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Using leaf $\delta^{13}C$ and photosynthetic parameters to understand acclimation to irradiance and leaf age effects during tropical forest regeneration

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ABSTRACT

Regenerating forests are important for the maintenance of tropical biodiversity. Forest management in fragments of Atlantic forest in Brazil includes removal of exotic eucalyptus trees that were once part of plantations, in order to reestablish native flora. However, it is unclear how native tree saplings regenerating under former plantations respond to abrupt changes in environmental conditions associated with exotic tree removal. We used leaf carbon isotope composition ($\delta^{13}C$) and photosynthetic parameters to evaluate physiological responses of native tree saplings to canopy opening. We analyzed young and mature leaves of the three most representative species of regenerating trees (*Byrsonima sericea*, *Siparuna guianensis*, *Xylopia sericea*) in one secondary forest fragment and three managed areas that form an irradiance gradient (9, 85, 230 and 550 μmol m^{-2} s^{-1}) in Brazilian Atlantic forest. Eucalyptus removal increased photosynthetic CO_2 assimilation and stomatal conductance in mature leaves of all species, but there was no change in intercellular CO_2 concentration. In young leaves, two species showed increasing *A* and one species showed increasing g_s in response to canopy opening. Leaf $\delta^{13}C$ did not vary significantly among species, but site and age affected $\delta^{13}C$, as leaves from shaded sites showed lower $\delta^{13}C$ values (around −33‰) than leaves from lighter sites (around −30‰), and young leaves showed higher $\delta^{13}C$ values (around −30‰) than mature leaves (around −32‰). Mature leaves showed greater photosynthesis and stomatal conductance than young leaves. The sensitivity of young leaf $\delta^{13}C$ to irradiance increases suggests that $\delta^{13}C$ in these organs is controlled not only through carbon imported to new leaves during growth, but also through direct responses of stomatal control and carboxylation as these young leaves develop their photosynthetic competency. Young and mature leaves showed decreased total chlorophyll/carotenoids with increasing irradiance, indicating acclimation capacity from early developmental phases. Young leaves in high irradiance sites showed susceptibility to irradiance stress (F_v/F_m around 0.7), but values for mature leaves did not show high irradiance stress (F_v/F_m around 0.8). In conclusion, forest management affected leaf $\delta^{13}C$ of the main regenerating understory species, with site effects being more important than species-specific features for photosynthetic performance. The data also indicate that these species are resilient to forest management that includes exotic eucalyptus canopy tree removal. In this context, carbon stable isotopes can be considered as recorders of ecological change and can be used to study the effects of management on forest regeneration and photosynthetic competency.

1. Introduction

Secondary forests are an important element of the forest biome in tropical landscapes and have been recognized for their biodiversity and conservation values, environmental services, and carbon sequestration potential (Bongers et al., 2015; Thompson et al., 2012). Secondary forests are defined as woody vegetation which develop where original vegetation had been destroyed by human activity (Finegan, 1992). In much of the tropics, mature native forest has been reduced to a fraction of its original extent, and secondary forest fragments have become an increasing

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proportion of total tropical forest cover (Bongers et al., 2015; Thompson et al., 2012; Wright and Muller-Landau, 2006). Yet, such secondary forest fragments sometimes contain a high diversity of species, so preserving even small forest fragments and connecting them through restored vegetation corridors is fundamental to the maintenance of biodiversity in disturbed tropical habitats (Morellato and Haddad, 2000).

A closer look at disturbed, degraded and secondary forests in the tropics reveals that in many cases, secondary forest fragments contain remnants of exotic species from former commercial plantations of eucalyptus and pine, in order to give financial return to the landowners (Baptista and Rudel, 2006). In other parts of the tropics, large areas have been reforested with fast-growing monocultures of a few exotic tree species to rapidly revegetate massively deforested watersheds (Lugo, 1988). In some of these cases, it was later found that early invasion of native species into the understory of exotic tree plantations in Central America and the Caribbean have the potential to facilitate forest restoration (Guariguata et al., 1995; Parrotta et al., 1997). Part of this phenomenon is due to the potential for exotic trees to act as nurse trees to native species regenerating in the understory (Feyera et al., 2002), through the maintenance of appropriate microenvironments with regard to irradiance, temperature, and relative humidity. Secondary forest regeneration can be similarly facilitated through mixed and pure stands of native species (Haggar et al., 1997). Therefore, as previous plantations are converted to new conservation areas, the environmental conditions that are created at various stages of this transition may have a large impact on the trajectory of forest development. Such conservation practices often involve removal of exotic tree species, leading to rapid changes in microclimatic conditions, including increased irradiance, vapor pressure deficit (VPD), and decreased soil moisture (Camargo and Kapos, 1995; Didham et al., 1999). Plants can acclimate to changing environmental conditions by adjusting their metabolism and performance (Goldstein et al., 2016), but it is not completely clear how these changes are manifested by previously suppressed trees and how such changes affect the ability of the forest to regenerate.

Studies concerning photosynthetic responses to irradiance, carried out in degraded or managed secondary forest, gaps or along irradiance gradients, have generally shown functional and structural acclimatation responses (Lage-Pinto et al., 2012; Rabelo et al., 2013; Silva et al., 2010; Teixeira et al., 2015; Vieira et al., 2015). Leaf functional responses to environmental variation can be evaluated as a time-integrated response of leaf carbon isotope composition ($\delta^{13}C$) (Dawson et al., 2002; Duarte et al., 2005; Leavitt and Long, 1986; Zimmerman and Ehleringer, 1990), because the supply of CO_2 at the site of carboxylation determines discrimination against $^{13}CO_2$ relative to $^{12}CO_2$ during photosynthesis (Farquhar and Richards, 1984). Under low irradiance conditions and abundant water, photosynthesis and consequently intercellular CO_2 concentration (C_i) depend strongly on irradiation (Pearcy and Pfitsch, 1991). When irradiance is low, the depletion of C_i by photosynthesis is low, so C_i remains relatively high favoring discrimination against $^{13}CO_2$ and resulting in ^{13}C-depleted leaf tissue (Duursma and Marshall, 2006; Terwilliger, 1997; Zimmerman and Ehleringer, 1990). In contrast, when irradiance availability is high, photosynthesis increases and C_i is reduced, limiting discrimination against $^{13}CO_2$ and resulting in ^{13}C-enriched tissue (Farquhar et al., 1989; Martinelli et al., 2009).

There are two other aspects that regulate leaf $\delta^{13}C$. The first is the $\delta^{13}C$ of source CO_2 for photosynthesis (Medina and Minchin, 1980; Domingues et al., 2005). It is well known that there is a vertical distribution of $\delta^{13}C$ of CO_2 in forested ecosystems (Medina and Minchin, 1980; Domingues et al., 2005), and that this is partly due to carbon that is derived from soil respiration with relatively depleted $\delta^{13}C$ values (around −27‰), compared to ambient air which is relatively enriched (−8‰) (Buchmann et al., 1997). Thus leaf material within 1–2 m of the soil surface tends to show more negative $\delta^{13}C$ values than leaves higher in the canopy (Domingues et al., 2005). The second aspect that can affect leaf $\delta^{13}C$ is fractionation within the plant as carbon moves among organs. Higher $\delta^{13}C$ in non-photosynthetic organs than in photosynthetic organs has been shown and suggests that as carbon is exported from autotrophic organs there is fractionation (Cernusak et al., 2009; Franco et al., 2005; Terwilliger, 1997). However, no difference between $\delta^{13}C$ in autotrophic and heterotrophic tissues, such as leaves and roots (Duarte et al., 2005; Franco et al., 2005) and between young and mature leaves (Sobrado, 2008; Terwilliger, 1997) has been observed as well. Species-specific variation in leaf expansion rates, use of imported carbon, and photosynthetic activity during leaf expansion could explain, in part, variation in leaf $\delta^{13}C$ values found in young leaves (Cernusak et al., 2009).

