

Sun-shade patterns of leaf carotenoid composition in 86 species of neotropical forest plants

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Abstract. A survey of photosynthetic pigments, including 86 species from 64 families, was conducted for leaves of neotropical vascular plants to study sun-shade patterns in carotenoid biosynthesis and occurrence of α -carotene (α -Car) and lutein epoxide (Lx). Under low light, leaves invested less in structural components and more in light harvesting, as manifested by low leaf dry mass per area (LMA) and enhanced mass-based accumulation of chlorophyll (Chl) and carotenoids, especially lutein and neoxanthin. Under high irradiance, LMA was greater and β -carotene (β -Car) and violaxanthin-cycle pool increased on a leaf area or Chl basis. The majority of plants contained α -Car in leaves, but the α - to β -Car ratio was always low in the sun, suggesting preference for β -Car in strong light. Shade and sun leaves had similar β , ϵ -carotenoid contents per unit Chl, whereas sun leaves had more β , β -carotenoids than shade leaves. Accumulation of Lx in leaves was found to be widely distributed among taxa: >5 mmol mol Chl⁻¹ in 20% of all species examined and >10 mmol mol Chl⁻¹ in 10% of woody species. In *Virola elongata* (Benth.) Warb, having substantial Lx in both leaf types, the Lx cycle was operating on a daily basis although Lx restoration in the dark was delayed compared with violaxanthin restoration.

Additional keywords: carotene, carotenoid biosynthesis, chlorophyll, leaf dry mass, *Virola*, xanthophyll cycle.

Introduction

Carotenoid composition in chloroplasts is highly conserved among vascular plants. Typically, leaves contain β -carotene (β -Car), lutein (L), violaxanthin (V) and neoxanthin (N) (Goodwin 1965; Young 1993). Part of V is converted to antheraxanthin (A) and zeaxanthin (Z) under excess light or other environmental stress conditions (Yamamoto *et al.* 1962). Together with chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*), most of the carotenoids are bound in the pigment-protein complexes of PSI and PSII, with the majority of xanthophylls (L, N, V, A and Z) found in the light-harvesting antenna complexes and β -Car in the core complexes (Yamamoto and Bassi 1996). Light-dependent de-epoxidation and epoxidation between V, A and Z, called xanthophyll cycle (or V cycle), play a central role in the protection of photosynthetic membranes against photooxidative damage (Demmig *et al.* 1987; Müller *et al.* 2001). The pool size of the V-cycle pigments (VAZ) exhibits pronounced sun-shade responses, being larger in sun than in shade leaves, which highlights the significance of the V cycle for photoprotection under strong light (Demmig-Adams and Adams 1992; Demmig-Adams 1998).

In higher plants, carotenoids are synthesised only in plastids (for reviews on carotenoid biosynthetic pathways in plants, see Cunningham and Gantt 1998; Hirschberg 2001; DellaPenna and

Pogson 2006). Cyclisation of lycopene, the last common precursor of the photosynthetic carotenoids mentioned above, gives rise to two branches of the biosynthetic pathway: β -cyclisation on both ends of lycopene leads to formation of β -Car and its derivatives, having two β -rings (β , β -carotenoids), and β -cyclisation and ϵ -cyclisation at each end of lycopene leads to formation of α -carotene (α -Car) and its derivatives, having one β - and one ϵ -ring (β , ϵ -carotenoids). Hydroxylation in the ring moieties of β - and α -Car produces Z and L, respectively, and subsequent stepwise epoxidation in the two 3-hydroxy- β -rings of Z leads to formation of A and V. Thus, the V cycle operates in the downstream of β -Car, reversing the flux in the β , β -branch of the biosynthetic pathway by light-induced de-epoxidation of V to A and Z. Finally, isomerisation and modification in an epoxy- β -ring of V give rise to N.

Unlike the β , β -branch that synthesises five carotenoids (β -Car, Z, A, V and N) commonly found in chloroplasts of higher plants, L is the only ubiquitous pigment produced in the β , ϵ -branch and accumulates in substantial amounts. Yet, accumulation of α -Car, the first product in the β , ϵ -branch and the precursor of L, has been reported for leaves of many different species, especially shade-tolerant or shade-grown plants (Thayer and Björkman 1990; Demmig-Adams and Adams 1992; Siefertmann-Harms 1994; Demmig-Adams 1998). Furthermore,

in the downstream of the β,ϵ -branch, epoxidation in the 3-hydroxy- β -ring of L can take place in leaves and other green tissues of certain taxa to result in substantial accumulation of lutein epoxide (Lx) and one-step xanthophyll cycling between L and Lx (Lx cycle) (García-Plazaola *et al.* 2007). Similar to the occurrence of α -Car, large Lx amounts have been found predominantly in shade leaves. Thus, possible functions of α -Car and Lx in light harvesting have been proposed for species adapted (or tolerant of) to shade environments (Krause *et al.* 2001; Matsubara *et al.* 2005, 2007, 2008). At the same time, enhanced accumulation of these pigments related to shade acclimation or adaptation may suggest general upregulation of carotenoid biosynthesis in the β,ϵ -branch, or changes in the balance between the fluxes down the β,ϵ - and β,β -branch.

Dense vegetations in tropical forests create contrasting light environments, ranging from deep shade on the forest floor to full sunlight in outer canopy. If α -Car and Lx contribute to efficient light harvesting in low-light environments, and if this confers an evolutionary advantage, leaves growing on the forest floor or inside the canopy would contain higher levels of α -Car and Lx than outer-canopy leaves in a wide variety of tropical forest species. Indeed, it has been documented that shade leaves of some neotropical forest plants have large amounts of α -Car and/or Lx (Königer *et al.* 1995; Krause *et al.* 2001, 2003, 2004; Matsubara *et al.* 2008), although the number of species examined in these studies was not large enough to see whether these pigments occur widely in many different species under shaded environments. Therefore, we conducted a pigment survey on leaf carotenoid composition in tropical forest plants, including 86 species from 64 families growing in different types of tropical forests in Panama. Leaf samples were collected from understory plants (shade) as well as inner and outer canopy (shade and sun, respectively) to compare levels of Chl as well as different β,ϵ - and β,β -carotenoids. In addition, leaf dry mass per area (LMA), an important leaf structural parameter (Reich *et al.* 1997; Wright *et al.* 2004), was determined to explore a possible relationship between LMA and carotenoid contents. Furthermore, operation of the Lx cycle was investigated in *Virola elongata* (Benth.) Warb, a tree species that showed high Lx amounts in both shade and sun leaves in the survey.

Materials and methods

Plant material and collection sites

Species were chosen randomly for leaf collection in different habitats in central Panama (9°N, 79–80°W) during the late wet season in November–December 2006 and 2007. Outer-canopy sun leaves were collected besides shade leaves when available and/or accessible. For two species (*Elaeis oleifera* (Kunth) Cortés and *Psychotria poeppigiana* Müll. Arg.), ‘sun leaves’ were taken from forest gaps and probably were not fully sun-acclimated. In total, 86 species from 64 plant families (two fern families, one gymnosperm family and 61 angiosperm families) were included in the survey. The angiosperm families considered represent ~30% of the monocotyledon and 28% of the dicotyledon families reported for Panama (Correa *et al.* 2004).

All species included in the study, their botanical naming authorities, life forms and collection sites are listed in Table 1. For nomenclature and authorities, the database of the

Missouri Botanical Garden (www.mobot.org, accessed 21 November 2008) was used. Tropical forest sites for leaf collection were (1) Soberania National Park and Gamboa area, seasonally dry lowland forest on the Caribbean side of the dividing range; (2) Metropolitan Nature Park, near Panama City, seasonally dry semi-deciduous lowland forest on the Pacific side of the dividing range; (3) Barro Colorado Island Nature Monument, Lake Gatún, Panama Canal area, moist, seasonally dry lowland forest; (4) San Lorenzo National Park, moist primary lowland forest on the Caribbean slope; (5) Galeta Island Nature Reserve, mangrove swamp at Caribbean coast; (6) Altos de Campana National Park, montane cloud forest (~800 m above sea level); (7) Chagres National Park, Cerro Jefe, montane cloud forest (~900 m above sea level). At sites (2) and (4), canopy leaves of mature trees and leaves of lianas were accessible from construction cranes. Climate data for some of the study sites can be obtained at http://striweb.si.edu/esp/physical_monitoring/index_phy_mon.htm (accessed 21 November 2008).

Diel course of Lx and V cycles in *Virola elongata* leaves

Diel variations in Lx and V were studied *in situ* in January 2007 (early dry season) on a mature tree of *Virola elongata* (Benth.) Warb. at collection site (4); the same tree was chosen for the pigment survey in the rainy season. Shade leaves were selected from the north-east side underneath the dense crown of the tree, where PAR remained $<100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Leaf discs (0.95 cm²) were sampled between early morning and midday and in the following morning. Because of strong wind, discs had to be taken from different leaves at each sampling time. The discs were frozen immediately in liquid nitrogen for pigment assay. At around midday, five sun leaves were collected and dark-adapted at ambient temperature. After 2 and 4.5 h of dark adaptation, sample discs were taken from these leaves to study restoration kinetics of Lx and V.

The ratio of maximal variable to maximal total of Chl fluorescence intensity, F_v/F_m , indicating the potential efficiency of PSII, was recorded with a MINI-PAM fluorometer (Walz, Effeltrich, Germany) after 10 min dark adaptation, as described by Krause *et al.* (2006). Photosynthetically active radiation was measured with a LI-190SA sensor connected to a LI-189 data logger (Li-Cor, Lincoln, NE, USA).

Analysis of photosynthetic pigments

For the pigment survey, whole leaves or, depending on leaf size, sections of leaf blades were detached and kept moist and dark at ambient temperature for at least 4 h before sampling of discs (0.95 cm²). The samples were frozen in liquid nitrogen, stored at –70 to –80°C and transported to Germany on dry ice for HPLC analysis of photosynthetic pigments. The major part of leaf samples was collected in November 2006 and analysed at the Institut für Phytosphäre, Forschungszentrum Jülich; a minor part was collected in November 2007 and analysed at the Institut für Biochemie der Pflanzen, Heinrich-Heine-Universität Düsseldorf. Pigments were extracted with acetone and quantified by HPLC using the method described by Matsubara *et al.* (2005) and by Krause *et al.* (2003) based on the method by Färber *et al.* (1997).

