Gap2m variables divided by the maximum mean value. The phenotypic plasticity index ranges from zero to one. For this, the results were presented as the median for each parameter and for the leaf traits (anatomical, morphological and physiological).

Results

Morphological leaf measurements

In general, the three species tended to have distinct values for all leaf variables (Fig. 1). The pioneer species, *S. terebinthifolius,* presented higher values for the leaf variables THIC, SUC and LMA, and the lowest values for highest leaf density (DEN) and higher leaf area (LA). The lowest values for THIC, SUC and LMA were observed in the early successional *A. verticillatus* leaves; however, this species had the DEN. Leaves from the late-successional *M. brevifolia* had LA and a tendency toward elevated succulence values.

When comparing the intraspecific difference (Fig. 1) between the studied sites (Forest and Gap2m), only *A. verticillatus* showed significant difference in the THIC, LMA and DEN variables, where leaves from the gap were thicker and had higher LMA values than those from the forest, while leaves from the forest were the densest. The leaf traits of *S. terebinthifolius* and *M. brevifolia* showed no significant differences for all studied variables.

Anatomical leaf measurements

The three species showed interspecific differences for all anatomical leaf measurements (Fig. 2). *S. terebinthifolius* leaves had the highest values for all of the variables studied, except the adaxial surface. *A. verticillatus* leaves presented the lowest values for the adaxial surface, palisade parenchyma, spongy parenchyma and leaf blade. *M. brevifolia* had a thinner cuticle and intermediate values for blade, spongy and palisade parenchyma.

For the intraspecific comparison between the studied sites (Forest and Gap2m), the adaxial surface and stomata density showed significant differences (Fig. 2), where the width of the adaxial surface of *M. brevifolia* was thicker in the forest site than the gap site and the stomata density of gap leaves from *A. verticillatus* was higher than the leaves collected in the forest. No significant differences were observed for *S. terebinthifolius.*
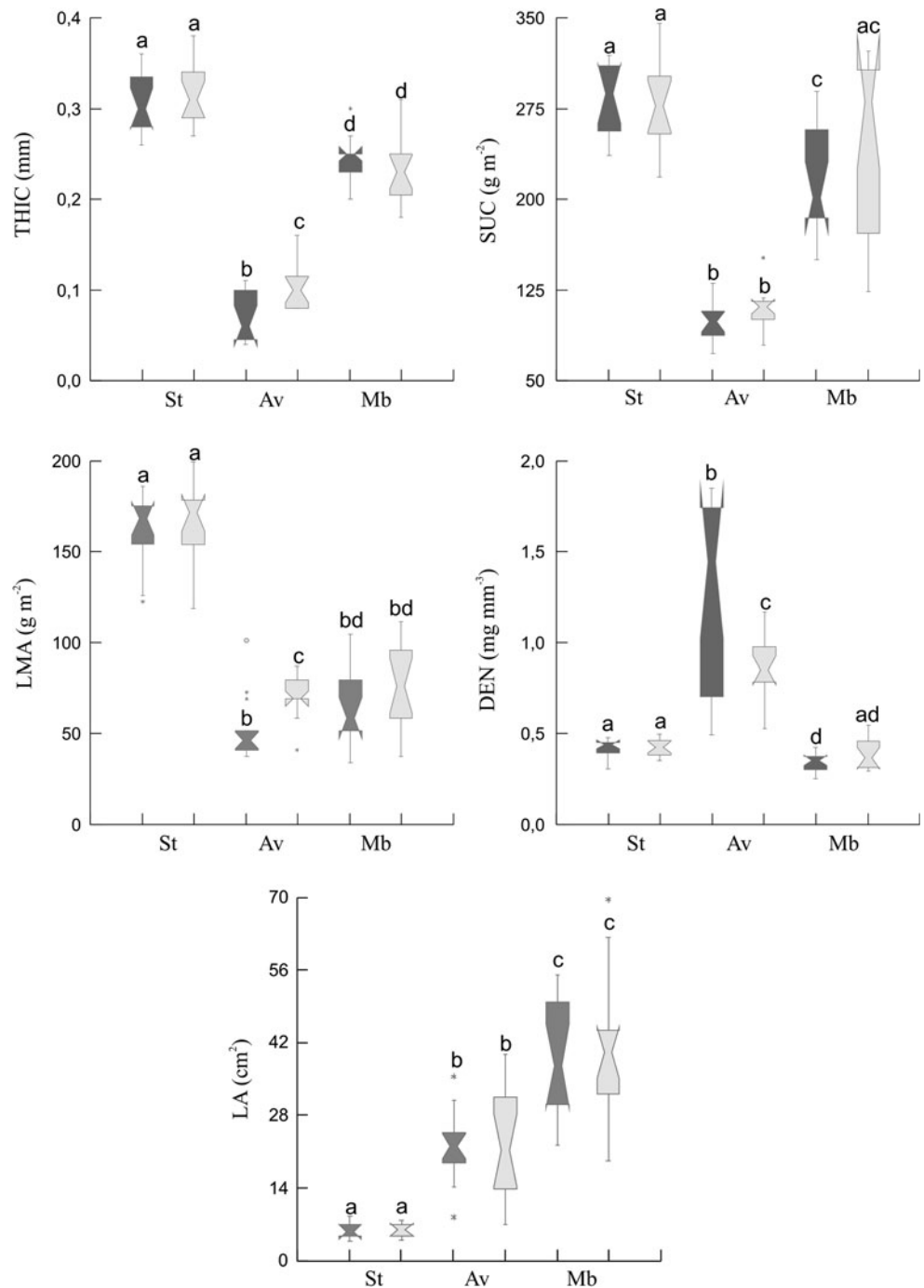
Chloroplast ultrastructure