We evaluated photosynthetic performance and leaf $\delta^{13}C$ in young and mature leaves in the three most abundant species (Evaristo et al., 2011), along an irradiance gradient in managed parcels of Brazilian Atlantic forest. The Atlantic forest of Brazil contains one of the most biodiverse and highly endemic floras in the world (Guedes-Bruni et al., 2009; Murray-Smith et al., 2009; Mutke and Barthlott, 2005). Originally occupying 150 million ha, today the Brazilian Atlantic forest has been reduced to 12–15% of its original extent (Ribeiro et al., 2009), 30–40% of which is secondary forests in early or medium stages of succession, combined with small fragments (<50 ha) and few large fragments (Morellato and Haddad, 2000). Four main questions guided our study: (1) Does forest management affect leaf $\delta^{13}C$ of regenerating understory vegetation? (2) Do the main regenerating species differ in their carbon isotope responses to prescribed canopy opening? (3) Do young and mature leaves show different responses of leaf $\delta^{13}C$ and photosynthesis to forest management? (4) How do the physiological characteristics of the main species of the regenerating understory affect the trajectory of forest regeneration?

2. Material and methods

2.1. Study area, species, and sampling period

This study was carried out in União Biological Reserve (ReBio União), state of Rio de Janeiro, Brazil (22°27′30″S, 42°02′15″W). ReBio União is 2550 ha with approximately 2200 ha of Atlantic forest and 220 ha of historical eucalyptus plantations (*Corymbia citriodora*). The vegetation has lowland and submontane formations and is classified as dense tropical rainforest (IBGE 2012). The climate in the region is tropical humid (*Aw* in the Köppen, 1948 climate classification), with an average annual temperature of 25 °C. Annual precipitation is 1920 mm with 85% occurring between October and April. The four areas studied present differences in management and preservation, forming a gradient in understory irradiance availability (Table 1, SM 1).

Study plants included *Siparuna guianensis* Aubl. (early secondary tree), Siparunaceae, *Xylopia sericea* A. St.-Hill (pioneer tree), Annonaceae, and *Byrsonima sericea* D.C. (pioneer tree), Malpighiaceae (Lorenzi, 2000). These species are among the most abundant species in the regenerating and management areas (Evaristo et al., 2011), and were found across all four study sites except *B. sericea*, which was found only in US 2 and US 3. These species also belong to the understory of the secondary forest site, but in lesser abundance than in the others three sites (Evaristo et al., 2011).

Study plant height ranged from 1 to 3 m. In each area surveyed, thirty individuals (ten of each species) were identified and monitored monthly, for seven months (May to November 2014). In May 2014, ends of branches were marked and in November

Table 1
Characterization of the four study sites in União Biological Reserve, Brazil, in November 2014. US: understory. – not applicable.

	Forest	US 1	US 2	US 3
Mean irradiance (μmol m^{-2} s^{-1})[a]	9	85	230	550
Canopy coverage (%)[b]	95.4	78.7	54.4	34.6
Eucalyptus presence	No	Yes	Yes	No
Eucalyptus trees spaced (m)	–	2 × 2	6 × 6	–
Understory developed conditions	Dense vegetation	Well-developed vegetation	Sparse vegetation	Sparse vegetation
Distance to the secondary forest (km)	–	0 (neighboring)	2	5
Time between eucalyptus removal and measurement (months)	–	–	5	19
Eucalyptus removal Management	No management	No management	Partial eucalyptus removal	Complete eucalyptus removal

[a] and [b]: values obtained at 1.30 m.
[a] Canopy cover was measured on a scale of 0–100% with a spherical densiometer (*Model-A*, *Forestry Suppliers, Inc.*, USA) at 10 points in each cardinal direction at study plants between 11:00 and 13:00 h on one sunny day.
[b] Irradiance was measured at 30 points near each study plant between 11:00 and 13:00 h on one sunny using a quantum sensor (*Li-190* coupled to *Li-250 A*, *Li-Cor*, USA).

2014, young (first pair) and mature (third pair) leaves were collected for analysis (SM 2). These young and mature leaves were formed between May and November 2014 except in the forest site where no leaves were formed in this period.

2.2. Leaf traits: Photosynthesis and Specific leaf area (SLA)

Six individuals of each species were analyzed in each area in November 2014. For each individual, one healthy leaf of the first (young) and third (mature) pair, were analyzed (SM 2). A portable infrared gas analyzer (CIRAS-2, PP Systems, UK) was used to measure net photosynthetic rate of 170 mm^2 of leaf under controlled chamber conditions of 25 °C, 80% relative humidity, 1600 μmol m^{-2} s^{-1} irradiance, and 400 μmol mol^{-1} CO_2 between 0730 and 1130 h, a period corresponding to maximum gas exchange rates. The parameters evaluated were photosynthetic rate per area (A_{area}; μmol CO_2 m^{-2} s^{-1}), internal CO_2 concentration (C_i; μmol mol^{-1}), stomatal conductance (g_s; mol m^{-2} s^{-1}), and transpiration (E; mmol m^{-2} s^{-1}).

Between 10 and 30 young and mature leaves per individual were collected for analysis of SLA, which involved measurement of area with a leaf area meter (Li-3100, Li-Cor, USA), drying at 60 °C for at least two days in a forced air circulation oven (MA 35, Marconi, Brazil) and measurement of dry mass on an analytical balance (AY 220, Shimadzu, Japan). SLA was calculated as leaf area per leaf mass. Photosynthetic rate per mass (A_{mass}) was calculated as A_{area} × SLA × 0.1 and was expressed in nmol CO_2 g^{-1} s^{-1}.

2.3. Chlorophyll a fluorescence

Chlorophyll fluorescence was measured in November 2014 on ten individuals, six of which were the same individuals and leaves used in photosynthetic measurements. Chlorophyll *a* fluorescence measurements were carried out between 1100 and 1300 h using a pulse amplitude modulation fluorimeter (FMS 2, Hansatech Instruments Ltd., Norfolk, UK). Young and mature leaves were dark acclimated with leaf-clips for 30 min before leaf surfaces were exposed to modulated weak far-red irradiance, approximately 6 μmol m^{-2} s^{-1} at 660 nm, followed by 0.8 s exposure to high-intensity (10,000 μmol m^{-2} s^{-1}) actinic white irradiance, according to Genty et al. (1989). The variables determined were: (1) maximal quantum yield of PSII (F_v/F_m); (2) quantum efficiency of photosystem II (Φ_{PSII}); (3) photochemical quenching (qP); (4) non-photochemical quenching (NPQ).

2.4. Photosynthetic pigments

Pigments were measured on 0.502 cm^2 disks punched from ten young and mature leaves of each species in November 2014 in each of the four study areas. The leaves used were the same as those used in photosynthetic and Chlorophyll *a* fluorescence measurements. The disks were kept in plastic tubes containing 5 mL of organic solvent (dimethyl sulfoxide, DMSO) protected from irradiance for 5 days. After this time, the organic solvents were analyzed in a spectrophotometer (TCC-240A, Shimadzu, Japan) at 480, 649, and 665 nm wavelengths. All laboratory procedures were carried out under a low-irradiance environment. Chlorophyll *a* (Chl *a*), Chl *b*, carotenoids (Car), total Chl ($a + b$), and the Chl a/b ratio were calculated according to Wellburn (1994).