Table 1. List of species, author names, life forms and collection sites of tropical plants studied

Collection sites are numbered as follows: (1) Soberania National Park and Gamboa area; (2) Metropolitan Nature Park, near Panama City; (3) Barro Colorado Island Nature Monument, Lake Gatún; (4) San Lorenzo National Park; (5) Galeta Island Nature Reserve; (6) Altos de Campana National Park; (7) Chagres National Park, Cerro Jefe

Species and botanical naming authorities	Life form	Site
<i>Aechmea magdalenae</i> (André) André ex Baker	Herb	1
<i>Albizia guachapele</i> (Kunth) Dugand	Tree	2
<i>Amphitecna latifolia</i> (Mill.) A.H.Gentry	Tree	5
<i>Anacardium excelsum</i> (Kunth) Skeels	Tree	2
<i>Anthurium cerrocampanense</i> Croat	Herb	6
<i>Aphelandra campanensis</i> Durkee	Herb	6
<i>Avicennia germinans</i> (L.) L.	Tree/mangrove	5
<i>Brassia caudata</i> (L.) Lindl.	Herb/epiphyte	6
<i>Bursera simaruba</i> (L.) Sarg.	Tree	1
<i>Calathea lutea</i> Schult.	Herb	1
<i>Carapa guianensis</i> Aubl.	Tree	4
<i>Carludovica palmata</i> Ruiz & Pav.	Herb	1
<i>Cassipourea elliptica</i> (Sw.) Poir.	Tree	6
<i>Cavendishia stenophylla</i> A.C.Sm.	Shrub/epiphyte	6
<i>Cecropia peltata</i> L.	Tree	2
<i>Cespedezia macrophylla</i> Seem.	Tree	4
<i>Chlorophora tinctoria</i> (L.) Gaudich. ex Benth.	Tree	2
<i>Chrysobalanus icaco</i> L.	Shrub	5
<i>Chusquea simpliciflora</i> Munro	Herb	1
<i>Clusia pratensis</i> Seem.	Shrub	1
<i>Coccoloba uvifera</i> (L.) L.	Tree	5
<i>Columnnea billbergiana</i> Beurl.	Shrub/epiphyte	6
<i>Combretum fruticosum</i> (Loefl.) Stuntz	Liana	2
<i>Connarus williamsii</i> Britton	Liana	3
<i>Costus villosissimus</i> Jacq.	Herb	1
<i>Cryosophila warscewiczii</i> (H.Wendl.) Bartlett	Tree	6
<i>Cyathea microdonta</i> (Desv.) Domin	Tree	1
<i>Cyclopeltis semicordata</i> (Sw.) J.Sm.	Herb	1
<i>Dalbergia monetaria</i> L.f.	Shrub/tree	5
<i>Daphnopsis americana</i> (Mill.) J.R.Johnst.	Shrub	6
<i>Dendropanax arboreus</i> (L.) Decne. & Planch.	Tree	6
<i>Dendrophthora ambigua</i> Kuijt	Shrub/mistletoe	7
<i>Desmopsis panamensis</i> (B.L. Rob.) Saff.	Shrub	3
<i>Dieffenbachia longispatha</i> Engl. & K. Krause	Herb	1
<i>Dioscorea racemosa</i> (Klotzsch) Uline	Liana	6
<i>Elaeis oleifera</i> (Kunth) Cortés	Tree	1
<i>Freziera candidans</i> Tul.	Tree	7
<i>Gouania lupuloides</i> (L.) Urb.	Liana	3
<i>Gustavia superba</i> (Kunth) O.Berg	Tree	1
<i>Hedyosmum bonplandianum</i> Kunth	Tree	6
<i>Heliconia irrasa</i> Lane ex R.R. Sm.	Herb	6
<i>Hybanthus prunifolius</i> (Humb. & Bonpl. ex Roem. & Schult.) Schulze-Menz	Shrub	3
<i>Hyeronima alchorneoides</i> Allemão	Tree	1
<i>Licania jefensis</i> Prance	Shrub	7
<i>Lindackeria laurina</i> C.Presl	Tree	1
<i>Lisianthus jefensis</i> A.Robyns & T.S.Elias	Shrub	7
<i>Luehea seemannii</i> Triana & Planch.	Tree	1
<i>Marila laxiflora</i> Rusby	Tree	4
<i>Miconia argentea</i> (Sw.) DC.	Tree	1
<i>Mikania leiostachya</i> Benth.	Liana	2
<i>Monstera dilacerata</i> (K.Koch & Sello) K.Koch	Liana	6
<i>Myrcia sylvatica</i> (G.Mey.) DC.	Tree	7
<i>Ormosia macrocalyx</i> Ducke	Tree	1
<i>Otoba novogranatensis</i> Mouldenske	Tree	6
<i>Parathesis bicolor</i> Lundell	Shrub	7
<i>Passiflora vitifolia</i> Kunth	Liana	1

Table 1. (continued)

Species and botanical naming authorities	Life form	Site
<i>Pera arborea</i> Mutis	Tree	1
<i>Petrea aspera</i> Turcz.	Liana	3
<i>Pharus latifolius</i> L.	Herb	1
<i>Phryganocydia corymbosa</i> (Vent.) Bureau ex K.Schum.	Liana	2
<i>Pilocosta campanensis</i> (Almeda & Wiffin)	Herb	6
<i>Piper reticulatum</i> L.	Shrub	1
<i>Pitcairnia atrorubens</i> (Beer) Baker	Herb	6
<i>Podocarpus oleifolius</i> D.Don ex Lamb.	Tree	6
<i>Psychotria poeppigiana</i> Müll. Arg.	Shrub	1
<i>Quassia amara</i> L.	Shrub	3
<i>Renealmia cernua</i> (Sw. ex Roem. & Schult.) J.F.Macbr.	Herb	6
<i>Rhizophora mangle</i> L.	Tree/mangrove	5
<i>Scleria latifolia</i> Sw.	Herb	6
<i>Serjania mexicana</i> (L.) Willd.	Liana	2
<i>Spondias mombin</i> L.	Tree	2
<i>Stigmaphyllon hypargyreum</i> Triana & Planch.	Liana	2
<i>Strychnos panamensis</i> Seem.	Shrub	3
<i>Tapirira guianensis</i> Aubl.	Tree	4
<i>Tectaria incisa</i> Cav.	Herb	1
<i>Terminalia amazonia</i> (J.F.Gmel.) Exell	Tree	7
<i>Tontelea ovalifolia</i> (Miers) A.C.Sm.	Liana	4
<i>Trichostigma octandrum</i> (L.) H.Walter	Liana	2
<i>Turnera panamensis</i> Urb.	Shrub	3
<i>Vanilla planifolia</i> Andrews	Liana	1
<i>Vantanea depleta</i> McPherson	Tree	4
<i>Virola elongata</i> (Benth.) Warb.	Tree	4
<i>Virola sebifera</i> Aubl.	Tree	4
<i>Vitis tiliifolia</i> Humb. & Bonpl. ex Roem. & Schult.	Liana	2
<i>Vochysia ferruginea</i> Mart.	Tree	4
<i>Xiphidium caeruleum</i> Aubl.	Herb	6

Determination of leaf dry mass

Additional leaf discs (0.95 cm²) collected from sun and shade leaves were used to determine dry weight. These discs were also frozen in liquid nitrogen immediately after the removal and stored at -70 to -80°C before and after transportation from Panama to Germany as described above for the samples of pigment analysis. The discs were lyophilised overnight and their dry mass determined by using an analytical balance (Explorer, Ohaus, NJ). Two replicate discs were measured for each species and leaf type except for *Freziera candidans* Tul. (sun), *Myrcia sylvatica* (G.Mey.) DC. (sun and shade) and *Parathesis bicolor* Lundell (sun), for which only one leaf disc was available.

Statistical analysis

Pigment data were statistically tested by using SigmaStat (SYSTAT Software GmbH, Erkrath, Germany). When datasets showed a normal distribution and similar variances between sun and shade, *t*-test (Student *t*-test) was used for analysis. When datasets failed to satisfy these conditions, a non-parametric rank sum test (Mann-Whitney Rank Sum Test) was used.

Results

Pigment survey

Total Chl contents (Chl *a+b*), Chl *a* to Chl *b* ratios (Chl *a/b*), carotenoid contents (based on Chl *a+b*) and ratios between α - and

β -Car (α -/ β -Car) in leaves of the species tested are presented in Table 2. Families of ferns, gymnosperms, monocotyledons and dicotyledons, respectively, have been ordered alphabetically. In 38 species, in which both sun and shade leaves were assayed (two species from forest gaps not considered), there was a strong tendency to higher Chl *a/b* ratios in sun compared with shade leaves (significant difference with $P < 0.05$ in 27 species); 29 species exhibited significantly higher VAZ in sun than in shade leaves. In 25 species, α -/ β -Car was significantly higher in shade compared with sun leaves. In contrast, no α -Car was detected in shade leaves of *Scleria latifolia* (Cyperaceae), *Vanilla planifolia* (Orchidaceae) and in both leaf types of *Bursera simaruba* (Burseraceae). The values of α -/ β -Car were very low in both leaf types of *Albizia guachapele* (Fabaceae) and *Lysanthus jefensis* (Gentianaceae); in five species, for which only shade leaves were tested, α -Car levels were very low (<5 mmol mol Chl⁻¹) or undetectable. For the family Rhizophoraceae, α -/ β -Car was very high in shade leaves of one of the two species tested (*Cassipourea elliptica*, from a montane cloud forest), whereas it was extremely low in the other (*Rhizophora mangle*, from a mangrove stand).

Of the 86 species studied, >10 mmol Lx mol Chl⁻¹ was found in shade leaves of eight species from Arecaceae, Annonaceae, Fabaceae, Hippocrateaceae and Myristicaceae families (Table 2). In the Fabaceae species, sun leaves exhibited lower Lx levels (5–10 mmol mol Chl⁻¹) than shade leaves, similar to

Table 2. Pigment contents in dark-adapted sun and shade leaves of tropical plant species

Means \pm s.e. are presented ($n = 3$, from individual leaves or leaflets) for Chl $a+b$ ($\mu\text{mol m}^{-2}$), Chl a/b ratio (mol mol^{-1}) and the following carotenoids (mmol mol^{-1} Chl $a+b^{-1}$): N, neoxanthin; VAZ, sum of viola-, anthera- and zeaxanthin; L, lutein; Lx, lutein epoxide; α -Car, α -carotene; β -Car, β -carotene; n.d., not detectable. Where appropriate and available, the ratio α -/ β -Car (mol mol^{-1}) and leaf dry mass per area (LMA, g m^{-2}) are also given. Values of LMA are means of two samples except for those marked with an asterisk (*), for which only one sample was analysed. The LMA data are meant to be used to study general trends among many different species, as in Figs 1–3; they are not for comparisons of individual species. Data mentioned in the text are shown in bold font