The ultrastructural organization of the chloroplasts revealed a remarkable tendency toward the accumulation of starch, mostly accompanied by an increase in the number and size of oil droplets and plastoglobuli in leaves exposed to high irradiation. In *S. terebinthifolius,* where leaves were exposed to high irradiance for long periods each day, the chloroplasts exhibited one or two starch grains and an accumulation of oil droplets (Fig. 3a); however, no plastoglobuli was observed. There were no difference between leaves fully exposed to sun and leaves less exposed to sun (Fig. 3a–c). In both leaf types, there was an enormous accumulation of tannins occupying most of the lumen (Fig. 3a–c).

Actinostemon verticillatus leaves from the forest exhibited chloroplasts of typical form, grana and stroma organization and presented few starch grains (Fig. 3d, e), while leaves from Gap2m (Fig. 3f, g) had an increased number of oil droplets and larger plastoglobuli, some changes grana organization of the thylakoids and a remarkable increase in stroma volume and relocation of the stroma.

The chloroplast from forest leaves of *M. brevifolia* had few oil droplets, typical grana and stroma organization and some

Fig. 1 Leaf morphology plasticity from forest and leaves formed after the gap formation. *Notched box plot* of leaf morphology features of *S. terebinthifolius* (St), *A. verticillatus* (Av) and *M. brevifolia* (Mb) measured in the forest and forest gap 2 months (Gap2m) after gap formation. *THIC* thickness (mm), *SUC* succulence (g m^{-2}), *LMA* leaf mass area (g m^{-2}), *DEN* Leaf density (mg mm^{-3}), *LA* leaf area (cm^2). They are notched at the median and return to full width at the lower and upper 95 % confidence interval values. Interquartile ranges define inner and outer fences. *Asterisks* are "outside values" and *circles* are "far outside values". The medians are statistically different (α = 0.95, represented by *different letters*) when the confidence intervals do not overlap. *Dark grey* = forest; *Light grey* = Gap2m

small plastoglobuli (Fig. 3h). Leaves from Gap2m had a disarranged thylakoid membrane, and an increased number of starch grains, oil droplets and plastoglobuli (Fig. 3i, j). The plastoglobuli content passing through the chloroplast membrane into the cellular lumen is shown in Fig. 3k.

Chlorophyll a fluorescence and pigments content

The parameters maximum quantum yield of photosystem II (F_v/F_m) and the maximum primary yield of the photochemistry of photosystem II (F_v/F_0) showed the same pattern of response for each of the studied species (Fig. 4). No significant differences were observed in *S. terebinthifolius* for both ratios. In general, both *A. verticillatus* and *M. brevifolia* presented no significant difference between the forest and Gap2m and lower values in the Gap1d and Gap7d for the three periods (9:00 a.m., 12:00 and 15:00 p.m.) measured. For Gap7d, *M. brevifolia* presented the lowest values during the three periods studied, while *A. verticillatus* had decreased values along the three
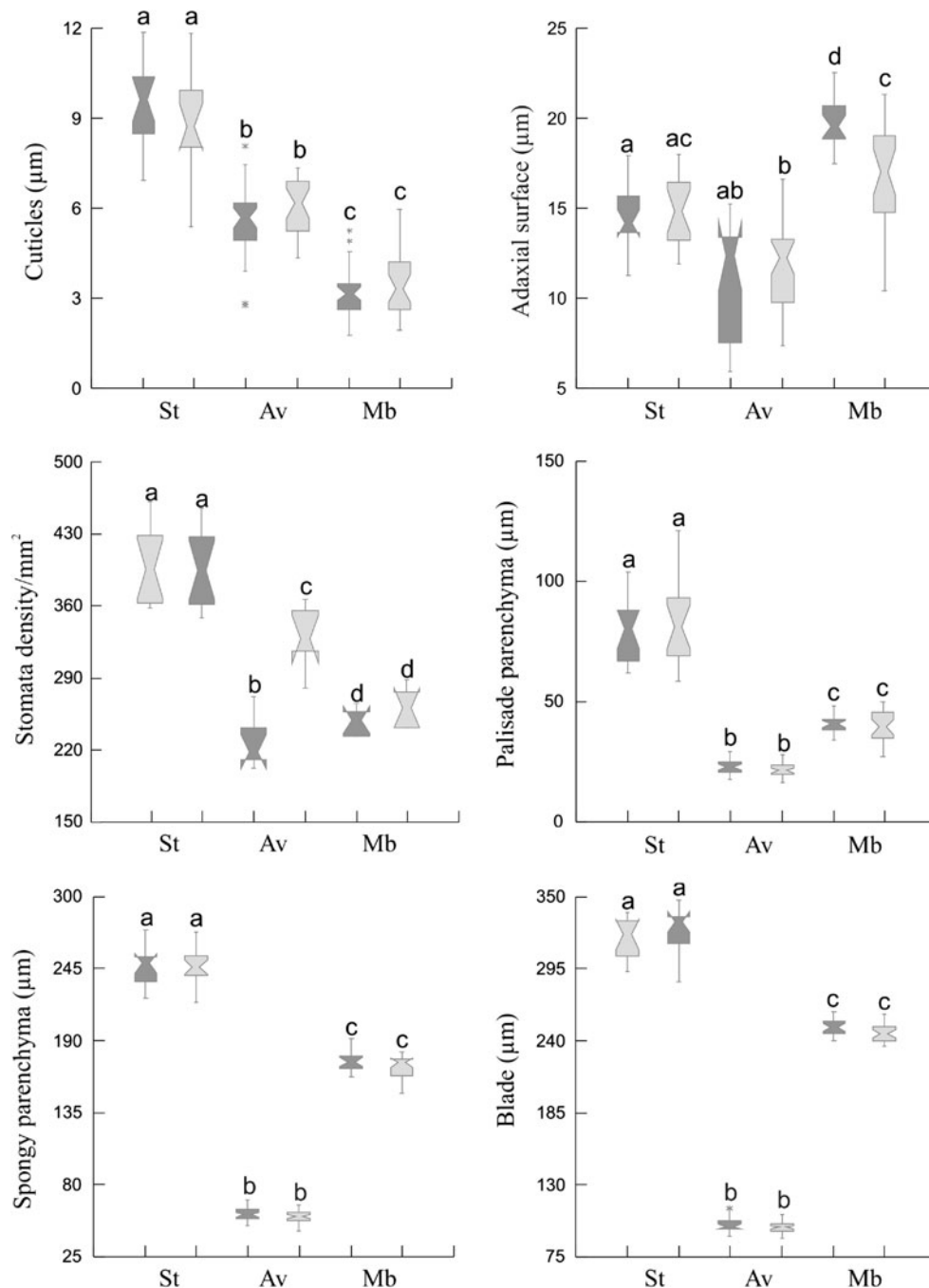
Fig. 2 Leaf anatomy plasticity from forest and leaves formed after the gap formation. *Notched box plot* of leaf anatomical features of *S. terebinthifolius* (*St*), *A. verticillatus* (*Av*) and *M. brevifolia* (*Mb*) measured in the forest and forest gap 2 months (Gap2m) after gap formation. They are notched at the median and return to full width at the lower and upper 95 % confidence interval values. Interquartile ranges define inner and outer fences. *Asterisks* are "outside values". The medians are statistically different ($\alpha = 0.95$, represented by *different letters*) when the confidence intervals do not overlap. *Dark grey* = forest; *Light grey* = Gap2m