2.5. Carbon stable isotope analyses

We collected young and mature leaves from 5 to 6 individuals per species. Leaves were dried at 60 °C for at least 2 days as described above and ground. Samples of *c.*1 mg were combusted on a continuous flow elemental analyzer (Carlo Erba, Milan, Italy) coupled to a stable isotope ratio mass spectrometer (IRMS Delta Plus, Finnigan Mat, San Jose, CA, USA) at the Laboratory of Isotope Ecology (Centro de Energia Nuclear na Agricultura, Piracicaba, Brazil). Carbon stable isotope values are reported in "delta" notation as δ values in parts per thousand (‰):

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R is the molar ratio of heavy to light carbon isotope ($^{13}C/^{12}C$), and R_{sample} is compared against a Pee Dee Belemnite (PDB) standard ($R_{standard}$). The precision of the measurements was 0.2‰. The analytical error was 0.05‰, calculated based in 10 analyses of a working standard (sugarcane) included at each 11 samples.

2.6. Statistical analyses

Differences between means were assessed by MANOVA (Statistica 6.0) followed by Tukey's test ($P \leqslant 0.05$). The factors considered were species, site, and age. Regression coefficients and correlations were calculated with Sigma Plot 11.0 software package (SPSS; Chicago, IL, USA).

3. Results

3.1. Responses of photosynthesis and leaf $\delta^{13}C$ to irradiance, leaf age and species

There was a tendency of increases in A, E and g_s with increasing irradiance for all species (Fig. 1). Intercellular CO_2 concentration (C_i) did not vary significantly with irradiance. Photosynthetic rate per area (A_{area}) differed significantly between leaf ages (Fig. 1), with higher A_{area} values observed in mature leaves for all sites

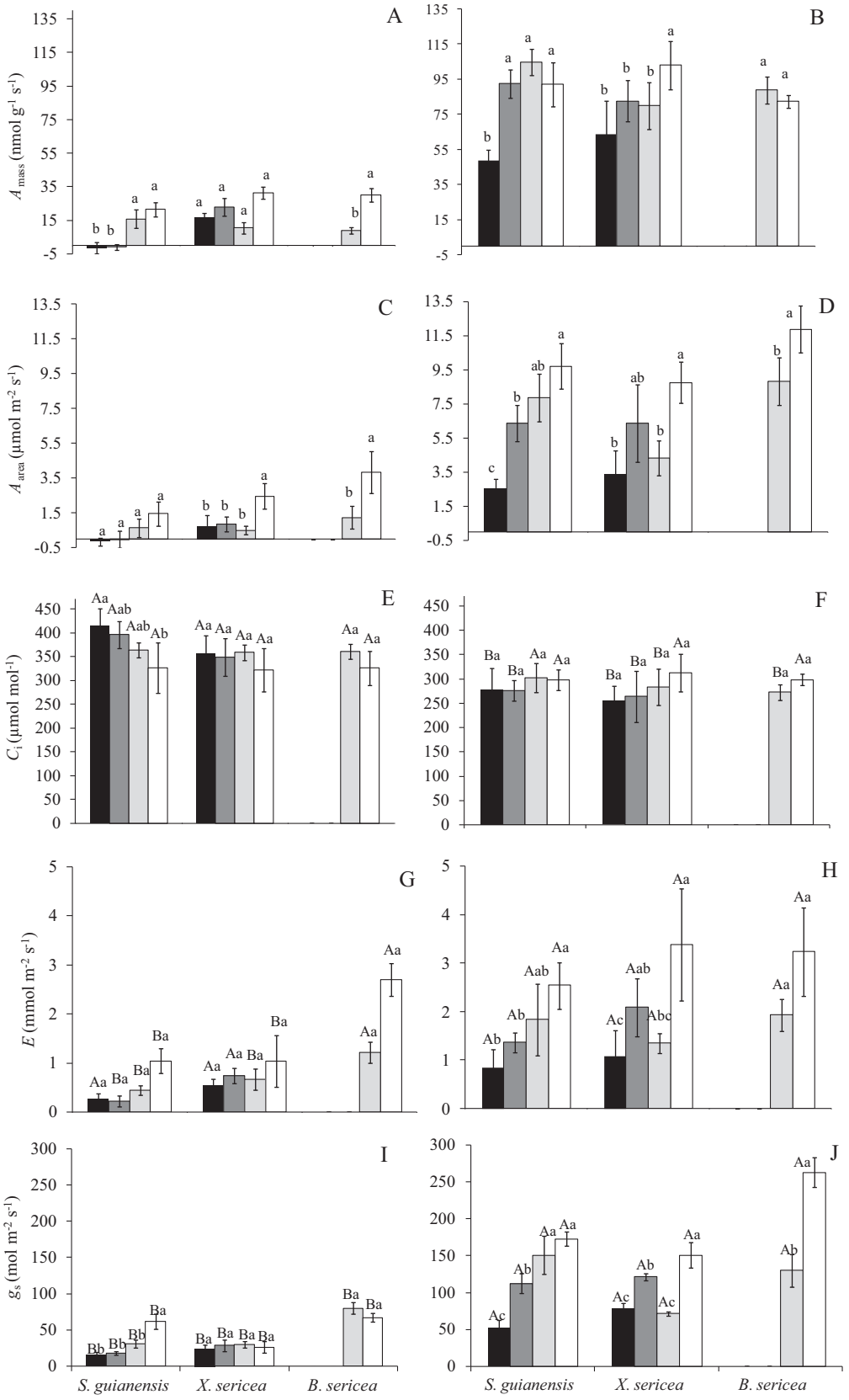

Fig. 1. Gas exchange of *Siparuna guianensis*, *Xylopia sericea*, and *Byrsonima sericea* in young (left column) and mature (right column) leaves in União Biological Reserve, Brazil, in November 2014. (A and B) photosynthetic rate per mass (A_{mass}, nmol g^{-1} s^{-1}), (C and D) photosynthetic rate per area (A_{area}, µmol m^{-2} s^{-1}), (E and F) internal CO_2 concentration (C_i, µmol mol^{-1}), (G and H) transpiration (E, mmol m^{-2} s^{-1}), (I and J) stomatal conductance (g_s, mol m^{-2} s^{-1}). Black: forest (9 µmol m^{-2} s^{-1}), dark grey: US 1 (85 µmol m^{-2} s^{-1}), light grey: US 2 (230 µmol m^{-2} s^{-1}), and white: US 3 (550 µmol m^{-2} s^{-1}). US: understory. Upper case letters compare the ages. Lower case letters compare the sites. The data represent mean ± standard error. Distinct letters indicate significant differences ($P \leqslant 0.05$). Lack of upper case letters indicates no significant differences for ages. n: 6.

Table 2
Relationship between photosynthetic rate per area (A_{area}, μmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}); and photosynthetic rate per area (A_{area}, μmol m^{-2} s^{-1}) and internal CO_2 concentration (C_i, μmol mol^{-1}) for young and mature leaves of *Siparuna guianensis*, *Xylopia sericea*, and *Byrsonima sericea* in four sites that form an irradiance gradient in União Biological Reserve, Brazil, in November 2014. Bold and italic types indicate significant correlations ($P \leqslant 0.05$). n: 6.

		A_{area} x g_s		A_{area} x C_i	
		Young	Mature	Young	Mature
Siparuna guianensis	r	0.843	0.886	−0.731	0.229
	P	***<0.001***	***<0.001***	***<0.001***	0.318
Xylopia sericea	r	0.117	0.752	−0.538	0.123
	P	0.613	***<0.001***	***0.012***	0.594
Byrsonima sericea	r	0.622	0.840	−0.673	0.609
	P	***0.041***	***0.001***	***0.023***	***0.047***

Table 3
Linear regression between $\delta^{13}C$ (‰) and photosynthetically active radiation (PAR) for young and mature leaves of *Siparuna guianensis*, *Xylopia sericea*, and *Byrsonima sericea* in four sites that form an irradiance gradient in União Biological Reserve, Brazil, in November 2014. Bold and italic types indicate significant correlations ($P \leqslant 0.05$). Equation y: mx + b. n: 6.