Family and species	Leaf type	Chl $a+b$	Chl a/b	N	VAZ	L	Lx	α -Car	β -Car	α -/ β -Car	LMA
FERNS											
Cyatheaceae											
<i>Cyathea microdonta</i>	Shade	524 \pm 11	3.17 \pm 0.05	34.9 \pm 0.7	24.6 \pm 0.4	119.3 \pm 2.3	n.d.	27.1 \pm 1.8	38.4 \pm 0.9	0.70 \pm 0.03	33.7
Dryopteridaceae											
<i>Cycloptelis semicordata</i>	Shade	477 \pm 80	2.99 \pm 0.11	34.7 \pm 0.2	23.0 \pm 1.0	115.9 \pm 1.3	n.d.	32.3 \pm 1.6	23.1 \pm 1.2	1.42 \pm 0.15	34.7
<i>Tectaria incisa</i>	Shade	411 \pm 32	2.53 \pm 0.21	32.2 \pm 2.8	24.2 \pm 1.9	111.8 \pm 6.2	n.d.	34.8 \pm 2.0	27.4 \pm 2.3	1.29 \pm 0.14	25.3
GYMNOSPERMS											
Podocarpaceae											
<i>Podocarpus oleifolius</i>	Shade	742 \pm 40	3.11 \pm 0.08	36.7 \pm 0.4	30.4 \pm 1.0	116.3 \pm 2.2	n.d.	47.4 \pm 0.6	26.4 \pm 0.1	1.80 \pm 0.03	83.2
ANGIOSPERMS-											
MONOCOTYLEDONS											
Araceae											
<i>Anthurium</i>	Shade	548 \pm 39	3.21 \pm 0.10	35.2 \pm 1.0	21.5 \pm 1.1	126.2 \pm 4.3	3.7 \pm 0.5	40.9 \pm 2.9	21.2 \pm 0.2	1.92 \pm 0.12	59.5
<i>cerrocampanense</i>											
<i>Dieffenbachia</i>	Shade	652 \pm 13	3.48 \pm 0.02	35.1 \pm 0.5	24.4 \pm 0.6	104.5 \pm 3.3	n.d.	55.0 \pm 2.0	20.5 \pm 0.5	2.69 \pm 0.15	50.0
<i>longispatha</i>											
<i>Monstera dilacerata</i>	Shade	657 \pm 28	3.13 \pm 0.09	40.3 \pm 1.1	22.8 \pm 2.1	120.4 \pm 4.5	1.5 \pm 0.3	37.3 \pm 1.2	29.9 \pm 0.8	1.24 \pm 0.01	53.2
Arecaceae											
<i>Cryosophila</i>	Shade	523 \pm 50	3.65 \pm 0.17	28.2 \pm 0.3	19.9 \pm 1.5	96.9 \pm 2.2	4.0 \pm 1.5	53.4 \pm 2.4	19.9 \pm 0.4	2.68 \pm 0.10	34.2
<i>warscewiczii</i>											
<i>Elaeis oleifera</i>	Sun (gap)	745 \pm 25	3.46 \pm 0.16	33.0 \pm 1.5	25.9 \pm 5.1	104.1 \pm 14.7	12.3 \pm 7.5	36.9 \pm 5.1	52.8 \pm 7.8	0.73 \pm 0.23	77.4
	Shade	684 \pm 26	3.32 \pm 0.02	34.1 \pm 0.5	16.1 \pm 0.7	99.2 \pm 1.7	11.0 \pm 2.9	40.9 \pm 2.7	45.0 \pm 0.5	0.91 \pm 0.07	80.3
Bromeliaceae											
<i>Aechmea magdalenae</i>	Shade	822 \pm 78	3.32 \pm 0.06	37.0 \pm 0.8	28.2 \pm 1.2	126.6 \pm 4.5	2.4 \pm 1.3	48.1 \pm 1.4	27.8 \pm 0.8	1.74 \pm 0.08	140.0
<i>Pitcairnia atrorubens</i>	Shade	776 \pm 36	3.08 \pm 0.01	31.9 \pm 1.9	33.4 \pm 2.5	108.5 \pm 2.6	n.d.	35.7 \pm 1.9	30.0 \pm 1.3	1.02 \pm 0.07	50.5
Cyclanthaceae											
<i>Carلودovica palmata</i>	Shade	592 \pm 44	3.14 \pm 0.10	32.5 \pm 2.0	17.5 \pm 0.4	117.1 \pm 10.4	n.d.	42.5 \pm 1.6	21.3 \pm 1.4	2.00 \pm 0.06	53.7
Cyperaceae											
<i>Scleria latifolia</i>	Shade	418 \pm 1	3.26 \pm 0.12	47.0 \pm 1.2	29.6 \pm 1.2	150.3 \pm 2.5	5.0 \pm 0.6	n.d.	81.1 \pm 2.1		32.6
Dioscoreaceae											
<i>Dioscorea racemosa</i>	Shade	462 \pm 28	3.05 \pm 0.05	29.0 \pm 0.9	24.9 \pm 0.1	100.0 \pm 0.5	n.d.	37.2 \pm 1.1	33.3 \pm 1.8	1.12 \pm 0.03	31.6
Haemodoraceae											
<i>Xiphidium caeruleum</i>	Shade	577 \pm 9	3.21 \pm 0.04	38.8 \pm 1.2	26.3 \pm 1.1	126.1 \pm 3.9	2.4 \pm 0.6	31.2 \pm 0.5	33.8 \pm 0.2	0.92 \pm 0.01	31.6
Heliconiaceae											
<i>Heliconia irrasa</i>	Shade	359 \pm 23	3.52 \pm 0.20	46.1 \pm 3.6	33.8 \pm 2.7	141.0 \pm 7.8	1.6 \pm 0.8	55.0 \pm 3.5	41.1 \pm 2.8	1.34 \pm 0.05	63.2
Marantaceae											
<i>Calathea lutea</i>	Shade	695 \pm 73	3.62 \pm 0.08	37.0 \pm 0.4	23.6 \pm 0.7	109.6 \pm 2.5	8.4 \pm 2.6	54.3 \pm 1.6	22.5 \pm 1.5	2.45 \pm 0.22	48.4
Orchidaceae											
<i>Brassia caudata</i>	Shade	545 \pm 15	2.69 \pm 0.07	39.3 \pm 1.8	12.6 \pm 0.6	145.8 \pm 4.4	7.1 \pm 0.9	3.9 \pm 1.2	47.7 \pm 1.1	0.08 \pm 0.02	44.7
<i>Vanilla planifolia</i>	Sun	309 \pm 33	3.41 \pm 0.07	43.7 \pm 1.7	85.0 \pm 3.9	249.2 \pm 7.6	2.2 \pm 1.2	3.1 \pm 0.5	69.7 \pm 8.2	0.05 \pm 0.01	116.8
	Shade	309 \pm 13	2.91 \pm 0.11	45.7 \pm 1.5	29.0 \pm 2.6	201.3 \pm 5.4	n.d.	n.d.	63.0 \pm 7.9		95.8
Poaceae											
<i>Chusquea simpliciflora</i>	Shade	375 \pm 17	3.47 \pm 0.04	36.1 \pm 0.6	19.4 \pm 0.6	111.5 \pm 2.5	n.d.	46.2 \pm 2.1	32.3 \pm 2.1	1.45 \pm 0.17	36.3
<i>Pharus latifolius</i>	Shade	316 \pm 23	4.21 \pm 0.47	21.5 \pm 0.5	23.1 \pm 1.4	88.6 \pm 1.9	n.d.	23.2 \pm 0.9	32.3 \pm 1.4	0.72 \pm 0.06	21.6
Zingiberaceae											
<i>Costus villosissimus</i>	Shade	192 \pm 14	2.39 \pm 0.16	51.6 \pm 1.9	26.6 \pm 0.8	174.6 \pm 5.0	5.4 \pm 0.1	73.1 \pm 4.3	37.5 \pm 2.0	1.96 \pm 0.18	31.1
<i>Renealmia cernua</i>	Shade	351 \pm 50	3.53 \pm 0.13	31.6 \pm 1.4	21.5 \pm 1.9	103.8 \pm 2.7	n.d.	59.0 \pm 3.9	34.3 \pm 1.7	1.73 \pm 0.12	34.7
ANGIOSPERMS-											
DICOTYLEDONS											
Acanthaceae											
<i>Aphelandra</i>	Shade	650 \pm 39	2.78 \pm 0.01	37.0 \pm 1.5	28.3 \pm 0.9	112.0 \pm 0.3	2.6 \pm 0.3	28.6 \pm 1.2	25.7 \pm 1.0	1.11 \pm 0.05	36.8
<i>campanensis</i>											
Anacardiaceae											
<i>Anacardium excelsum</i>	Sun	485 \pm 26	4.04 \pm 0.03	35.5 \pm 0.4	52.7 \pm 1.7	110.4 \pm 2.1	2.6 \pm 0.0	11.5 \pm 0.9	68.3 \pm 1.3	0.17 \pm 0.01	
	Shade	739 \pm 25	3.27 \pm 0.07	38.6 \pm 0.4	19.9 \pm 0.3	125.7 \pm 2.0	6.0 \pm 0.6	30.5 \pm 1.1	33.6 \pm 2.9	0.93 \pm 0.10	
<i>Spondias mombin</i>	Sun	475 \pm 26	3.48 \pm 0.09	35.8 \pm 1.1	34.3 \pm 0.4	124.8 \pm 2.2	1.2 \pm 0.6	3.2 \pm 1.7	72.5 \pm 1.6	0.04 \pm 0.02	90.0
	Shade	574 \pm 6	3.24 \pm 0.06	37.5 \pm 1.6	26.0 \pm 1.8	132.9 \pm 1.5	n.d.	15.0 \pm 4.3	67.6 \pm 2.6	0.27 \pm 0.09	81.1
<i>Tapirira guianensis</i>	Sun	612 \pm 11	3.76 \pm 0.01	29.5 \pm 0.3	50.8 \pm 1.1	115.8 \pm 0.7	n.d.	8.7 \pm 0.3	73.0 \pm 0.4	0.12 \pm 0.00	104.2
	Shade	821 \pm 17	3.08 \pm 0.06	34.0 \pm 0.5	28.5 \pm 1.2	135.8 \pm 3.9	n.d.	15.8 \pm 2.8	53.7 \pm 2.5	0.30 \pm 0.07	110.0

Table 2. (continued)