periods. On the other hand, *M. brevifolia* presented a linear F_v/F_m and F_v/F_0 response for the three periods, while *A. verticillatus* showed a remarkable slope at 12:00 p.m. in Gap1d.

The qP value increased in function in exposed environments of forest gaps (Table 2); however, no significant differences were observed for *S. terebinthifolius*. The qP values for *A. verticillatus* showed no significant differences at 12:00 p.m. At 9:00 a.m.; the only difference was the higher qP values in Gap2m compared to the forest traits. At 15:00 p.m., Gap2m still had higher qP values, while no significant difference were found among the forest, Gap1d

and Gap7d. The *qP* values for *M. brevifolia* presented the lowest values in the forest when compared to all other stages of the gap.

The *NPQ* values of *S. terebinthifolius* presented no significant difference for the three periods measured. *A. verticillatus* and *M. brevifolia* showed higher *NPQ* values in the gaps than in the forest for the three periods (Table 2).

The *Chl a/b* and *carotenoids* contents were measured at 12:00 p.m. (Table 3). Only *M. brevifolia* presented a significant difference in the *Chl a/b* rate, where Gap2m showed the highest values. There were no variations in the concentrations of carotenoids, due to the environment, for the species studied.

PCA and plasticity index

For PCA, the first two axes explain about 69 % of the total variation (Fig. 5a). The ordination along with the principal components 1 and 2 show the separation of the five groups of plants into distinct clusters where even intraspecific groups (Fig. 5a, dashed circles) composed of *A. verticillatus* and *M. brevifolia* were separated clearly as plants from the forest and plants from gaps. Individuals from *S. terebinthifolius* (SG and SF) could not be segregated.

In Fig. 5b, the first axis, which explains 40.01 % of the total variation, shows variables that are more important in separating the groups. Most of these variables that exhibit high factor loadings are related to morpho-anatomical leaf characteristics (Thickness, Succulence, Blade, Palisade, LSM, Chl a/b and Density). Seven variables (Spongy, Adaxial ep., Leaf area, F_v/F_0, F_v/F_m, Cuticle and *NPQ*) were found to be highly associated with the second principal component, which explains 29.32 % of the variation (Fig. 5b).

The different leaf traits (morpho-anatomical and physiological) were compared in order to present the plasticity index (sensu Valladares et al. 2000) (Table 4). The highest median was for a morphological leaf trait (leaf area 0.60) and the lowest was for a physiological trait (F_v/F_m: 0.01). Cuticle, leaf area, F_v/F_0 and carotenoids showed the highest plasticity index among anatomical, morphological and physiological traits, respectively, while sponginess and thickness showed higher disparity.

When comparing species, the highest plasticity index for the morphological and physiological traits was observed in *A. verticillatus* (0.47 and 0.20, respectively), while *S. terebinthifolius* had the lowest (0.27 and 0.15, respectively). For anatomical traits, *S. terebinthifolius* presented the highest plasticity index (0.32) and *M. brevifolia* the lowest (0.24).