		$\delta^{13}C$ x PAR	
		Young	Mature
Siparuna guianensis	r^2	0.431	0.551
	P	***0.001***	***<0.001***
	m	0.00276	0.00418
Xylopia sericea	r^2	0.487	0.646
	P	***<0.001***	***<0.001***
	m	0.00286	0.0058
Byrsonima sericea	r^2	0.528	0.495
	P	***0.011***	***0.016***
	m	0.00417	0.00431

and species. However, C_i values were higher in young than in mature leaves in more shaded sites (forest and US 1) for *S. guianensis* and *X. sericea* and in US 2 for *B. sericea*. Photosynthetic rate per area (A_{area}) was positively correlated with g_s in most cases, except in young leaves of *X. sericea* (Table 2), and negatively correlated with C_i in all young leaves for all species and only positively correlated in mature leaves of *B. sericea* (Table 2).

Young and mature leaves showed an increase in $\delta^{13}C$ values with increasing irradiance (Fig. 2). In general, samples from US 3, the understory site with highest irradiance, showed a tendency for greater $\delta^{13}C$ values, with young leaves ranging between −28.79‰ and −30.76‰ (mean of −29.80‰), and mature leaves ranging between −29.07‰ and −31.74‰ (mean of −30.32‰).

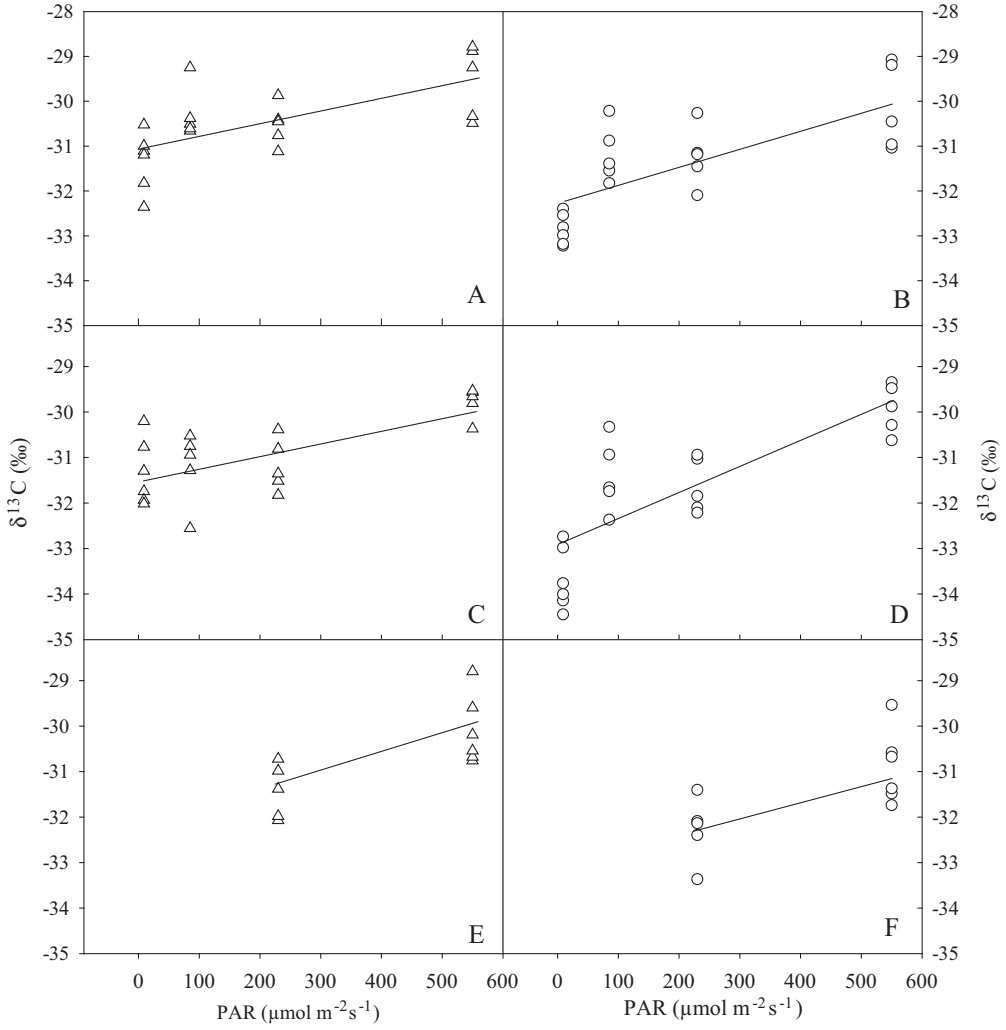

Fig. 2. Linear regression between carbon isotope composition ($\delta^{13}C$, ‰) and photosynthetically active radiation (PAR, μmol m^{-2} s^{-1}) for young (left column, triangles) and mature leaves (right column, circle) of *Siparuna guianensis* (A and B), *Xylopia sericea* (C and D), and *Byrsonima sericea* (E and F) in four sites that form an irradiance gradient in União Biological Reserve, Brazil, in November 2014. n: 5–6.

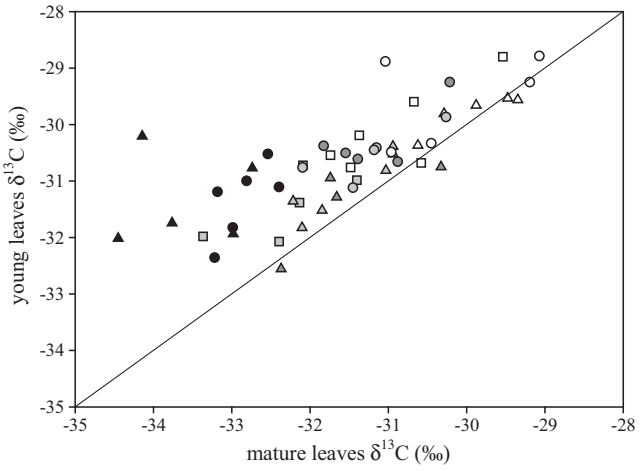

Fig. 3. Carbon isotope composition ($\delta^{13}C$, ‰) of young leaves plotted against mature leaves from the same individuals of three species: *Siparuna guianensis* (circle), *Xylopia sericea* (triangle), and *Byrsonima sericea* (square) in four sites in União Biological Reserve, Brazil, in November 2014. Black: forest (9 μmol m^{-2} s^{-1}), dark grey: US 1 (85 μmol m^{-2} s^{-1}), light grey: US 2 (230 μmol m^{-2} s^{-1}), and white: US 3 (550 μmol m^{-2} s^{-1}). US: understory. The solid line is the one-to-one line. n: 5–6.

There was a positive and significant relationship between $\delta^{13}C$ and irradiance (PAR) for all species (Table 3 and Fig. 2).

For the majority of the individuals of all species, young leaves showed greater $\delta^{13}C$ values than mature leaves (Fig. 3), with $\delta^{13}C$ values of young and mature leaves increasing with increasing irradiance (*S. guianensis*, r = 0.769; *X. sericea*, r = 0.693; *B. sericea*, r = 0.888; $P \leqslant 0.001$ for all). Differences in $\delta^{13}C$ values between young and mature leaves was greatest in the forest (*S. guianensis* = −1.53‰ and *X. sericea* = −2.35‰), compared to sunny sites (for US 3, *S. guianensis* = −0.59‰ and *X. sericea* = −0.13‰), indicating that differences in $\delta^{13}C$ values between young and mature leaves were gradually decreasing with increasing irradiance (see different color symbols in Fig. 3).

Along the irradiance gradient, positive correlations were observed between $\delta^{13}C$ and A_{area}, and between $\delta^{13}C$ and g_S for young and mature leaves of *S. guianensis* and between $\delta^{13}C$ and g_S for mature leaves of *X. sericea* (Fig. 4, Table 4). Correlations between $\delta^{13}C$ and C_i were negative for young leaves of *S. guianensis* and *X. sericea*, and no correlations were observed for mature leaves of any of the species. The $\delta^{13}C$ of *B. sericea* did not show correlations with *A*, C_i or g_S in young and mature leaves (Fig. 4, Table 4).