Family and species	Leaf type	Chl a+b	Chl a/b	N	VAZ	L	Lx	α -Car	β -Car	α - β -Car	LMA
Annonaceae											
<i>Desmopsis panamensis</i>	Shade	353 \pm 12	3.37 \pm 0.08	34.6 \pm 0.8	19.3 \pm 0.3	129.9 \pm 1.3	10.0 \pm 1.4	47.3 \pm 0.1	26.6 \pm 0.4	1.78 \pm 0.03	32.6
Araliaceae											
<i>Dendropanax arboreus</i>	Shade	720 \pm 15	2.99 \pm 0.06	31.1 \pm 1.3	25.1 \pm 0.7	108.4 \pm 2.8	1.4 \pm 0.7	27.7 \pm 2.2	29.8 \pm 0.5	0.93 \pm 0.06	44.2
Asteraceae											
<i>Mikania leiostachya</i>	Sun	548 \pm 18	3.54 \pm 0.05	27.6 \pm 0.3	56.3 \pm 1.0	110.8 \pm 1.0	n.d.	5.7 \pm 0.3	66.3 \pm 0.6	0.09 \pm 0.00	71.1
	Shade	520 \pm 31	3.38 \pm 0.03	30.1 \pm 0.6	31.9 \pm 0.6	107.4 \pm 1.8	n.d.	9.0 \pm 0.8	54.3 \pm 0.8	0.17 \pm 0.02	60.5
Bignoniaceae											
<i>Amphitecna latifolia</i>	Shade	643 \pm 64	3.25 \pm 0.03	35.4 \pm 0.3	27.7 \pm 0.5	127.4 \pm 1.3	n.d.	31.5 \pm 0.9	36.7 \pm 1.6	0.86 \pm 0.06	74.2
<i>Phryganocydia corymbosa</i>	Sun	683 \pm 43	4.17 \pm 0.08	26.3 \pm 0.2	47.0 \pm 2.6	98.7 \pm 2.4	1.0 \pm 0.5	13.0 \pm 0.8	48.8 \pm 2.1	0.27 \pm 0.02	91.1
	Shade	502 \pm 21	3.50 \pm 0.09	30.7 \pm 0.1	37.2 \pm 1.9	113.3 \pm 0.9	0.6 \pm 0.6	18.4 \pm 2.8	42.7 \pm 1.9	0.44 \pm 0.09	80.0
Burseraceae											
<i>Bursera simaruba</i>	Sun	372 \pm 39	4.47 \pm 0.12	30.9 \pm 1.7	43.5 \pm 2.4	108.9 \pm 2.2	5.6 \pm 0.9	n.d.	82.6 \pm 0.8		88.4
	Shade	405 \pm 7	3.55 \pm 0.02	33.4 \pm 0.6	29.2 \pm 0.9	107.8 \pm 1.0	1.6 \pm 0.1	n.d.	64.9 \pm 0.3		42.1
Cecropiaceae											
<i>Cecropia peltata</i>	Sun	529 \pm 18	4.36 \pm 0.09	32.9 \pm 1.2	56.8 \pm 6.3	89.1 \pm 3.8	0.7 \pm 0.7	4.8 \pm 1.7	74.9 \pm 2.3	0.07 \pm 0.03	74.7
	Shade	470 \pm 53	4.08 \pm 0.11	33.0 \pm 1.7	40.4 \pm 2.7	95.1 \pm 0.4	1.0 \pm 0.5	12.1 \pm 2.8	65.2 \pm 3.8	0.19 \pm 0.05	64.2
Chloranthaceae											
<i>Hedyosmum bonplandianum</i>	Shade	649 \pm 11	3.00 \pm 0.01	31.9 \pm 1.6	21.8 \pm 2.3	115.3 \pm 6.0	n.d.	31.1 \pm 1.1	25.5 \pm 0.7	1.22 \pm 0.07	45.3
Chrysobalanaceae											
<i>Chrysobalanus icaco</i>	Shade	526 \pm 12	3.08 \pm 0.00	38.5 \pm 1.0	25.1 \pm 0.6	150.3 \pm 0.9	2.7 \pm 0.3	17.7 \pm 0.9	54.5 \pm 3.2	0.33 \pm 0.03	91.1
<i>Licania jefensis</i>	Sun	243 \pm 11	5.15 \pm 0.07	32.7 \pm 1.0	82.7 \pm 6.2	153.0 \pm 5.3	n.d.	24.0 \pm 1.2	76.9 \pm 2.2	0.31 \pm 0.02	140.5
	Shade	381 \pm 5	3.92 \pm 0.02	33.1 \pm 0.3	33.1 \pm 1.7	136.9 \pm 3.6	n.d.	45.4 \pm 1.7	44.9 \pm 3.0	1.02 \pm 0.10	128.9
Clusiaceae											
<i>Clusia pratensis</i>	Sun	251 \pm 17	2.44 \pm 0.11	45.8 \pm 2.3	63.1 \pm 8.9	211.7 \pm 11.9	n.d.	n.d.	103 \pm 5.5		
<i>Marila laxiflora</i>	Sun	377 \pm 9	3.61 \pm 0.05	35.9 \pm 1.1	54.7 \pm 2.6	138.7 \pm 2.4	n.d.	19.6 \pm 1.6	61.4 \pm 2.1	0.32 \pm 0.03	115.3
	Shade	512 \pm 33	3.32 \pm 0.08	39.1 \pm 0.8	31.6 \pm 1.8	137.3 \pm 4.5	n.d.	47.6 \pm 4.3	41.2 \pm 4.0	1.20 \pm 0.22	106.3
Combretaceae											
<i>Combretum fruticosum</i>	Sun	594 \pm 26	4.00 \pm 0.11	33.2 \pm 0.1	53.0 \pm 1.9	118.2 \pm 0.9	2.7 \pm 0.2	14.5 \pm 1.5	55.4 \pm 0.8	0.26 \pm 0.03	62.1
	Shade	619 \pm 41	3.21 \pm 0.11	32.6 \pm 1.7	32.6 \pm 0.4	102.7 \pm 1.8	9.3 \pm 0.7	24.6 \pm 1.8	34.6 \pm 1.0	0.71 \pm 0.07	90.5
<i>Terminalia amazonia</i>	Sun	542 \pm 80	3.57 \pm 0.06	31.1 \pm 1.3	37.9 \pm 2.8	121.0 \pm 3.0	n.d.	9.0 \pm 1.1	59.8 \pm 3.2	0.15 \pm 0.03	118.4
	Shade	685 \pm 54	2.88 \pm 0.04	38.1 \pm 1.6	29.6 \pm 1.5	113.9 \pm 2.9	1.3 \pm 0.7	19.4 \pm 1.9	29.2 \pm 0.3	0.50 \pm 0.05	95.8
Connaraceae											
<i>Connarus williamsii</i>	Shade	373 \pm 12	3.63 \pm 0.07	34.6 \pm 0.4	21.7 \pm 0.4	128.0 \pm 1.6	1.7 \pm 0.9	55.6 \pm 1.3	31.3 \pm 0.3	1.78 \pm 0.02	46.3
Ericaceae											
<i>Cavendishia stenophylla</i>	Shade	558 \pm 9	2.88 \pm 0.02	31.1 \pm 0.7	21.9 \pm 0.3	115.3 \pm 2.2	3.5 \pm 0.3	0.7 \pm 0.1	53.6 \pm 1.2	0.01 \pm 0.00	76.8
Euphorbiaceae											
<i>Hyeronima alchorneoides</i>	Sun	622 \pm 19	3.36 \pm 0.15	29.5 \pm 1.2	33.3 \pm 1.0	99.4 \pm 4.6	0.4 \pm 0.4	26.6 \pm 4.0	44.0 \pm 2.5	0.62 \pm 0.13	51.6
	Shade	509 \pm 38	2.97 \pm 0.14	31.7 \pm 1.7	27.7 \pm 0.9	101.8 \pm 2.1	3.0 \pm 0.4	30.5 \pm 0.6	34.8 \pm 2.1	0.88 \pm 0.04	43.2
<i>Pera arborea</i>	Sun	248 \pm 4	4.58 \pm 0.05	29.4 \pm 0.2	26.2 \pm 1.0	165.5 \pm 4.5	n.d.	5.9 \pm 0.5	95.9 \pm 3.5	0.06 \pm 0.00	72.1
	Shade	681 \pm 17	3.58 \pm 0.08	34.5 \pm 0.5	24.3 \pm 0.6	110.0 \pm 2.5	1.5 \pm 0.4	43.0 \pm 0.9	33.6 \pm 1.7	1.29 \pm 0.07	53.2
Fabaceae-Mimosoideae											
<i>Albizia guachapele</i>	Sun	322 \pm 14	5.22 \pm 0.14	43.8 \pm 1.2	57.8 \pm 9.3	150.0 \pm 4.6	6.2 \pm 0.7	5.5 \pm 0.4	90.8 \pm 1.7	0.06 \pm 0.00	60.0
	Shade	507 \pm 39	3.79 \pm 0.02	35.8 \pm 1.3	26.4 \pm 1.9	121.2 \pm 8.6	30.9 \pm 6.3	5.4 \pm 1.3	81.9 \pm 5.9	0.07 \pm 0.02	78.9
Fabaceae-Papilionoideae											
<i>Dalbergia monetaria</i>	Sun	809 \pm 75	3.95 \pm 0.06	33.0 \pm 0.4	39.3 \pm 2.0	95.4 \pm 1.6	5.9 \pm 0.5	5.1 \pm 0.5	86.1 \pm 2.1	0.06 \pm 0.01	103.2
	Shade	934 \pm 89	3.16 \pm 0.13	38.2 \pm 1.6	21.1 \pm 2.5	116.8 \pm 5.6	16.3 \pm 2.4	18.0 \pm 4.4	58.3 \pm 4.9	0.32 \pm 0.10	80.3
<i>Ormosia macrocalyx</i>	Sun	637 \pm 37	3.75 \pm 0.12	36.8 \pm 0.9	36.2 \pm 10.2	150.8 \pm 6.8	0.7 \pm 0.7	9.9 \pm 2.7	63.4 \pm 4.1	0.16 \pm 0.05	92.1
	Shade	740 \pm 28	3.09 \pm 0.04	38.5 \pm 0.6	15.0 \pm 0.7	138.6 \pm 1.7	6.7 \pm 0.6	20.9 \pm 1.9	40.8 \pm 1.8	0.52 \pm 0.07	65.8
Flacourtiaceae											
<i>Lindackeria laurina</i>	Sun	484 \pm 19	4.13 \pm 0.09	38.6 \pm 0.5	41.0 \pm 2.3	125.6 \pm 4.3	1.6 \pm 1.6	15.5 \pm 1.1	53.8 \pm 0.5	0.29 \pm 0.02	57.9
	Shade	575 \pm 26	3.88 \pm 0.14	37.2 \pm 0.9	34.9 \pm 1.3	119.5 \pm 1.5	3.5 \pm 0.9	23.0 \pm 3.7	53.0 \pm 5.8	0.46 \pm 0.12	65.3
Gentianaceae											
<i>Lisianthus jefensis</i>	Sun	757 \pm 59	3.51 \pm 0.09	45.5 \pm 1.2	58.3 \pm 3.9	143.1 \pm 2.1	n.d.	1.5 \pm 0.2	57.4 \pm 5.1	0.03 \pm 0.00	105.8
	Shade	722 \pm 42	3.35 \pm 0.06	41.2 \pm 1.5	20.9 \pm 1.9	122.6 \pm 1.7	n.d.	1.5 \pm 0.2	48.1 \pm 3.8	0.03 \pm 0.01	52.1
Gesneriaceae											
<i>Columnnea billbergiana</i>	Shade	439 \pm 33	3.15 \pm 0.02	31.4 \pm 1.0	32.2 \pm 0.2	105.6 \pm 5.0	n.d.	13.1 \pm 3.4	40.8 \pm 3.1	0.31 \pm 0.06	93.2
Hippocrateaceae											
<i>Tontelea ovalifolia</i>	Sun	635 \pm 76	3.47 \pm 0.19	38.1 \pm 1.5	72.0 \pm 4.3	158.2 \pm 10.3	3.6 \pm 0.5	17.2 \pm 2.1	44.2 \pm 3.2	0.39 \pm 0.02	
	Shade	819 \pm 41	3.39 \pm 0.08	40.0 \pm 1.0	31.5 \pm 1.9	115.5 \pm 1.6	11.5 \pm 1.3	40.7 \pm 2.6	21.4 \pm 2.5	2.00 \pm 0.39	
Humiriaceae											
<i>Vantanea depleta</i>	Sun	478 \pm 24	4.04 \pm 0.02	32.7 \pm 0.3	62.1 \pm 3.0	119.9 \pm 2.7	n.d.	10.1 \pm 1.1	77.5 \pm 1.6	0.13 \pm 0.02	112.6
	Shade	900 \pm 27	3.03 \pm 0.07	37.5 \pm 0.5	25.3 \pm 0.8	122.5 \pm 1.6	3.3 \pm 0.5	48.5 \pm 1.0	29.8 \pm 1.5	1.63 \pm 0.05	130.5
Lecythidaceae											
<i>Gustavia superba</i>	Shade	846 \pm 53	3.28 \pm 0.03	39.7 \pm 0.5	23.5 \pm 0.1	110.6 \pm 2.3	5.2 \pm 0.4	53.9 \pm 0.6	20.9 \pm 0.8	2.59 \pm 0.07	53.7

Table 2. (continued)