## Discussion

Morpho-anatomical plasticity

Leaves of the *S. terebinthifolius* were the THIC, and together with *M. brevifolia* were more SUC than the leaves of *A. verticillatus*. Leaf thickness is related to an improvement in the photosynthetic mesophyll cell layer because high irradiance promotes elongation of the palisade parenchyma and development of more subepidermal layers which causes an increase in thickness (Lambers et al. 1998; Niinemets et al. 1999). High photosynthetic performance requires an improvement in water apportion in leaf tissues, and the succulence of leaves represents a water storage mechanism that possibly promotes the efficient use of water during moments when water is not available (Lamont and Lamont 2000; Schwinning and Ehleringer 2001).

According to Oguchi et al. (2003, 2005), there is a significant relationship between LMA and THIC. Higher LMA and THIC are typically associated with species, genotypes, and phenotypes from xeric or exposed sites (Sobrado 1986; Myers et al. 1987; Abrams 1990). Higher LMA of *S. terebinthifolius* leaves might reflect better efficiency in water absorption by maintaining higher osmotic pressure and stomata conductance (Abrams et al. 1994).

The lowest LA values were found in *S. terebinthifolius* and the highest in *M. brevifolia*. This might represent two microclimatic extremes that these species colonize, where the reduction of leaf area in *S. terebinthifolius* corresponds to an adaptation to counteract the negative effects of overheating and high transpiration rates (Gates 1980). On the other hand, *M. brevifolia* prioritizes an investment in photosynthetic tissues, and, as a consequence, this species maintains a high leaf area to maximize the irradiation flux above the leaves.

The leaves of *A. verticillatus* had the lowest THIC, SUC and LMA and DEN that are commonly related to an increase in fibers and sclereids and thicker cell walls, which promotes changes in tissue elasticity (Niklas 1989) and increases water tolerance limitations (Niinemets 2001). Such modifications, according to Witkowski and Lamont (1991), also promote reduction in herbivory.

Only *A. verticillatus* presented plasticity in leaves that was related to the study sites (Forest and Gap2m), where it adjusted its leaf thickness and LMA in the high irradiance environment of the gap and grew the densest leaves in the forest. The high LMA and thickness of *A. verticillatus* leaves allow for a higher photosynthetic ratio, and for this, consequently, an increase in water flux is required. If the density of the leaves of this species causes a decrease in intercellular conductance of CO_2 (Reich et al. 1999) then
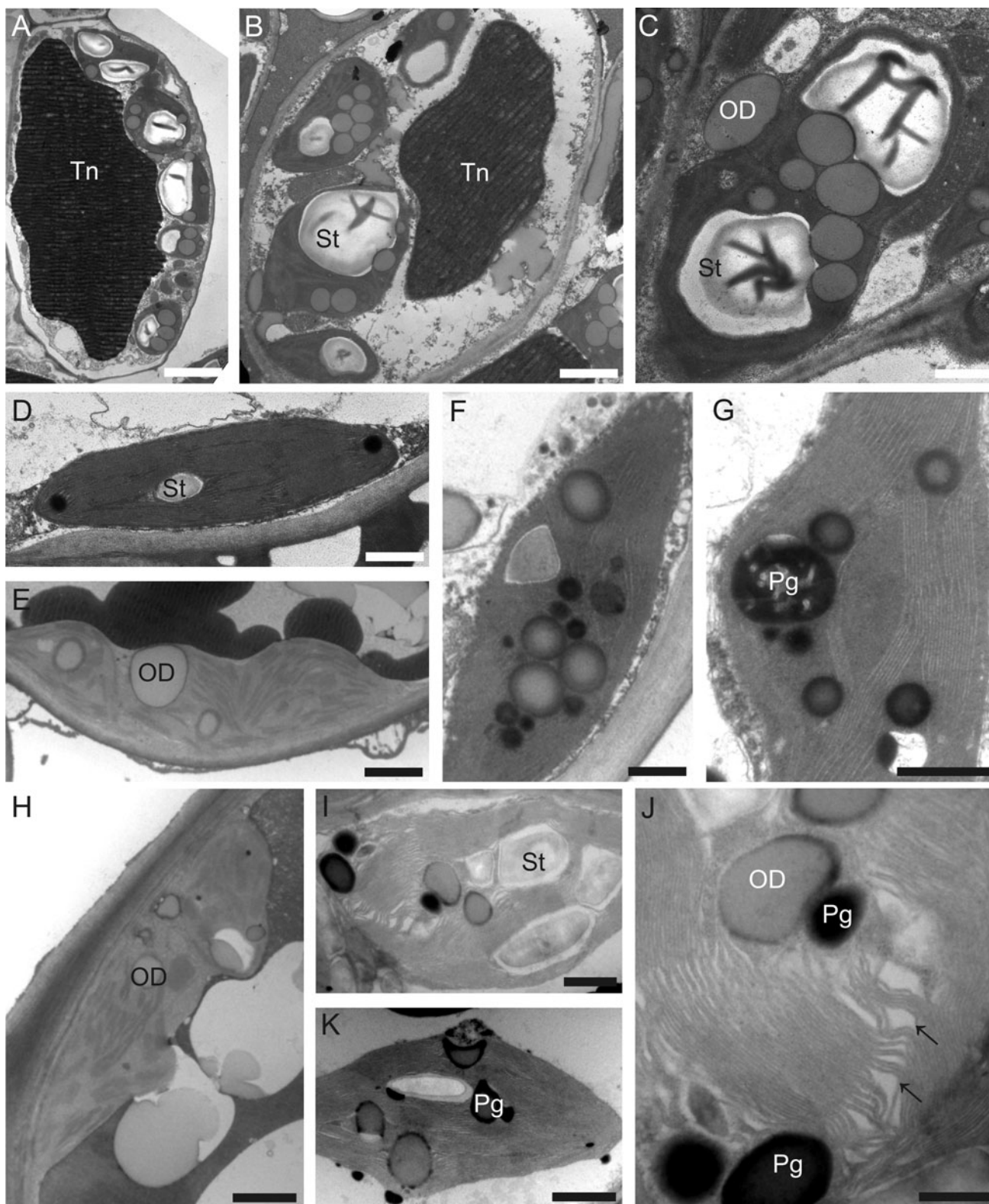
the uptake of water can increase (Niinemets 2001). In addition, the plasticity of the stomata density allows for efficient gas exchange, which counteracts the negative effects of an increase in leaf density.