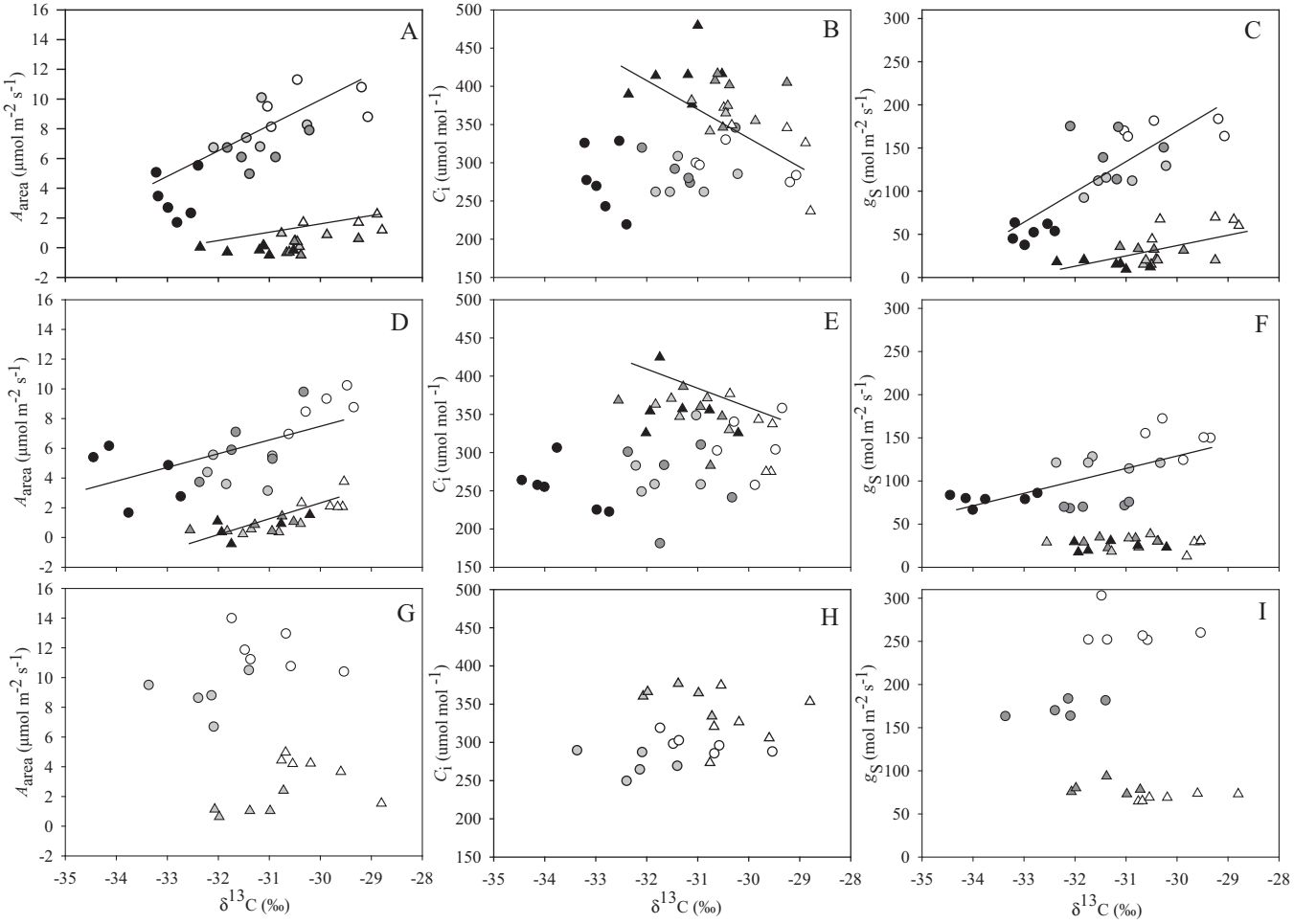

Fig. 4. Relationship between leaf carbon isotope composition ($\delta^{13}C$, ‰) and photosynthetic rate per area (A_{area}, μmol m^{-2} s^{-1}, left column), internal CO_2 concentration (C_i, μmol mol^{-1}, middle column), and stomatal conductance (g_S, mol m^{-2} s^{-1}, right column) for young (triangles) and mature (circles) leaves of *Siparuna guianensis* (A, B and C), *Xylopia sericea* (D, E and F), and *Byrsonima sericea* (G, H and I) in four sites in União Biological Reserve, Brazil, in November 2014. Black: forest (9 μmol m^{-2} s^{-1}), dark grey: US 1 (85 μmol m^{-2} s^{-1}), light grey: US 2 (230 μmol m^{-2} s^{-1}), and white: US 3 (550 μmol m^{-2} s^{-1}). US: understory. Line indicates significant correlations ($P \leqslant 0.05$). n: 5–6.

Table 4
Relationship between leaf carbon isotope composition ($\delta^{13}C$, ‰) and photosynthetic rate per area (A_{area}, μmol m^{-2} s^{-1}); internal CO_2 concentration (C_i, μmol mol^{-1}); and stomatal conductance (g_s, mol m^{-2} s^{-1}) for young and mature leaves of *Siparuna guianensis*, *Xylopia sericea*, and *Byrsonima sericea* in four sites forming an irradiance gradient in União Biological Reserve, Brazil, in November 2014. Bold and italic types indicate significant correlations ($P \leqslant 0.05$). US = understory. Equation y: $mx + b$. n: 6.

		$\delta^{13}C$ x A_{area}		$\delta^{13}C$ x C_i		$\delta^{13}C$ x g_s	
		Young	Mature	Young	Mature	Young	Mature
Siparuna guianensis	r	0.676	0.803	−0.5813	−0.1474	0.5659	0.8001
	P	***0.0008***	***<0.0001***	***0.0057***	0.5238	***0.0075***	***<0.0001***
	m	0.5918	1.8198	−31.2427	3.8615	11.3882	33.6121
Xylopia sericea	r	0.778	0.688	−0.5539	0.3982	0.015	0.6898
	P	***<0.0001***	***0.0006***	***0.0092***	0.0738	0.947	***0.0005***
	m	0.8442	1.0796	−23.1946	11.4128	−0.1568	15.2289
Byrsonima sericea	r	0.392	0.388	−0.3325	−0.2250	0.363	0.6685
	P	0.232	0.237	0.3192	0.5060	0.2714	0.245
	m	0.6778	0.7838	−11.3192	4.2175	−3.0924	32.3468

3.2. No stress conditions in mature leaves according to chlorophyll *a* fluorescence and photosynthetic pigments

The F_v/F_m ratio of mature leaves ranged from 0.75 to 0.83 (Fig. 5). Lower values for this ratio were observed in young leaves compared to mature leaves (Fig. 5, range = 0.68–0.82). There were no significant differences in F_v/F_m ratio among sites for mature leaves except for *X. sericea* in the forest. For young leaves, the F_v/F_m ratio was lower in sunny sites. The Φ_{PSII} values for mature leaves (around of 0.75) were generally higher than for young leaves (range = 0.50–0.66). However, the Φ_{PSII} values between sites did not show significant differences except for young