Family and species	Leaf type	Chl <i>a</i> + <i>b</i>	Chl <i>a</i> / <i>b</i>	N	VAZ	L	Lx	α-Car	β-Car	α-/β-Car	LMA
Loganiaceae											
<i>Strychnos panamensis</i>	Shade	330 ± 10	3.28 ± 0.03	26.9 ± 0.3	14.8 ± 0.2	103.2 ± 2.2	4.0 ± 1.2	36.2 ± 0.5	18.9 ± 1.3	1.93 ± 0.11	41.1
Malpighiaceae											
<i>Stigmaphyllon hypargyreum</i>	Sun	499 ± 24	3.85 ± 0.05	38.1 ± 1.5	52.1 ± 3.8	113.6 ± 4.4	n.d.	11.2 ± 0.4	66.7 ± 1.6	0.17 ± 0.01	
Melastomataceae											
<i>Miconia argentea</i>	Sun	454 ± 13	3.45 ± 0.09	29.7 ± 0.8	33.1 ± 4.0	130.6 ± 14.3	n.d.	28.9 ± 6.8	47.0 ± 8.0	0.70 ± 0.25	
	Shade	486 ± 16	2.89 ± 0.10	34.1 ± 1.0	15.0 ± 0.6	117.7 ± 6.7	1.4 ± 0.4	54.1 ± 1.0	22.5 ± 1.1	2.42 ± 0.07	
<i>Pilocosta campanensis</i>	Shade	298 ± 6	3.22 ± 0.06	29.5 ± 1.3	21.9 ± 0.3	112.9 ± 1.5	n.d.	14.8 ± 0.4	45.0 ± 1.5	0.33 ± 0.02	28.9
Meliaceae											
<i>Carapa guianensis</i>	Sun	406 ± 19	4.45 ± 0.23	34.0 ± 5.5	56.5 ± 5.9	126.3 ± 5.3	n.d.	9.9 ± 0.8	67.8 ± 0.5	0.15 ± 0.01	102.1
	Shade	467 ± 19	3.83 ± 0.24	36.1 ± 1.0	33.2 ± 2.3	122.5 ± 1.7	2.5 ± 0.3	15.8 ± 1.3	54.0 ± 2.6	0.29 ± 0.03	112.1
Moraceae											
<i>Chlorophora tinctoria</i>	Sun	699 ± 30	4.09 ± 0.04	36.1 ± 0.6	42.5 ± 1.5	138.9 ± 1.1	2.2 ± 0.3	3.0 ± 0.4	76.8 ± 1.1	0.04 ± 0.01	82.6
	Shade	632 ± 29	3.61 ± 0.07	37.3 ± 0.3	24.9 ± 0.4	129.9 ± 4.7	2.5 ± 0.2	10.4 ± 2.7	54.6 ± 1.6	0.19 ± 0.06	75.3
Myristicaceae											
<i>Otoba novogranatensis</i>	Sun	426 ± 37	3.35 ± 0.04	31.5 ± 0.4	37.3 ± 1.5	102.3 ± 7.6	10.4 ± 0.7	17.6 ± 3.5	59.5 ± 4.0	0.30 ± 0.08	104.2
	Shade	519 ± 41	2.85 ± 0.07	34.6 ± 1.0	16.2 ± 1.2	85.3 ± 3.1	25.1 ± 0.9	46.0 ± 2.2	21.4 ± 1.1	2.15 ± 0.12	60.7
<i>Virola elongata</i>	Sun	565 ± 8	3.46 ± 0.16	33.4 ± 1.2	35.6 ± 4.8	94.4 ± 1.0	57.9 ± 2.4	15.6 ± 4.7	67.7 ± 4.9	0.24 ± 0.09	148.0
	Shade	929 ± 99	2.91 ± 0.06	39.0 ± 1.8	13.6 ± 2.3	101.3 ± 7.0	37.6 ± 5.8	45.4 ± 0.8	26.7 ± 2.2	1.71 ± 0.13	134.7
<i>Virola sebifera</i>	Sun	241 ± 24	2.33 ± 0.25	43.0 ± 1.7	59.1 ± 4.3	128.3 ± 5.5	53.4 ± 6.9	47.8 ± 5.1	93.7 ± 7.5	0.51 ± 0.08	66.6
	Shade	541 ± 46	2.14 ± 0.02	50.9 ± 0.6	18.8 ± 1.4	129.1 ± 2.9	46.7 ± 0.6	68.5 ± 2.3	31.9 ± 2.0	2.15 ± 0.07	63.2
Myrsinaceae											
<i>Parathesis bicolor</i>	Sun	238 ± 15	4.59 ± 0.13	33.2 ± 1.0	60.6 ± 4.9	117.8 ± 2.2	n.d.	7.4 ± 0.8	72.2 ± 1.1	0.10 ± 0.01	155.8*
	Shade	455 ± 71	3.64 ± 0.16	37.5 ± 0.9	33.9 ± 1.0	120.2 ± 1.9	n.d.	21.6 ± 4.0	52.6 ± 4.3	0.43 ± 0.10	125.8
Myrtaceae											
<i>Myrcia sylvatica</i>	Sun	384 ± 14	3.76 ± 0.12	30.1 ± 0.4	28.8 ± 2.5	122.7 ± 7.5	n.d.	17.8 ± 1.9	53.3 ± 3.3	0.34 ± 0.06	132.6*
	Shade	568 ± 39	3.16 ± 0.08	39.6 ± 1.0	31.6 ± 1.9	133.4 ± 5.7	1.6 ± 0.2	28.1 ± 3.5	39.5 ± 7.2	0.78 ± 0.20	182.1*
Ochnaceae											
<i>Cespedezia macrophylla</i>	Sun	652 ± 67	3.62 ± 0.04	35.3 ± 2.9	30.8 ± 2.7	145.3 ± 7.2	0.9 ± 0.9	33.2 ± 4.2	45.6 ± 1.9	0.73 ± 0.08	90.5
	Shade	553 ± 26	3.76 ± 0.05	39.0 ± 1.7	23.9 ± 3.7	129.0 ± 3.8	0.6 ± 0.6	40.8 ± 5.0	36.7 ± 5.9	1.21 ± 0.31	68.9
Passifloraceae											
<i>Passiflora vitifolia</i>	Sun	205 ± 12	4.79 ± 0.30	28.5 ± 0.9	80.9 ± 6.2	114.4 ± 3.6	n.d.	4.5 ± 0.4	81.0 ± 2.8	0.06 ± 0.01	
	Shade	373 ± 3	3.44 ± 0.00	34.4 ± 0.3	25.0 ± 1.1	117.2 ± 0.9	n.d.	36.2 ± 2.6	32.9 ± 0.7	1.11 ± 0.10	
Phytolaccaceae											
<i>Trichostigma octandrum</i>	Sun	551 ± 31	4.13 ± 0.09	39.4 ± 0.3	66.5 ± 4.1	136.2 ± 1.2	n.d.	24.5 ± 1.4	67.3 ± 0.6	0.36 ± 0.07	61.1
	Shade	718 ± 97	3.98 ± 0.11	33.1 ± 0.6	21.8 ± 0.6	100.0 ± 5.2	0.4 ± 0.4	37.6 ± 2.7	37.1 ± 1.7	1.02 ± 0.11	61.6
Piperaceae											
<i>Piper reticulatum</i>	Shade	754 ± 73	3.19 ± 0.08	37.6 ± 1.9	34.3 ± 0.4	101.4 ± 3.7	1.3 ± 0.0	47.3 ± 2.1	22.1 ± 0.1	2.14 ± 0.11	42.1
Polygonaceae											
<i>Coccoloba uvifera</i>	Shade	678 ± 27	3.24 ± 0.05	34.9 ± 0.5	32.7 ± 0.1	142.5 ± 1.3	n.d.	15.4 ± 2.2	47.7 ± 3.0	0.33 ± 0.06	86.3
Rhamnaceae											
<i>Gouania lupuloides</i>	Shade	214 ± 20	3.30 ± 0.07	29.2 ± 1.3	27.3 ± 3.5	126.8 ± 6.1	n.d.	43.7 ± 2.8	31.0 ± 1.5	1.42 ± 0.16	51.1
Rhizophoraceae											
<i>Cassipourea elliptica</i>	Shade	514 ± 59	2.96 ± 0.05	28.6 ± 3.0	25.5 ± 2.2	94.9 ± 6.8	0.4 ± 0.4	46.2 ± 1.0	18.1 ± 1.2	2.58 ± 0.22	49.5
<i>Rhizophora mangle</i>	Shade	561 ± 14	3.61 ± 0.02	34.0 ± 0.8	27.8 ± 0.9	133.6 ± 4.0	n.d.	2.6 ± 0.2	66.0 ± 1.9	0.04 ± 0.00	73.7
Rubiaceae											
<i>Psychotria poeppigiana</i>	Sun (gap)	431 ± 96	3.59 ± 0.17	35.6 ± 2.7	29.5 ± 1.4	118.2 ± 9.2	n.d.	41.9 ± 1.2	35.6 ± 5.2	1.23 ± 0.17	36.8
	Shade	338 ± 37	3.51 ± 0.12	37.2 ± 2.2	21.4 ± 2.1	121.8 ± 5.1	n.d.	49.2 ± 0.4	25.4 ± 1.8	1.96 ± 0.14	28.9
Sapindaceae											
<i>Serjania mexicana</i>	Sun	470 ± 30	4.01 ± 0.05	33.7 ± 1.1	45.7 ± 4.9	128.6 ± 7.4	n.d.	10.7 ± 2.7	71.9 ± 1.6	0.15 ± 0.04	83.7
	Shade	500 ± 42	3.46 ± 0.09	34.4 ± 1.2	24.0 ± 1.3	111.7 ± 3.6	2.5 ± 0.2	31.6 ± 1.8	40.4 ± 1.8	0.78 ± 0.05	75.3
Simaroubaceae											
<i>Quassia amara</i>	Shade	523 ± 21	3.08 ± 0.01	36.6 ± 0.2	14.2 ± 0.3	129.5 ± 1.3	3.9 ± 0.7	44.0 ± 0.3	26.1 ± 0.7	1.69 ± 0.04	31.1
Theaceae											
<i>Freziera candicans</i>	Sun	370 ± 31	4.32 ± 0.11	34.1 ± 1.1	58.1 ± 0.8	126.0 ± 2.5	n.d.	2.3 ± 0.1	80.7 ± 3.0	0.03 ± 0.00	183.2*
	Shade	438 ± 66	3.61 ± 0.09	29.7 ± 0.8	24.7 ± 0.7	108.9 ± 1.5	n.d.	6.1 ± 0.4	52.8 ± 4.4	0.12 ± 0.02	157.9
Thymelaeaceae											
<i>Daphnopsis americana</i>	Shade	605 ± 29	3.18 ± 0.09	40.7 ± 1.1	20.8 ± 0.7	144.1 ± 3.2	1.8 ± 0.3	0.9 ± 0.2	65.1 ± 1.4	0.01 ± 0.00	35.3
Tiliaceae											
<i>Luehea seemannii</i>	Sun	581 ± 53	3.03 ± 0.59	33.1 ± 5.4	44.8 ± 8.8	124.7 ± 7.1	n.d.	24.8 ± 4.9	50.8 ± 11.8	0.50 ± 0.07	
	Shade	543 ± 13	1.53 ± 0.04	26.8 ± 0.7	25.1 ± 1.4	106.3 ± 4.6	n.d.	22.8 ± 2.6	13.3 ± 1.4	1.72 ± 0.15	42.1
Turneraceae											
<i>Turnera panamensis</i>	Shade	404 ± 15	3.01 ± 0.05	34.7 ± 1.8	21.5 ± 1.5	127.5 ± 7.0	2.4 ± 1.2	0.4 ± 0.4	59.8 ± 2.7	0.01 ± 0.01	32.6
Verbenaceae											
<i>Avicennia germinans</i>	Shade	461 ± 37	3.35 ± 0.04	33.7 ± 1.2	32.2 ± 1.5	121.1 ± 3.3	0.6 ± 0.6	2.1 ± 0.2	63.9 ± 7.7	0.03 ± 0.00	67.4
<i>Petrea aspera</i>	Shade	516 ± 20	3.14 ± 0.05	34.3 ± 1.3	17.4 ± 0.6	122.0 ± 1.8	2.3 ± 0.5	47.9 ± 0.6	22.1 ± 0.9	2.18 ± 0.07	53.7

Table 2. (continued)

Family and species	Leaf type	Chl <i>a+b</i>	Chl <i>a/b</i>	N	VAZ	L	Lx	α -Car	β -Car	α -/ β -Car	LMA
Violaceae											
<i>Hybanthus prunifolius</i>	Shade	348 \pm 40	3.62 \pm 0.05	23.6 \pm 3.3	21.7 \pm 7.3	105.1 \pm 6.8	n.d.	52.2 \pm 8.7	42.2 \pm 10.6	0.75 \pm 0.31	22.1
Viscaceae											
<i>Dendrophthora ambigua</i>	Sun	250 \pm 2	4.48 \pm 0.19	35.9 \pm 0.9	85.2 \pm 5.4	174.4 \pm 9.5	n.d.	n.d.	56.7 \pm 3.5		243.2
Vitaceae											
<i>Vitis tiliifolia</i>	Sun	257 \pm 3	3.88 \pm 0.20	36.2 \pm 1.3	63.2 \pm 3.3	119.7 \pm 0.1	n.d.	5.2 \pm 0.5	75.1 \pm 0.9	0.07 \pm 0.01	74.7
	Shade	457 \pm 23	3.08 \pm 0.13	40.3 \pm 2.2	26.2 \pm 1.3	138.8 \pm 3.0	5.3 \pm 1.9	23.1 \pm 1.8	46.8 \pm 4.3	0.51 \pm 0.09	66.8
Vochysiaceae											
<i>Vochysia ferruginea</i>	Sun	365 \pm 27	4.55 \pm 0.21	24.6 \pm 1.6	18.9 \pm 3.3	103.4 \pm 3.9	3.0 \pm 0.5	10.3 \pm 1.9	67.0 \pm 2.0	0.16 \pm 0.03	83.7
	Shade	857 \pm 20	3.53 \pm 0.01	32.3 \pm 1.0	25.4 \pm 0.6	106.8 \pm 0.8	6.5 \pm 0.7	29.1 \pm 1.1	51.2 \pm 0.8	0.57 \pm 0.01	116.8

Inga species (Fabaceae-Mimosoideae) studied previously (Matsubara *et al.* 2008). Very high amounts of Lx were detected in both sun and shade leaves of *Virola elongata* and *V. sebifera* (Myristicaceae), as has been reported for *V. surinamensis* (Matsubara *et al.* 2008). Intermediate levels of Lx (5–10 mmol mol Chl⁻¹) were found in shade leaves of 11 species and 36 species contained low levels of Lx (<5 mmol mol Chl⁻¹) in shade and/or sun leaves. In 31 species, Lx was not detectable.