The presence of thicker cuticles and high stomata density in *S. terebinthifolius* was probably a response to higher exposure to light (Cutler et al. 1982; Bastos et al. 1993; Rôças et al. 1997, 2001), where thicker cuticles act to

◄ **Fig. 3** Leaf chloroplasts from forest and leaves formed after the gap formation. Transmission electron microscopy (TEM) of the mesophyll chloroplasts of *S. terebinthifolius*, *A. verticillatus* and *M. brevifolia* collected in the forest and forest gap, 2 months (Gap2m) after gap formation. *Schinus terebinthifolius* collected in the Forest (**a**) and Gap2m (**b–c**). *Actinostemon verticillatus* collected in the forest (**d–e**) and Gap2m (**f–g**, note the increase of the number and size of oil droplets, plastoglobuli, stroma volume and dislodgement of the stroma). **h** *M. brevifolia* from the forest. **i–k** *M. brevifolia* collected in the Gap2m presented an increase of starch grains, oil droplets and plastoglobuli and disarrangement of the thylakoid membrane (*arrow*), note a content pass through the chloroplast membrane to the lumen cellular (l). *St* Starch grain; *Tn* Tannin; *OD* Oil droplets; *Pg* Plastoblobuli. *Scale bar* **a–b**, **d–e**, **h**, **k** = 2 μm; **c**, **i** = 1 μm; **f–g**, **j** = 500 nm

minimize uncontrolled water loss (Schönherr and Riederer 1989) and high stomata density allows for efficient gas exchange (Lleras 1977), better control of transpiration, and

possibly to avoid excessive water loss (Weyers and Meidner 1990). As with other late-successional species, *M. brevifolia* showed the thinnest cuticle, which is an adjustment to the shady environment of the forest.

The thicker palisade and spongy parenchyma found in *S. terebinthifolius* leaves represents a structural mechanism that maximizes light absorption and carbon fixation. The tubular shape of palisade cells aids in the penetration of direct sunlight, and thicker spongy parenchyma helps scatter the light, thus increasing the chance that light will be absorbed for photosynthesis (Vogelmann and Martin 1993; Vogemann et al. 1996). The palisade and spongy parenchyma are the two main characteristics responsible for an increase in blade thickness and are considered an improvement to the photosynthetic structural mechanism, because thicker mesophyll facilitates CO_2 dissolution into
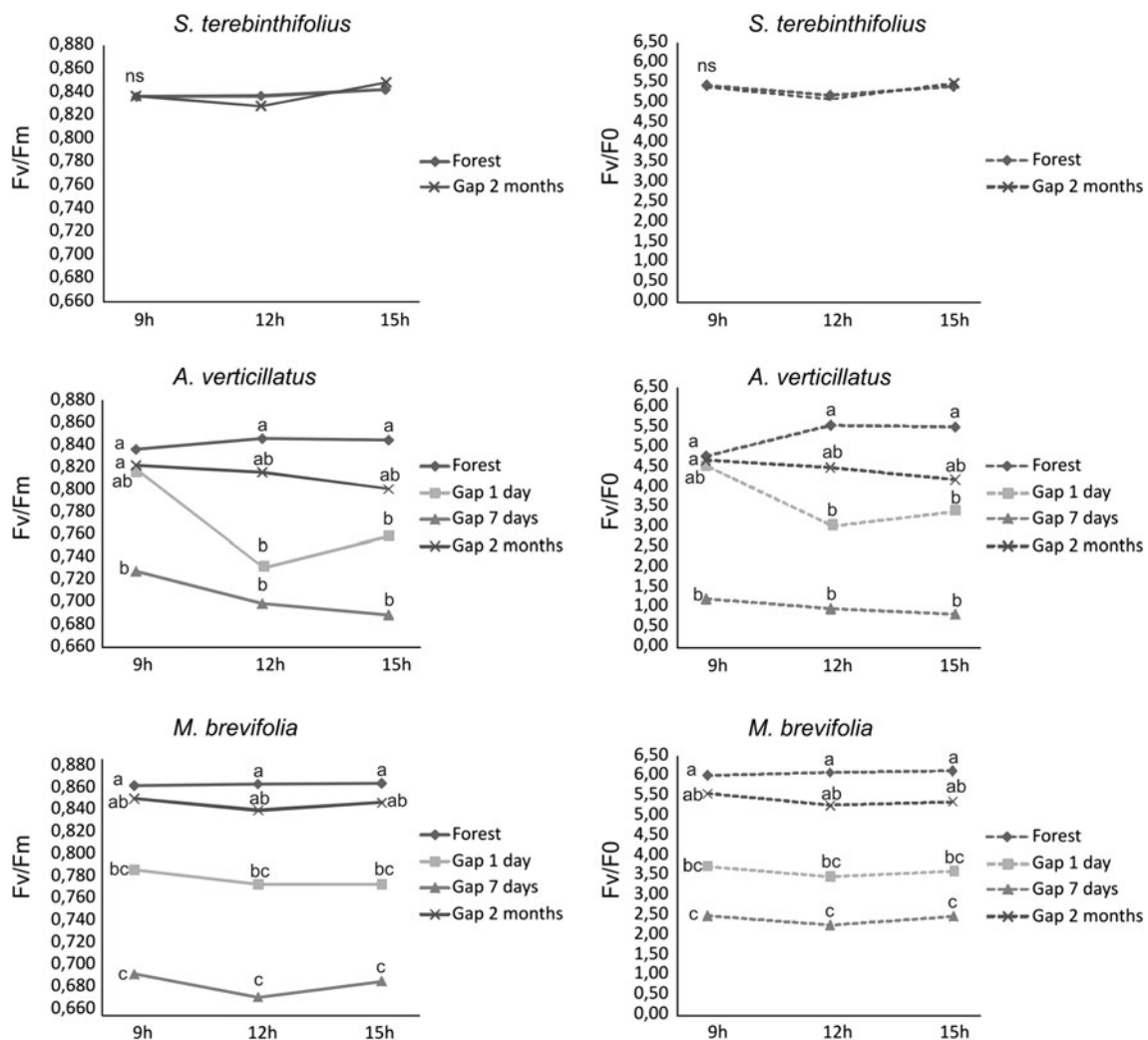