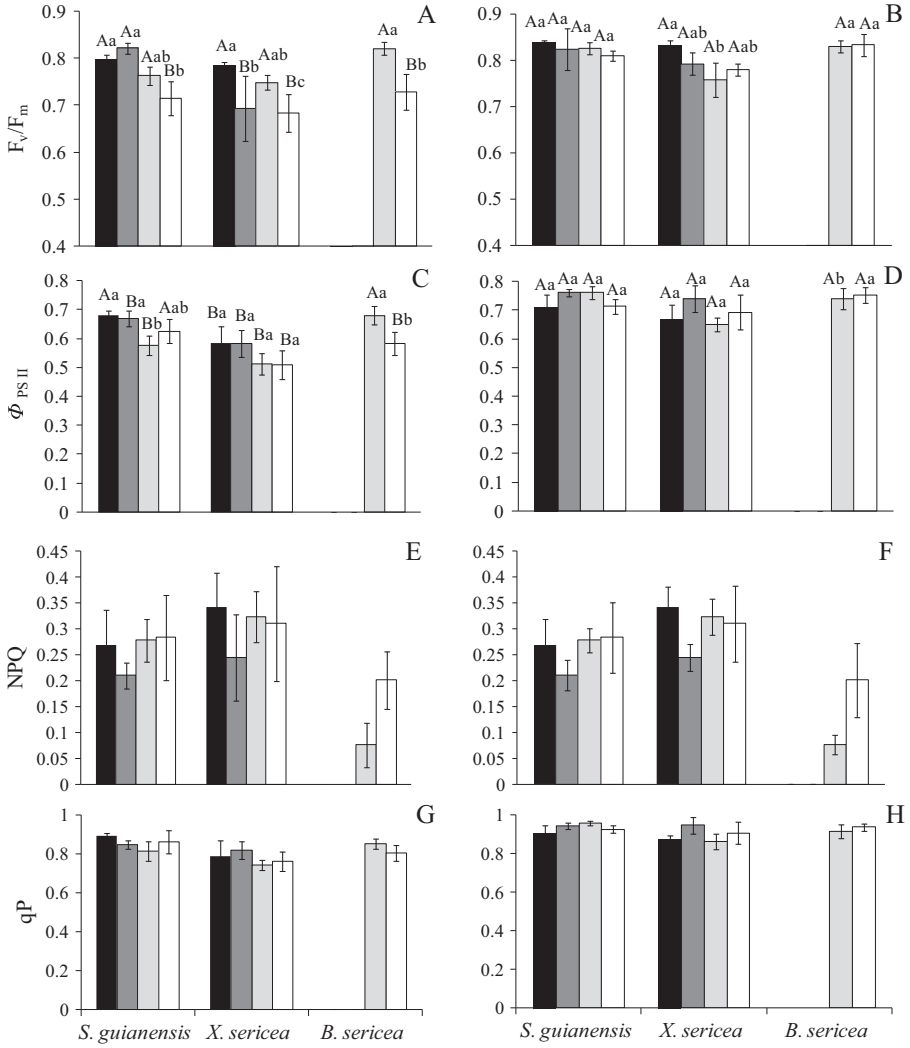

Fig. 5. Chlorophyll *a* fluorescence parameters of *Siparuna guianensis*, *Xylopia sericea*, and *Byrsonima sericea* in young (left column) and mature (right column) leaves in União Biological Reserve, Brazil, in November 2014. (A and B) F_v/F_m, (C and D) Φ_{PSII}, (E and F) NPQ, and (G and H) qP. Black: forest (9 μmol m^{-2} s^{-1}), dark grey: US 1 (85 μmol m^{-2} s^{-1}), light grey: US 2 (230 μmol m^{-2} s^{-1}), and white: US 3 (550 μmol m^{-2} s^{-1}). US: understory. Φ_{PSII}: quantum efficiency of photosystem II. qP: Photochemical quenching. NPQ: Non-photochemical quenching. Upper case letters compare the ages. Lower case letters compare the sites. The data represent mean ± standard error. Distinct letters indicate significant differences ($p \leqslant 0.05$). No letters, no significant differences. n: 10.

Table 5
Photosynthetic pigment contents (nmol cm^{-2}) and SD (standard deviation) in young and mature leaves of *Siparuna guianensis*, *Xylopia sericea* and *Byrsonima sericea* in four sites forming an irradiance gradient in União Biological Reserve, Brazil, in November 2014. US: understory. Upper case letters compare between ages. Lower case letters compare between sites. $P \leqslant 0.05$. n: 10.

	Chlorophyll *a*				Chlorophyll *b*				Chlorophyll *a*/*b*			
	Young		Mature		Young		Mature		Young		Mature	
S. guianensis												
Forest	16,97 ± 1,83	Ba	45,06 ± 3,85	Aa	5,80 ± 0,93	Ba	12,54 ± 0,89	Aab	2,95 ± 0,24	Aa	3,60 ± 0,35	Aa
US 1	19,16 ± 3,75	Ba	46,37 ± 7,19	Aab	6,95 ± 1,24	Ba	14,59 ± 1,61	Aa	2,77 ± 0,34	Aa	3,17 ± 0,23	Aab
US 2	17,87 ± 3,26	Ba	37,27 ± 7,80	Abc	6,44 ± 1,15	Ba	12,50 ± 2,57	Aab	2,79 ± 0,26	Aa	2,98 ± 0,13	Ab
US 3	16,17 ± 3,50	Ba	36,48 ± 5,04	Ac	5,47 ± 1,35	Ba	11,05 ± 1,52	Ab	3,00 ± 0,29	Aa	3,31 ± 0,15	Aab
X. sericea												
Forest	7,70 ± 1,32	Ba	41,48 ± 6,43	Aa	2,87 ± 0,43	Ba	11,68 ± 1,84	Aa	2,68 ± 0,11	Bab	3,56 ± 0,32	Aa
US 1	9,44 ± 1,68	Ba	29,51 ± 7,46	Ab	3,35 ± 0,50	Ba	9,00 ± 2,26	Aa	2,81 ± 0,20	Aab	3,28 ± 0,13	Aa
US 2	9,33 ± 1,35	Ba	22,44 ± 6,01	Ab	4,14 ± 1,05	Ba	7,60 ± 2,01	Ab	2,33 ± 0,44	Bb	2,97 ± 0,32	Aa
US 3	10,21 ± 2,86	Ba	19,95 ± 2,33	Ac	3,68 ± 1,39	Aa	5,95 ± 2,14	Ab	2,88 ± 0,35	Aa	3,30 ± 0,64	Aa
B. sericea												
US 2	14,23 ± 2,89	Ba	30,84 ± 5,06	Aa	6,82 ± 1,30	Aa	9,88 ± 1,41	Aa	2,11 ± 0,42	Ba	3,12 ± 0,12	Aa
US 3	12,95 ± 3,15	Ba	25,76 ± 3,42	Aa	4,94 ± 1,51	Ba	7,98 ± 1,46	Aa	2,70 ± 0,42	Ba	3,31 ± 0,58	Aa
	Total chlorophyll				Carotenoids				Total chlorophyll /carotenoids			
S. guianensis												
Forest	22,76 ± 2,73	Ba	57,59 ± 4,10	Aab	5,14 ± 0,79	Ba	11,75 ± 2,14	Aa	4,45 ± 0,20	Aa	4,99 ± 0,61	Aa
US 1	26,10 ± 4,77	Ba	60,96 ± 8,68	Aa	6,35 ± 0,92	Ba	15,13 ± 2,18	Aa	4,09 ± 0,20	Aa	4,04 ± 0,23	Ab
US 2	24,32 ± 4,33	Ba	49,77 ± 10,33	Aa	7,01 ± 1,16	Ba	14,11 ± 3,07	Aa	3,47 ± 0,26	Aa	3,55 ± 0,36	Ab
US 3	21,64 ± 4,80	Ba	47,52 ± 6,51	Ab	6,85 ± 0,79	Ba	15,28 ± 1,94	Aa	3,13 ± 0,39	Ab	3,14 ± 0,48	Ac
X. sericea												
Forest	10,57 ± 1,74	Ba	53,15 ± 8,01	Aa	2,54 ± 0,51	Ba	12,02 ± 2,34	Aa	4,19 ± 0,23	Aa	4,46 ± 0,29	Aa
US 1	12,79 ± 2,16	Ba	38,51 ± 9,70	Ab	3,48 ± 0,46	Ba	9,45 ± 2,13	Aab	3,67 ± 0,29	Aa	4,06 ± 0,25	Ab
US 2	13,47 ± 2,03	Ba	30,04 ± 7,89	Abc	3,94 ± 0,48	Ba	7,99 ± 1,82	Ab	3,43 ± 0,46	Ab	3,75 ± 0,28	Ab
US 3	13,89 ± 4,22	Ba	25,90 ± 3,50	Ac	4,85 ± 1,13	Ba	9,20 ± 3,17	Aab	2,83 ± 0,29	Ac	3,01 ± 0,58	Ac
B. sericea												
US 2	21,05 ± 3,79	Ba	40,71 ± 6,45	Aa	9,00 ± 1,53	Aa	12,10 ± 1,96	Aa	2,34 ± 0,27	Ba	3,37 ± 0,20	Aa
US 3	17,89 ± 4,49	Ba	33,74 ± 3,72	Aa	7,69 ± 2,20	Ba	11,48 ± 2,53	Aa	2,37 ± 0,41	Ba	3,04 ± 0,57	Aa