In order to compare sun-acclimation responses of photosynthetic pigments in the two major life forms of tropical forest canopies, i.e. trees and lianas, data of sun leaves were combined for 12 tree species and nine liana species from seasonally dry forests (Table 3). In sun leaves lianas accumulated significantly larger amounts of the V-cycle pigments on a Chl basis than trees ($P \leq 0.001$). Levels of other pigments did not differ significantly between the two groups.

Sun-shade leaf characteristics

Leaf dry mass, Chl *a+b* and carotenoid contents (all on a leaf area basis) were compared between shade and sun leaves (Fig. 1). Data are presented in box plots, illustrating species distribution for each leaf type. Generally, shade leaves had lower LMA than sun leaves (Fig. 1A; $P < 0.001$). Sun leaves of *Dendrophthora ambigua* (mistletoe, Viscaceae) and *Freziera candicans* (Theaceae) and shade leaves of *Myrcia sylvatica* (Myrtaceae) and *F. candicans* showed the highest LMA values for each leaf type. Shade leaves tended to contain higher amounts of Chl per unit leaf area than sun leaves (Fig. 1B; $P = 0.015$), but there was no significant difference in the level of total carotenoids (Fig. 1C).

The amounts of pigments on a leaf dry mass basis were plotted against the parameter of leaf structural trait, LMA, in Fig. 2. Although data for shade and sun leaves are strongly overlapping, those with very low LMA and high pigment contents on a mass basis mostly belong to shade leaves. Similar observations for Chl *a+b* and total carotenoids (Fig. 2A, B, respectively) indicate a common trend between LMA and accumulation of these photosynthetic pigments: the lower the LMA, the higher the pigment contents per leaf dry mass. In the lowest range of LMA, leaves of different species varied mainly in pigment contents and barely in LMA. *Vice versa*, in the lowest range of pigment contents, species variations were largely due to different LMA. Notably, all data, representing both leaf types of a broad range of tropical forest species, formed a single correlation between LMA and mass-based pigment levels.

Figure 3 depicts data for different carotenoids based on leaf area (Fig. 3A), Chl *a+b* (Fig. 3B) and leaf dry mass (Fig. 3C). On a leaf area basis, shade leaves, having more Chl (Fig. 1B), also contained more N than sun leaves (Fig. 3A). Likewise, α -Car accumulated more strongly in shade leaves, whereas VAZ and β -Car exhibited an opposite trend, i.e. higher in sun than in shade leaves. Regardless of the leaf type, species varied strongly in the amounts of L, or L plus Lx (LxL). When the data were expressed on a Chl basis (Fig. 3B), sun-shade patterns remained unchanged for VAZ, α - and β -Car whereas the differences between the two leaf types disappeared for N and variation was greatly reduced for LxL. The two highest values of LxL were found in shade leaves of *Vanilla planifolia* (Orchidaceae) and *Costus villosissimus* (Zingiberaceae) and sun leaves of

Table 3. Pigment comparison between sun leaves of 12 tree species and nine liana species collected in seasonally dry forests

Samples of sun leaves ($n = 3$, from individual leaves or leaflets) were collected in site 1 and 2 (Table 1). Pigment contents are given on a leaf area basis ($\mu\text{mol m}^{-2}$) for total chlorophylls (Chl *a+b*) and total carotenoids (Σ Caro) or on a Chl basis (mmol mol Chl *a+b*⁻¹) for different carotenoids. N, neoxanthin; VAZ, sum of the violaxanthin-cycle pigments; LxL, sum of lutein and lutein epoxide, α -Car, α -carotene; β -Car, β -carotene. Values are mean \pm s.e. Means of VAZ are significantly different (*) between trees and lianas ($P \leq 0.001$ by Student *t*-test)

	Leaf area basis ($\mu\text{mol m}^{-2}$)		N	Chlorophyll basis (mmol mol Chl <i>a+b</i> ⁻¹)			
	Chl <i>a+b</i>	Σ Caro		VAZ*	LxL	α -Car	β -Car
Trees	500 \pm 56	140 \pm 15	34 \pm 2	41 \pm 5	128 \pm 10	12 \pm 5	68 \pm 7
Lianas	457 \pm 31	135 \pm 8	34 \pm 1	61 \pm 3	133 \pm 9	10 \pm 1	67 \pm 2

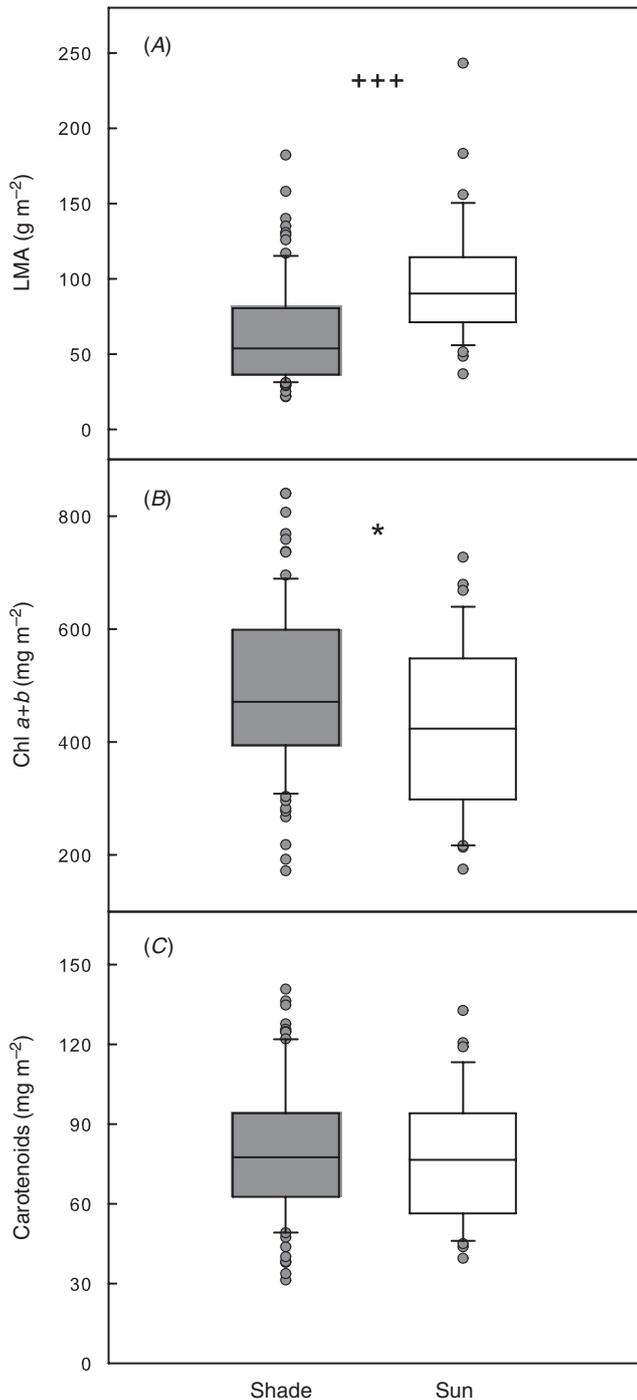


Fig. 1. (A) Leaf dry mass per area (LMA), (B) total chlorophyll (Chl *a+b*), and (C) total carotenoid contents per area of shade (grey boxes) and sun leaves (white boxes). Mean values from individual species were used in the analysis. The lower boundary of each box indicates the 25th percentile, a line within the box marks the median, and the upper boundary of the box indicates the 75th percentile. Whiskers below and above the box denote the 10th and 90th percentiles, respectively. Symbols beyond whiskers represent outlying data. Shade and sun datasets were significantly different for LMA ($P < 0.001$ by rank sum test, significance denoted by three plus signs) and for Chl *a+b* ($P = 0.015$ by *t*-test, significance denoted by an asterisk). For methods of statistical analysis, see 'Materials and methods'.

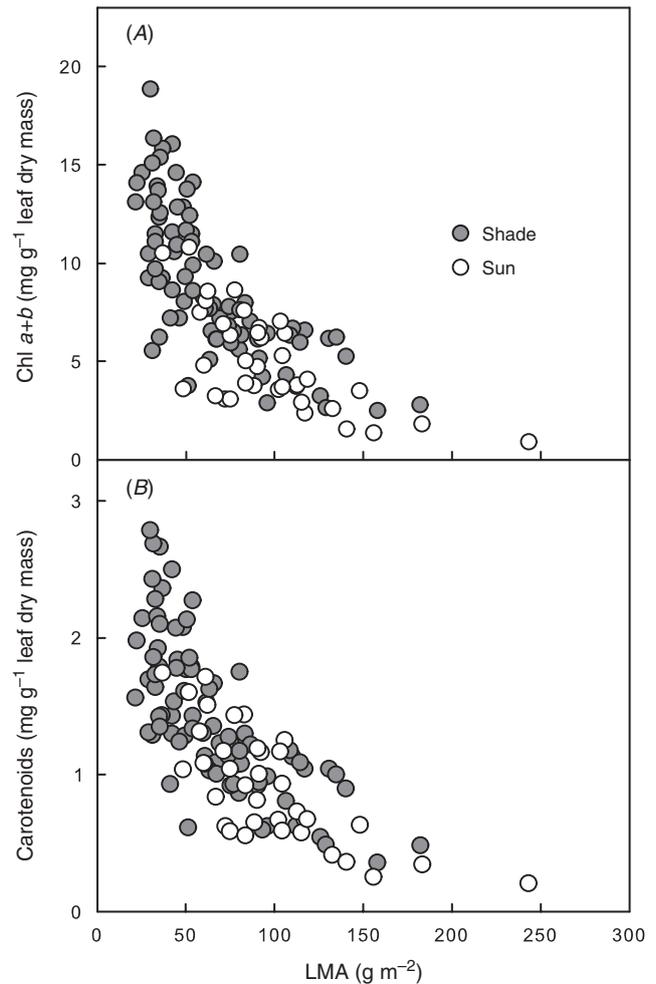


Fig. 2. Relationship between leaf dry mass per area (LMA) and (A) total chlorophyll (Chl *a+b*) and (B) total carotenoid contents on a leaf dry mass basis in shade and sun leaves. Each symbol represents a mean value ($n = 3$ for pigments, $n = 2$ for LMA) of each species and leaf type. Open symbols, sun leaves; closed symbols, shade leaves.

Vanilla planifolia and *Clusia pratensis* (Clusiaceae) (see also Table 2).