Fig. 4 Photosynthetic efficiency during the acclimation process. Mean values of the maximum quantum yield of photosystem II (F_v/F_m) and the maximum primary yield of photochemistry of photosystem II (F_v/F_0) measured at 9, 12 and 15 h for different traits: forest and forest gap at 1 day (Gap1d), 7 days (Gap7d) and 2 months (Gap2m) after gap formation. *Different letters* indicate significant differences at $p < 0.05$ (HSD Tukey test). *NS* not significantly different

Table 2 Mean and standard deviation of photochemical (*qP*) and non-photochemical quenching (*NPQ*) measured at 9, 12 and 15 h for different traits: forest and forest gap at 1 day (Gap1d), 7 days (Gap7d) and 2 months (Gap2m) after formation

Hour		Traits	*S. terebinthifolius*	*A. verticillatus*	*M. brevifolia*
9 h	*qP*	Forest	0.9303 ± 0.0433*	0.7652 ± 0.1148b	0.7993 ± 0.0273c
		Gap1d	–	0.8233 ± 0.0799ab	0.8457 ± 0.0562bc
		Gap7d	–	0.8048 ± 0.1437ab	0.9610 ± 0.0272a
		Gap2m	0.9485 ± 0.0692*	0.8920 ± 0.0376a	0.8769 ± 0.0253b
	NPQ	Forest	0.7660 ± 0.0440*	0.5244 ± 0.0544b	0.5226 ± 0.0519b
		Gap1d	–	0.7386 ± 0.1017a	0.6986 ± 0.0750a
		Gap7d	–	0.7246 ± 0.0548a	0.7618 ± 0.0684a
		Gap2m	0.7521 ± 0.0328*	0.6239 ± 0.0670b	0.6921 ± 0.0290a
12 h	*qP*	Forest	0.8841 ± 0.0364*	0.7833 ± 0.0915*	0.7721 ± 0.0322b
		Gap1d	–	0.8928 ± 0.0660*	0.8819 ± 0.0421a
		Gap7d	–	0.8484 ± 0.2027*	0.8912 ± 0.0764a
		Gap2m	0.8968 ± 0.8841*	0.8809 ± 0.0466*	0.8524 ± 0.0252a
	NPQ	Forest	0.8103 ± 0.0355*	0.5083 ± 0.0957b	0.4907 ± 0.0305b
		Gap1d	–	0.6607 ± 0.0412a	0.6827 ± 0.0787a
		Gap7d	–	0.6900 ± 0.0619a	0.7621 ± 0.0781a
		Gap2m	0.8051 ± 0.0304*	0.6719 ± 0.0591a	0.7181 ± 0.0660a
15 h	*qP*	Forest	0.8559 ± 0.0499*	0.8133 ± 0.0841ab	0.7350 ± 0.0375b
		Gap1d	–	0.6546 ± 0.1084b	0.6984 ± 0.0805b
		Gap7d	–	0.6433 ± 0.2308b	0.7871 ± 0.0879ab
		Gap2m	0.8702 ± 0.0364*	0.8806 ± 0.0368a	0.8456 ± 0.0162a
	NPQ	Forest	0.7534 ± 0.0610*	0.5113 ± 0.0869b	0.4939 ± 0.0480b
		Gap1d	–	0.5407 ± 0.1045ab	0.6229 ± 0.0962a
		Gap7d	–	0.6069 ± 0.1093ab	0.6750 ± 0.1229a
		Gap2m	0.7421 ± 0.0408*	0.6587 ± 0.0578a	0.7106 ± 0.0593a

Mean ± SD (ANOVA, $p < 0.05$). Different letters within columns indicate significant differences at $p < 0.05$ (HSD Tukey test)

(*) Not significantly different, (–) Not measured

the water within the cell wall, which decreases the resistance to CO_2 diffusion from the intercellular space to the chloroplast stroma (Evans 1999; Terashima et al. 2001). *M. brevifolia* also presented high leaf blade values, mainly because of the thickness of the spongy parenchyma. These results associated with the higher SUC values could corroborate with the hypothesis of adaptation mechanisms with investments in tissues that inhibit water shortage and maximize gas exchange.