leaves of *S. guianensis* in US 2, and *B. sericea* in US 4. The quenching values (non-photochemical, NPQ; and photochemical, qP) did not show significant differences between ages and sites for any of the species (Fig. 5).

Photosynthetic pigment (chlorophylls *a* and *b*, and carotenoids) levels were higher in mature than in young leaves for most species at most sites (Table 5). Plants of all species presented lower levels of chlorophyll and total chlorophyll/carotenoid ratios with increases in irradiance availability. In general, the chlorophyll *a*/*b* ratio did not differ significantly along the irradiance gradient (Table 5).

3.3. SLA decreases with increasing in irradiance

The SLA did not vary between leaf ages for any species or sites, except for *S. guianensis* in the forest. On the other hand, SLA values decreased with increasing irradiance, independently of leaf age (Fig. 6).

4. Discussion

4.1. Leaf $\delta^{13}C$ as tracer to the forest management

Our data indicate a sensitivity of leaf $\delta^{13}C$ to forest management in the most representative species of regenerating Brazilian Atlantic forest trees, suggesting that carbon stable isotopes can be used as recorders of ecological changes for future studies of forest ecology. The $\delta^{13}C$ of leaves in regenerating vegetation leaves behind a signal that can be detected after the modifications in the environment (West et al., 2001) and $\delta^{13}C$ has been described as an important tool for assessing the integrated response of the ecosystem that can be followed through the carbon cycle (Dawson et al., 2002). In restoration ecology and forest management, plant $\delta^{13}C$ has been used to determine past regeneration environments (West et al., 2001), the effects of gaps on forest dynamics (van der Sleen et al., 2014), and selection of shade-tolerant tree species (Kennedy et al., 2006; West et al., 2001). We suggest that

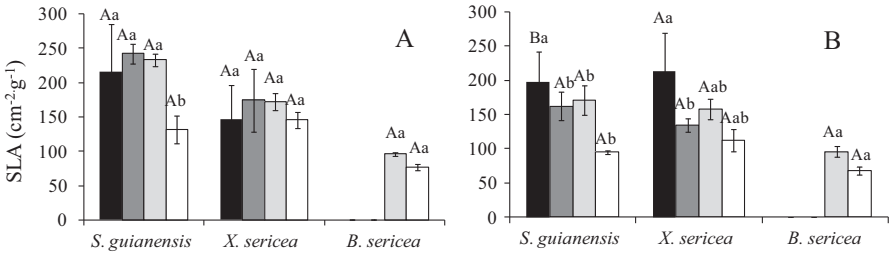

Fig. 6. Specific leaf area (SLA) of *Siparuna guianensis*, *Xylopia sericea*, and *Byrsonima sericea* in young (A) and mature leaves (B) in União Biological Reserve, Brazil, in November 2014. Black: forest (9 μmol m^{-2} s^{-1}), dark grey: US 1 (85 μmol m^{-2} s^{-1}), light grey: US 2 (230 μmol m^{-2} s^{-1}), and white: US 3 (550 μmol m^{-2} s^{-1}). US: understory. Upper case letters compare the ages. Lower case letters compare the sites. The data represent mean ± standard error. Distinct letters indicate significant differences ($P \leqslant 0.05$). n: 10–30.

leaf $\delta^{13}C$ can also be used to document changes in the rate of carbon gain of remaining vegetation after canopy removal, and to determine how alterations in canopy environment affect the ability of understory vegetation to assimilate carbon for growth. Overall, changes in forest $\delta^{13}C$ after natural and/or anthropogenic disturbance have been associated with a number of abiotic factors, but respond most strongly to changes in irradiance, nutrient and water availability (van der Sleen et al., 2014).

Two principal factors that affect leaf $\delta^{13}C$ during carbon assimilation are: (1) the isotope composition of atmospheric source CO_2 (air $\delta^{13}C$), and (2) the ratio of partial pressures of intercellular CO_2 (C_i) and ambient CO_2 (C_a), C_i/C_a at the site of carboxylation (Farquhar et al., 1982, 1989). For a tropical forest in French Guiana, it was described that approximately 20% of variation in leaf $\delta^{13}C$ was due to source air effects and 80% due to changes in C_i/C_a (Buchmann et al., 1997). Along forest height gradients, differences are expected between the $\delta^{13}C$ of air closest to forest soil (^{13}C-depleted) and the canopy (^{13}C-enriched) (Berry et al., 1997; Buchmann et al., 1997; Martinelli et al., 1998; Ometto et al., 2006). Simultaneous variation in source CO_2 and irradiance have been described as responsible for ^{13}C spatial height gradients in forests (Berry et al., 1997; Ometto et al., 2006). However, some studies have suggested other factors responsible for $\delta^{13}C$ variation, such as leaf water potential and/or VPD (Coble and Cavaleri, 2015), leaf nitrogen content (Duursma and Marshall, 2006), or a mix of several factors (Ometto et al., 2006; Kranabetter et al., 2010; Cornwell et al., 2016). Overall, there is agreement that photosynthetic capacity is the central process that coordinates carbon isotope discrimination, with more photosynthetically active leaves being relatively ^{13}C-enriched (Leavitt and Long, 1986; Zimmerman and Ehleringer, 1990; Terwilliger, 1997; Le Roux et al., 2001; Kranabetter et al., 2010).

4.2. Similar leaf $\delta^{13}C$ and photosynthetic performance of the main regenerating species

Whereas a comparison between the three main regenerating species showed similar values in their leaf $\delta^{13}C$, the carbon stable isotopes data revealed site effects. This was due to similarities in the photosynthetic performance between species in the same site. Besides differences between sites, leaf $\delta^{13}C$ found in this study was similar to average values reported for other tropical forests in Brazil with values of −32.1‰ (Martinelli et al., 1998), between −31.7 and −27.5‰ (Duarte et al., 2005), −32.3‰ (Ometto et al., 2006), and between −38.5‰ to −24.6‰ (Martinelli et al., 2007), in French Guiana with values between −34.4‰ and −26.6‰ (Buchmann et al., 1997), between −34.8‰ and −27.5‰ (Bonal et al., 2000a,b), and in Venezuela with a mean value of −30.3‰ (Sobrado, 2008). These values are generally consistent with expected $\delta^{13}C$ values for C_3 plants with high photosynthetic carbon isotope discrimination (Farquhar et al., 1989). Our data shows that g_s was the physiological process limiting photosynthesis (Table 2). This parameter was controlled by abiotic factors of the sites, probably irradiance, since water availability was generally high in this ecosystem (Braga et al., 2016). Irradiance levels and their relationship to g_s have been suggested as an important sources of variation in leaf $\delta^{13}C$ (Zimmerman and Ehleringer, 1990). We observed positive correlations between g_s and leaf $\delta^{13}C$, and A_{area} and leaf $\delta^{13}C$ according to the irradiance gradient for *S. guianensis* and *X. sericea*. *Byrsonima sericea* was found only in more sunny sites (US 2 and 3), and this contracted gradient is a possible reason for absence of correlations for this species. However, a clear tendency for positive correlations in leaf $\delta^{13}C$-A_{area}, and leaf $\delta^{13}C$-g_s relationships for *B. sericea* was observed (Fig. 4A and C), thus, emphasizing the effect of site.