The amounts of different carotenoids were also compared on a leaf dry mass basis (Fig. 3C). Shade leaves generally had greater amounts of N as well as LxL per unit leaf dry mass than sun leaves. The species with extremely high values of LxL on a leaf dry mass basis were *Daphnopsis americana* (Thymelaeaceae), *Xiphidium caeruleum* (Haemodoraceae) and *Quassia amara* (Simaroubaceae) for shade leaves and *Psychotria poeppigiana* (Rubiaceae) for sun leaves. On a dry mass basis, the sun-shade difference in α -Car became even more evident than was seen on a leaf area or Chl *a+b* basis, with the levels in sun leaves extremely low in most species. Further, shade and sun leaves were found to be comparable in terms of VAZ and β -Car relative to leaf dry mass, contrary to the typical sun-shade behaviour of these pigments on a leaf area or Chl *a+b* basis (Fig. 3A–C). Shade leaves of *D. americana* and

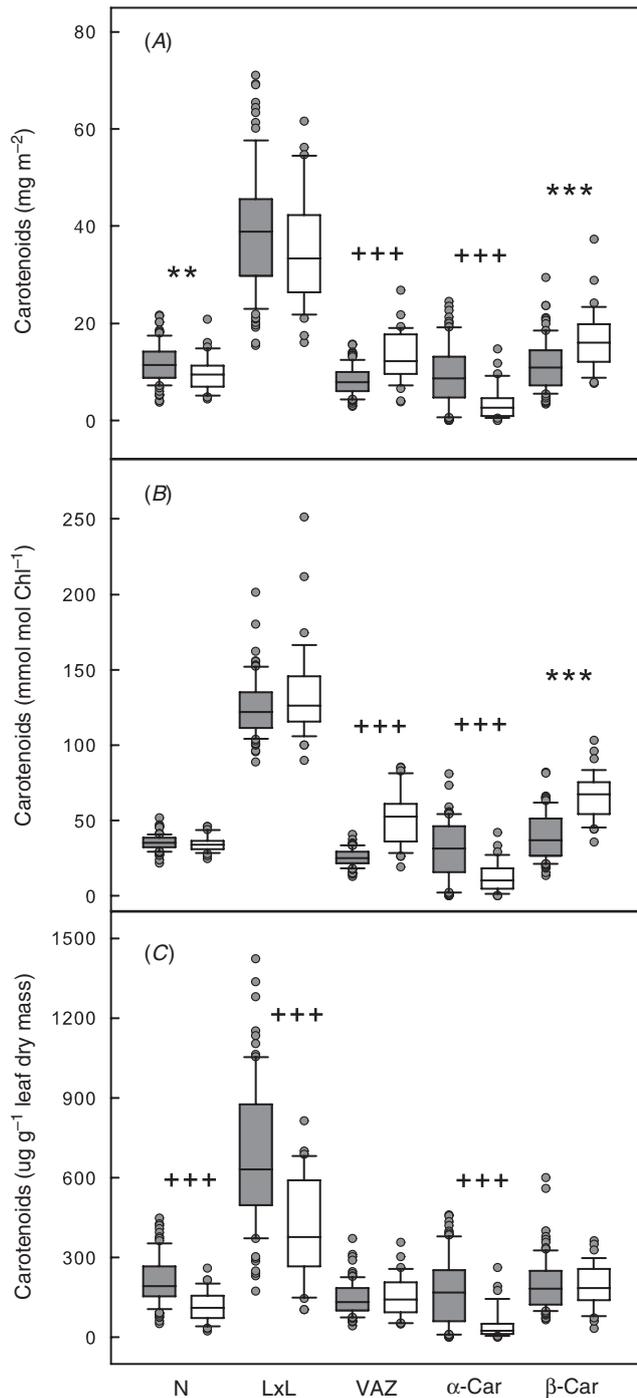


Fig. 3. Contents of different carotenoids in shade (grey boxes) and sun leaves (white boxes) based on (A) leaf area, (B) Chl $a+b$, and (C) leaf dry mass. N, neoxanthin; LxL, sum of lutein and lutein epoxide; VAZ, sum of violaxanthin and zeaxanthin; α -Car, α -carotene; β -Car, β -carotene. For explanation of box plots, see legend to Fig. 1. Asterisks and plus signs indicate significant differences between shade and sun leaves tested by t -test and rank sum test, respectively. *** and +++, $P < 0.001$; **, $P = 0.006$.

Scleria latifolia (Cyperaceae) contained extremely high levels of β -Car on a dry mass basis.

Interrelation between carotenoids in shade and sun leaves

The relationship between carotenoids synthesised in the β,β - and β,ϵ -branch is depicted in Fig. 4. On a leaf area basis (Fig. 4A), both shade and sun leaves exhibited large variations in the levels of β,β - and β,ϵ -carotenoids. Nevertheless, for a given amount of β,ϵ -carotenoids, sun leaves tended to accumulate more β,β -carotenoids than shade leaves. Very high levels of β,ϵ -carotenoids were found mostly in shade leaves. The apparently linear correlation from lower left to upper right in Fig. 4A probably reflects differences in LMA among species (Fig. 1A). When expressed on a Chl basis (Fig. 4B), the sum of β,ϵ -carotenoids was rather uniform among sun and shade leaves, centred at around $150 \text{ mmol mol Chl}^{-1}$, except for some species showing extreme values ($>200 \text{ mmol mol Chl}^{-1}$). Higher β,β -carotenoid levels in sun compared with shade leaves are clearly seen on a leaf area basis as well as on a Chl basis (Fig. 4A, B).

Figure 5 shows the relationship between the ratios $\beta,\epsilon/\beta,\beta$ -carotenoids and α/β -Car. Data from sun leaves were confined within the region of low to intermediate values of $\beta,\epsilon/\beta,\beta$ -carotenoids (~ 0.5 to 1.5) and low α/β -Car (mostly < 0.5). Among shade leaves, in contrast, pronounced variability was found for both $\beta,\epsilon/\beta,\beta$ -carotenoids and α/β -Car. There was a linear correlation between the two parameters, which is stronger for shade than for sun leaves. The slope of the regression line of shade leaves (1.75) suggests that variations in $\beta,\epsilon/\beta,\beta$ -carotenoids are accompanied by larger differences in α/β -Car. In comparison, sun leaves exhibited smaller variations in α/β -Car than in $\beta,\epsilon/\beta,\beta$ -carotenoids (slope 0.79).

The striking divergence in α/β -Car found in sun and shade leaves of different species was largely attributable to substitution between these two carotenes (Fig. 6). In leaves with high amounts of β -Car, the α -Car levels were low and *vice versa*. The sum of α - and β -Car varied little between sun and shade leaves (see legend to Fig. 6). Although a wide range of α -Car levels was found in shade leaves (from zero to $\sim 80 \text{ mmol mol}^{-1} \text{ Chl}$), sun leaves rarely reached α -Car levels $> 45 \text{ mmol mol Chl}^{-1}$ (see also Table 2).

Figure 7 illustrates the relationship between α/β -Car and VAZ. The sum of the V-cycle pigments reached highest levels in sun leaves having low α/β -Car (< 0.5). There was little variation in VAZ among shade leaves, regardless of α/β -Car. It is noteworthy that all data points, without a single exception, were scattered either at the bottom of the panel along the x-axis (mostly shade leaves) or on the left-hand side along the y-axis (mostly sun leaves), indicating that high α/β -Car and a large VAZ pool did not co-exist in any of the leaves examined.

Lx and V cycles in *Virola elongata* in situ

Given the high Lx contents in sun leaves of *Virola* species (Table 2; Matsubara *et al.* 2008), we examined for canopy leaves of *V. elongata* whether the Lx and V cycles operate in parallel in a diel manner *in situ* (Fig. 8). In the early morning, the Lx pool size was similar in sun and shade leaves but that of V differed markedly. As PAR rose to $\sim 2000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Fig. 8A), a large proportion of

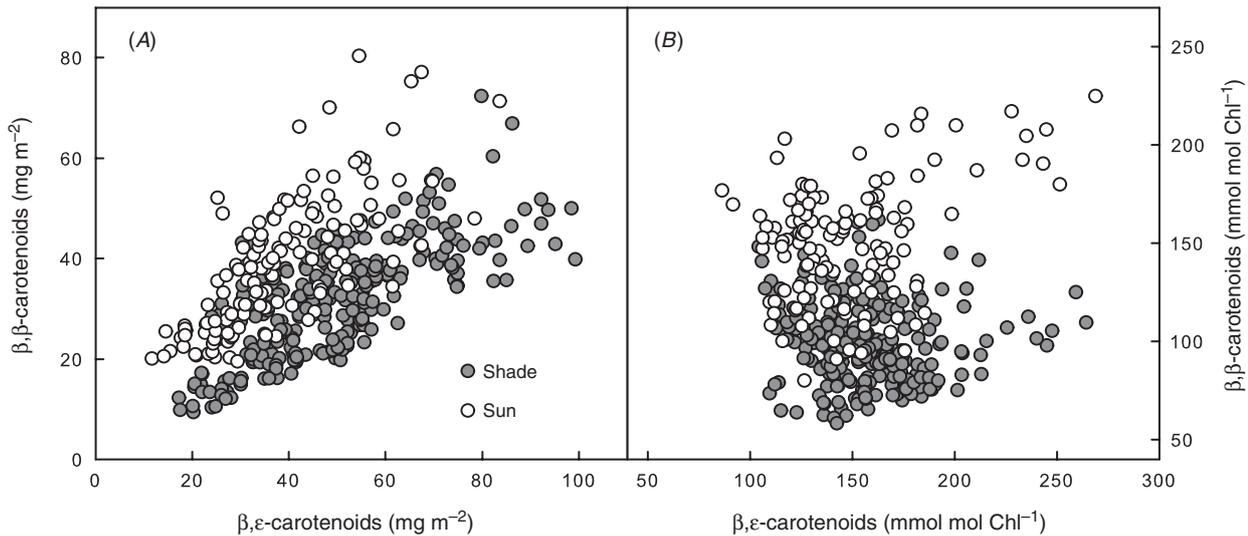


Fig. 4. Relationship between the sum of β,β - and β,ϵ -carotenoids in shade and sun leaves. Data are shown on a (A) leaf area, and (B) $Chl\ a+b$ basis. Symbols represent individual leaves or leaflets. Open symbols, sun leaves; closed symbols, shade leaves.

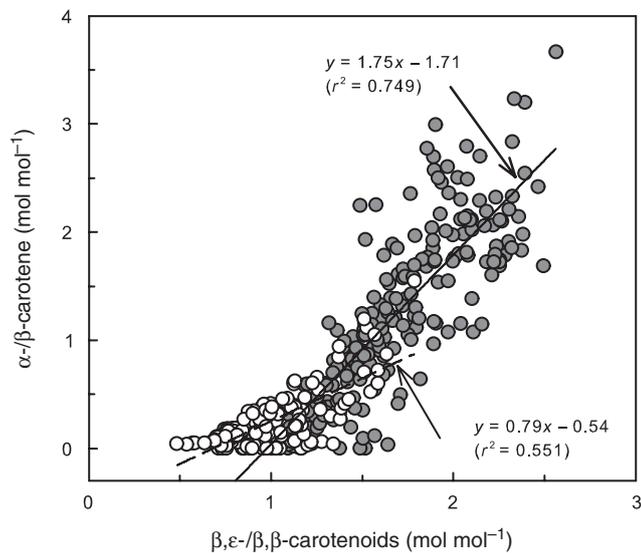


Fig. 5. Relationship between the ratios β,ϵ -/ β,β -carotenoids and α -/ β -carotene. Symbols represent individual leaves or leaflets. Open symbols, sun leaves; closed symbols, shade leaves. Solid line, linear regression of shade leaves; dashed line, linear regression of sun leaves.