Ultrastructure, chlorophyll *a* fluorescence and pigments

It is known that changes in the grana organization of the thylakoids, an increase in stroma volume, oil droplets and plastoglobuli are commonly related to plants subjected to environmental conditions that increase oxidative stress on the photosynthetic apparatus (Molas 2002; Vitória et al. 2003, 2006; Munné-Bosch and Alegre 2004; Austin et al. 2006; Lage-Pinto et al. 2008). All of these ultrastructural changes were observed in the *A. verticillatus* and *M. brevifolia* chloroplasts from the Gap2m site. *M. brevifolia* also

showed a noticeable increase of starch grains and plastoglobuli content. According to Molas (2002), disturbances and inhibitions of carbohydrate transport from leaves to the other parts of plant were accompanied by an increase in plastoglobuli content.

The F_v/F_m and F_v/F_0 of the *M. brevifolia* revealed that maximum stress occurred 7 days after the gap formed, and that the photosynthetic process stabilized after 2 months (Gap2m). A similar response occurred for *A. verticillatus,* but this species showed more sensitivity at 12:00 p.m., suggesting a "midday depression" of net CO_2 uptake, which is observed when the photon flux density (PFD) and leaf temperatures reached their daily maximum (Muraoka et al. 2000; Franco and Lüttge 2002; Lichtenthaler et al. 2005). In addition, thin leaves of *A. verticillatus* did not allow an efficient dissipation of large amounts of energy that occur in forest gaps.

According to Jakl and Bolhar-Nordenkampf (1991), values of F_v/F_m between 0.75 and 0.85 reflect an efficient photosynthetic apparatus, while a decrease in the F_v/F_0 ratio is an indication of structural damage, which occurs in the

Table 3 Mean and standard deviation of photosynthetic pigments measured at 1200 hours of different sites: forest and a forest gap at 2 months (Gap2m) after your formation

Photosynthetic pigments	Traits	*S. terebinthifolius*	*A. verticillatus*	*M. brevifolia*
Chl a/b	Forest	20.85 ± 0.23*	12.74 ± 0.15*	11.77 ± 0.19b
	Gap2m	20.36 ± 0.20*	14.99 ± 0.19*	19.51 ± 0.12a
Carotenoids	Forest	153.80 ± 26.87*	178.74 ± 21.63*	145.13 ± 24.33*
	Gap2m	151.92 ± 24.06*	196.93 ± 34.05*	143.04 ± 17.92*

Mean ± SD (ANOVA, $p < 0.05$). Different letters within columns indicate significant differences at $p < 0.05$ (HSD Tukey test)

(*) Not significantly different

thylakoids and affects the photosynthetic transport of electrons (Vernay et al. 2007). The trend of lower F_v/F_m and F_v/F_0 in *A. verticillatus,* and mainly in the late-successional *M. brevifolia* in the early stage of gap formation, suggests problems in photosynthetic performance (Krause and Weis 1991; Krause et al. 2001) or a lower efficiency of PSII when exposed to high irradiance (Critchley and Russell 1994).

The reduction state of the primary stable quinone acceptor of PSII can be estimated as photochemical quenching (*qP*). The *qP* values for *A. verticillatus* and *M. brevifolia* increased in the exposed environments of forest gaps, where large amounts of absorbed energy were driven to photochemical reactions of photosynthesis (Krause and Weis 1991; Bolhàr-Nordenkampf and Öquist 1993).

Non-photochemical quenching (*NQP*) monitors the constant rate of heat loss. The increase in NPQ of *A. verticillatus* and *M. brevifolia* may indicate a control mechanism in the thylakoid membrane that adjusts thermal dissipation of excess excitation energy that exists because there is more light in a gap (Demmig-Adams et al. 1996).

The chlorophyll *a/b* ratio of the *M. brevifolia* also had higher values in the gap, and, according to Brugnoli et al. (1998), *NPQ* is linearly related to the chlorophyll *a/b* ratio. A higher chlorophyll *a/b* ratio may indicate a higher ratio of PSI to PSII, which was observed by Takabayashi et al. (2005) as an adaptive stress mechanism.

The ultrastructural (*M. brevifolia*) and morpho-anatomy (*A. verticillatus*) differences found between the Gap2m and forest sites, and mainly the consequent stabilization in photosynthesis after 2 months, suggest some acclimation mechanisms that were sufficient enough to maintain the normal photosynthetic process.