4.3. Influence of forest management on photosynthetic performance of young and mature leaves

Forest management affected young and mature leaves differently, with direct influence on leaf $\delta^{13}C$ values and causing reversible photodamage in young leaves. The sensitivity of leaf $\delta^{13}C$ to irradiance in young leaves suggests that $\delta^{13}C$ in these organs is controlled not only by carbon imported to new leaves through phloem sap, but also by stomatal control and carboxylation as these young leaves develop their photosynthetic competency. In fact, the smallest differences between $\delta^{13}C$ in young and mature leaves were observed in the sunniest understory site (US 3), although mature leaves continue to be ^{13}C-depleted compared to young leaves. Young leaves begin their development supported by carbon in phloem sap, which is often ^{13}C-enriched relative to autotrophic tissue, and change status to become a photosynthetic organ, absorbing carbon that is more ^{13}C-depleted with time. As young leaves gain a degree of photosynthetic competency, CO_2 fixation begins and isotope discrimination by Rubisco gradually changes the carbon isotope composition of these young leaves adding relatively more ^{12}C. Once these leaves are able to fix CO_2, the respired CO_2 (^{13}C-enriched) will be refixed, mainly under low-irradiance (Ubierna Lopez and Marshall, 2007). According to our data, the increase in irradiance enabled young leaves to develop photosynthesis, resulting in a direct relationship between irradiance and leaf ^{13}C-enrichment (Fig. 2), as observed for mature leaves. In mature leaves, the $\delta^{13}C$ signal is related mostly to structural carbon accumulated during leaf growth and values may be different from phloem sap.

Although more irradiance can accelerate the process of maturity in young leaves, it can also cause photodamage. In the present study, the lowest values of F_v/F_m in young leaves of sunny sites (US 2 and 3) can be interpreted as more susceptibility than mature leaves to increasing irradiance due to incomplete development of the antenna. In addition, photodamage occurred not as function of the timing of management (5 or 19 months, respectively in US 2 and US 3), although photodamage is expected immediately following canopy opening, but rather according to the degree of irradiance. In general, individuals of all species exposed to more irradiance during 5 months (US 2, 230 μmol m^{-2} s^{-1}) had higher F_v/F_m values than individuals exposed during 19 months (US 3, 500 μmol m^{-2} s^{-1}). There are two possible explanations for these data in young leaves: (1) the required time for these species to acclimate to more irradiance is less than 5 months; (2) nineteen months at 500 μmol m^{-2} s^{-1} decreases the capacity to recover F_v/F_m values. Recent investigations of short-term photochemical response to irradiance of 1500 μmol m^{-2} s^{-1} in mature leaves of these species suggests that all are able to photoacclimate in 3 months (Teixeira et al., 2015). Based on this previous study and the present data, we suggest that the increase in irradiance was more important than time of management in determining photoacclimation in this study.

Lower chlorophyll and carotenoid contents in young leaves compared to mature leaves support the idea of incomplete development of the antenna (Sobrado, 2008). However, both young and mature leaves show decreases in total chlorophyll/carotenoids with increasing irradiance, suggesting an acclimation capacity from the early developmental phase. The mature leaves analyzed were once young leaves that tolerated and grew in these new environmental conditions, and did not show photosynthetic stress and/or differences in chlorophyll *a* parameters once mature. This suggests that photodamage is reversible in young leaves. Although these species show photoacclimation capacity (Silva et al., 2010; Lage-Pinto et al., 2012; Teixeira et al., 2015; Vieira et al., 2015), dynamic photoinhibition had been observed 1 year after management for *S. guianensis* and *X. sericea* in this area (Lage-Pinto et al.,

2012). All species are able to photoacclimate, but *B. sericea* is the fastest species to recuperate initial conditions after forest management (Teixeira et al., 2015). In fact, *B. sericea* is the most light-demanding species among the studied species and was found only in high irradiance sites. Higher convexity of adaxial epidermis was observed as an anatomical strategy to potentially concentrate irradiance in mesophyll cells of this species under low irradiance conditions (Silva et al., 2010), but it is also possible that this plastic response is unable to sustain the occurrence of this species inside the forest.

4.4. Forest regeneration and physiological characteristics of the main species

The data show that the main species regenerating in the understory can increase carbon gain and tolerate more irradiance after management. These data in addition to previous studies (Evaristo et al., 2011; Lage-Pinto et al., 2012; Lage-Pinto et al., 2015; Silva et al., 2010; Teixeira et al., 2015; Vieira et al., 2015) show the photoacclimation performance of these species to more irradiance, that could explain their abundance in the open and management areas. Thus, there is a possibility that the management may affect the trajectory of forest regeneration, including potential dominance of these species. More irradiance after selective logging or in open understories during forest regeneration favor high light–demanding species (Darrigo et al., 2016; Ostertag et al., 2008). This causes changes in species composition and species turnover in regenerating understories, important components for maintenance of functionality and biodiversity in tropical forest. Restoring native biodiversity in degraded or regenerating areas has become an important question in conservation biology and the use of non-native species has helped. However, controversies about the use and maintenance of non-native species during forest regeneration has been reported (Evaristo et al., 2011; Ostertag et al., 2008; Parrotta et al., 1997; Podadera et al., 2015).

The positive effects of using exotic species are fast growth, providing favorable conditions for eventual germination and establishment of native species (Parrotta et al., 1997). On the order hand, non-native species may become invasive, inhibiting or even preventing the growth and establishment of some native species (Evaristo et al., 2011). In the specific case of eucalyptus, allelopathy, slow decomposition (Batish et al., 2006), and risk of fire due to large amounts of flammable leaf litter (Schneider, 2003) have been described as limiting forest regeneration (Evaristo et al., 2011). In fact, the removal of non-native species has been shown to improve diversity and accelerate the succession process (Podadera et al., 2015). However, selective logging changes the distribution of resources, such as irradiance and nutrients, modifying the performance and abundance of the species that remain (Darrigo et al., 2016). Thus, ecological knowledge of exotic species and native species living in the same environment is fundamental to orient forest managers. Our study highlights the photosynthetic acclimation and the rapid photosynthetic maturity of the main species of the understories to irradiance increases after management, and show that $\delta^{13}C$ can be used to monitor this performance. Studies of different phases following forest management are essential to best understand the beneficial effect of this practice.

5. Conclusion

Forest management affected leaf $\delta^{13}C$ of the main regenerating understory species, with changes in site being more important than species-specific features for photosynthetic performance. The present study found evidence that photosynthetic performance is a strong determinant of leaf $\delta^{13}C$ in regenerating forest, and must be considered for interpretation of forest regeneration processes under spatial irradiance variation.

The increase in irradiance due to forest management affected the physiology of young and mature leaves differently, showing that $\delta^{13}C$ in young leaves along an irradiance gradient is controlled not only through carbon imported to new leaves through the phloem sap, but also through direct responses of stomatal control and carboxylation as these young leaves develop their photosynthetic competency. However, young leaves show susceptibility to photodamage, but were able to adjust to new environmental conditions, suggesting an acclimation capacity from early developmental phases.

The forest management practices provided more irradiance, causing increases in the photosynthetic rates of the main native species of the understories. Although this is a positive response for the regeneration of the forest, this could promote dominance by a few species in this important hot spot of biodiversity. Thus, continuous monitoring is necessary to understand regeneration trajectories.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2016.07.048.

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