The multivariate analysis distinguished the three species and two sub-clusters formed by the intraspecific variation between *A. verticillatus* and *M. brevifolia,* mostly as a consequence of the morpho-anatomical differences of leaf traits. The individuals of *S. terebinthifolius* could not be segregated. The plasticity index analysis (Valladares et al. 2000) was performed to simplify the comparison of leaf responsiveness to gap formation among the three species.
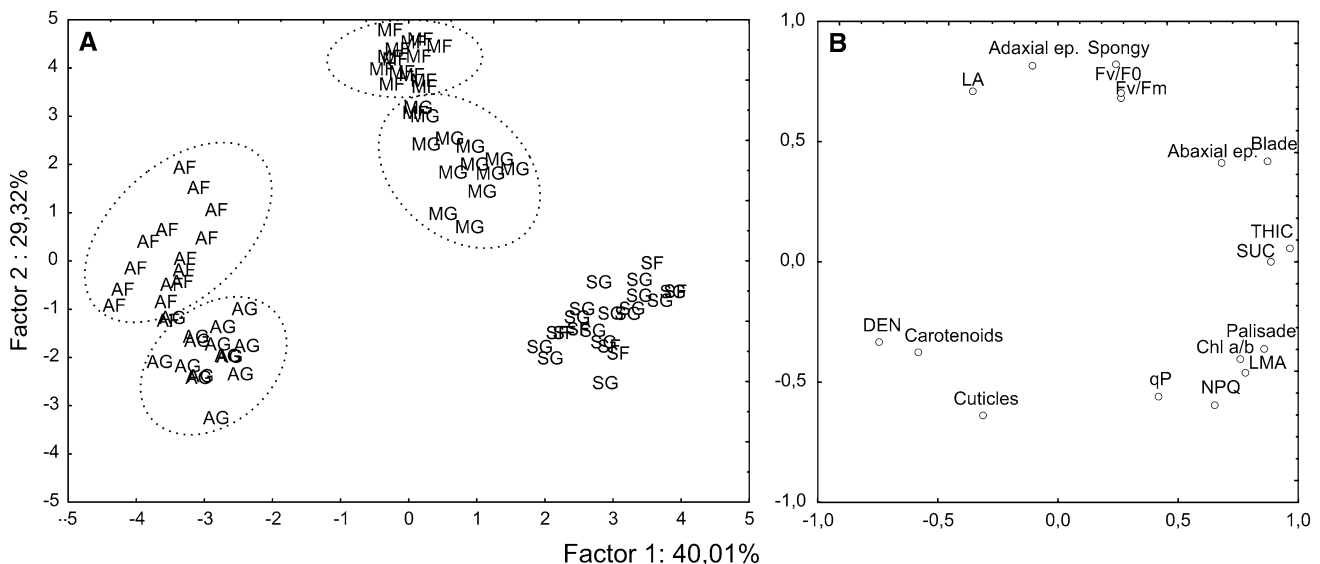

Fig. 5 Principal component analysis of *S. terebinthifolius* from the forest (*SF*) and Gap2m (*SG*); *A. verticillatus* from the Forest (*AF*) and Gap2m (*AG*); *M. brevifolia* from the forest (*MF*) and Gap2m (*MG*). **a** Projection of the species on the factor-plane. **b** Projection of the variables on the factor-plane

Table 4 Median of the phenotypic plasticity index for the anatomical, morphological and physiological traits of *S. terebinthifolius* (St), *A. verticillatus* (Av), *M. brevifolia* (Mb) species

Plasticity index	St	Av	Mb
Anatomical traits			
Cuticle (Adaxial)	0.45	0.38	0.40
Adaxial ep.	0.34	0.31	0.18
Abaxial ep.	0.28	0.30	0.30
Palisade	0.30	0.23	0.22
Spongy	0.40	0.14	0.11
Blade	0.09	0.06	0.05
Anatomical median	**0.32**	**0.26**	**0.24**
Morphological traits			
Thickness	0.16	0.50	0.22
Succulence	0.30	0.33	0.48
LSM	0.17	0.18	0.31
Density	0.28	0.56	0.35
Leaf area	0.47	0.60	0.55
Morphological median	**0.27**	**0.47**	**0.36**
Physiological traits			
F_v/F_m	0.05	0.05	0.01
F_v/F_0	0.25	0.25	0.10
qP	0.06	0.06	0.07
NQP	0.09	0.09	0.16
Carotenoids	0.33	0.33	0.29
Chl a/b	0.24	0.24	0.17
Physiological median	**0.15**	**0.20**	**0.18**

Bold case means the total median for traits

Higher morphological and physiological plasticity in the early successional *A. verticillatus* suggests two important adaptations to high irradiance in this species; morphological plasticity implies an efficient capacity to resource acquisition of the plant (Crick and Grime 1987) and higher physiological plasticity is related to a greater capacity to exploit high light environments (Strauss-Debenedetti and Bazzaz 1991; Yamashita et al. 2000). In comparison to *A. verticillatus,* the lower anatomical plasticity in the late-successional *M. brevifolia,* in association with higher costs of leaf construction and maintenance (higher SUC), revealed limited leaf acclimation. This suggests that this species, as other later secondary, has a metabolism adapted to constantly low levels of flux resource (Bazzaz and Pickett 1980; Strauss-Debenedetti and Bazzaz 1991; Rabelo et al. 2012).

Conclusions

This study, based on field conditions, describes the leaf morpho-anatomy, ultrastructure and ecophysiology of tree species of different successional status, and how the status can favor the acclimation of species to forest gaps. All of the features studied reinforce how the pioneer species *S. terebinthifolius* is adjusted well to open environments; however, the absence of this species just inside the forest edge suggests other abilities are required, especially for germination and seedling establishment, to grow in the forest (Whitmore 1989, 1996).

Higher morphological and ecophysiological plasticity of the early successional *A. verticillatus* reveals the opportunistic behavior of this species to take advantage of high light environments when a forest gap is created. The differences in leaf acclimation of *A. verticillatus* suggest an investment in mechanisms that promote more efficient photosynthesis by increasing leaf thickness and LMA, and in water absorption by increasing leaf density at the expense of gas exchange, which is compensated by a high density of stomata. *M. brevifolia* compensates for the higher costs of leaves and the lowest leaf plasticity with ultrastructural changes that adjust the photosynthetic process and this, consequently, promotes shorter leaf pay-back time (time required to compensate for leaf construction) (Niinemets 2001). Complementary greenhouse studies of plastic response to single types of stress, such as irradiance or water stress, might shed additional light on the adaptive strategies of these species.

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