Lx and V was de-epoxidised rapidly in sun leaves (Fig. 8B), accompanied by a significant reduction in F_v/F_m (see legend to Fig. 8). No substantial xanthophyll turnover was detected in shade leaves, for which PAR remained $<100\ \mu mol\ photons\ m^{-2}\ s^{-1}$ and F_v/F_m stayed constant (Fig. 8A, C, see legend to Fig. 8 for F_v/F_m). Apparent fluctuations of Lx and V in shade leaves presumably resulted from variations between individual leaves collected at different time points.

In sun leaves detached and dark-adapted at around midday, $\sim 50\%$ of V was restored by 1440 hours and 1710 hours (grey

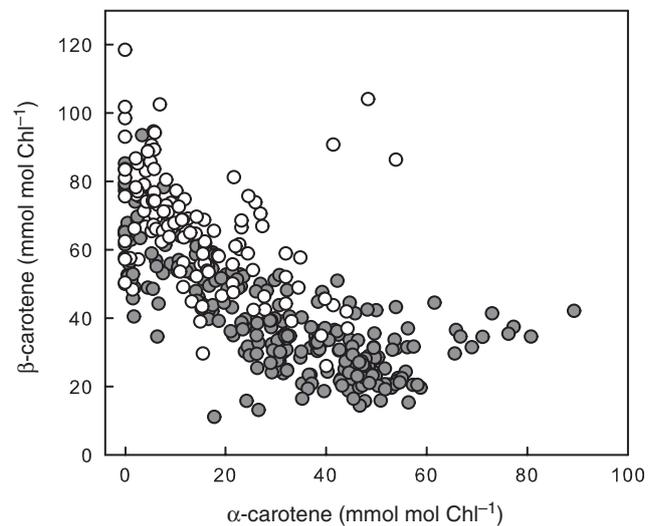


Fig. 6. Relationship between the contents of α - and β -carotene. Data are given on a $Chl\ a+b$ basis. Symbols represent individual leaves or leaflets. Open symbols, sun leaves; closed symbols, shade leaves. The sum of α - and β -carotene was 81 ± 15 and $70 \pm 13\ mmol\ mol^{-1}\ Chl$ for sun and shade leaves, respectively.

symbols in Fig. 8B), corresponding to a partial recovery of F_v/F_m in these leaves (see legend to Fig. 8). Restoration of Lx occurred more slowly than that of V in the detached and dark-adapted sun leaves, but both Lx and V were found to be fully restored by the next morning in sun leaves *in situ*. Obviously, both xanthophyll cycles were operating in a 24-h cycle in sun leaves of *V. elongata*, albeit with different restoration (epoxidation) kinetics. In this canopy experiment performed during the dry season, the amounts of Lx found in sun leaves early in the morning were considerably lower than those in dark-adapted sun leaves collected from the same tree during the rainy season (Table 2).

Discussion

Carotenoid contents as a leaf functional trait in neotropical forest species

Large-scale studies of plants from different biomes indicate a universal correlation between different leaf functional traits, such as LMA, leaf life-span, rates of photosynthesis, respiration, and nitrogen content (Reich *et al.* 1997; Wright *et al.* 2004). Correlations are generally stronger when traits are expressed on a leaf dry mass basis, which emphasises leaf economics (cost and benefit) than on a leaf area basis which considers fluxes of PAR, CO₂, and H₂O. For example, rates of photosynthesis per unit dry mass decline as LMA increases. We observed a similar trend between leaf chlorophyll and carotenoid contents, respectively, and LMA (Fig. 2). At low values of LMA, characteristic of shade leaves (Fig. 1A), pigment

content was particularly sensitive to changes in LMA, and pigment content decreased to a lesser extent as LMA increased to higher values (Fig. 2). Data points for sun and shade leaves from diverse tropical plant taxa fitted the same relationship (Fig. 2), an observation which is not necessarily true for relationships between photosynthesis rate and LMA, or leaf nitrogen content and LMA in sun and shade leaves (Santiago and Wright 2007).

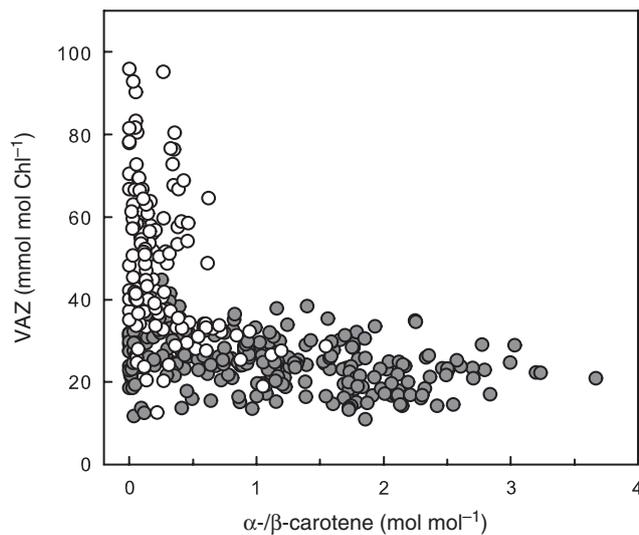
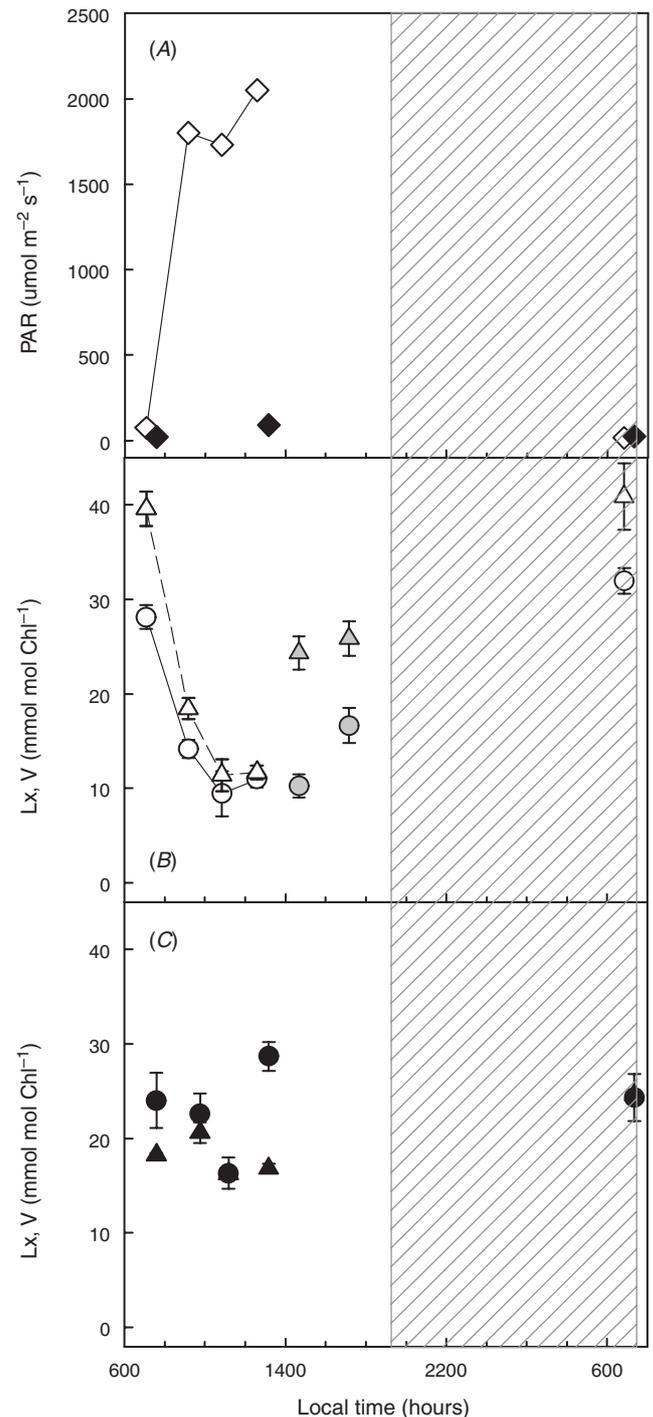
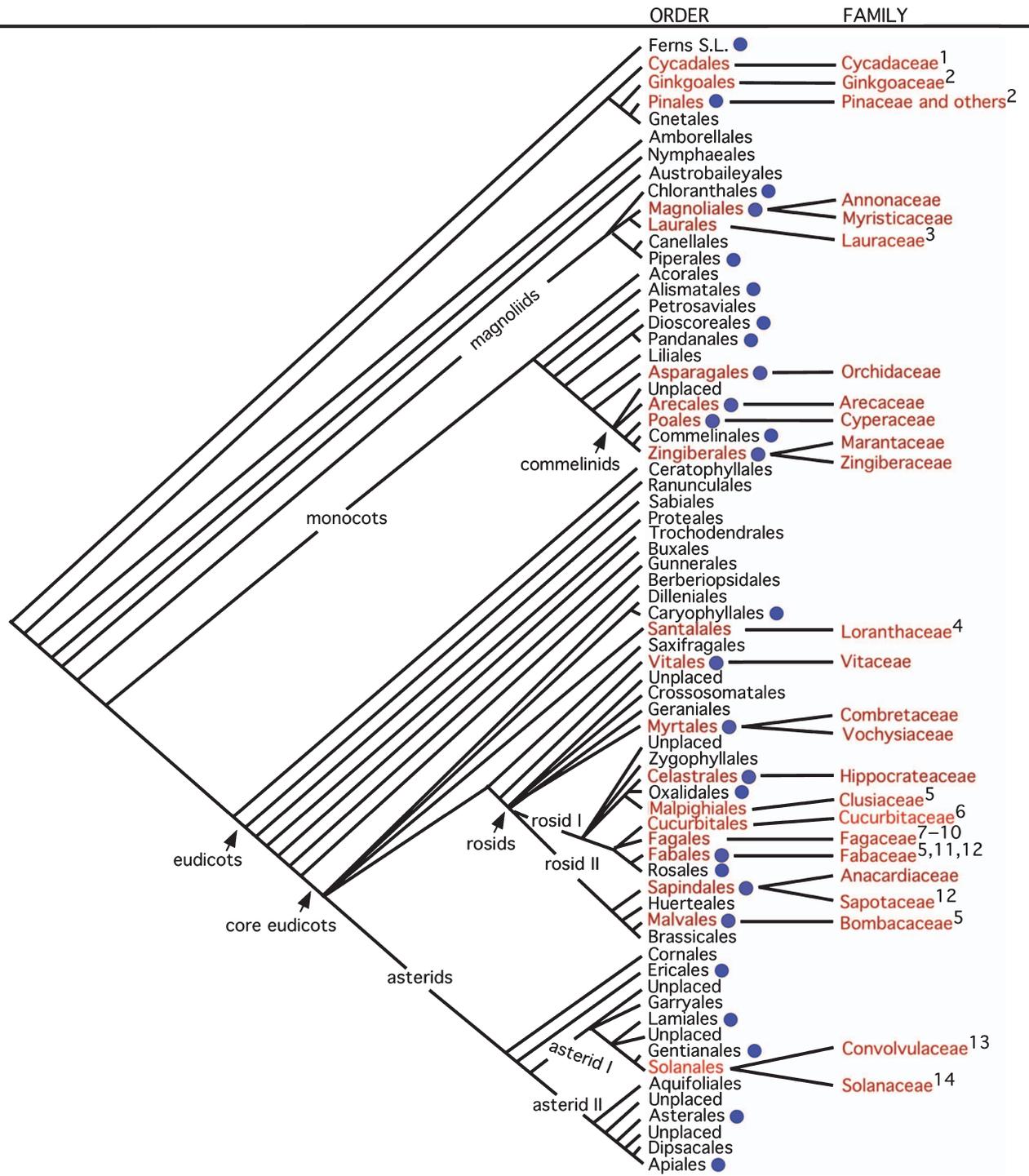


Fig. 7. Relationship between the ratio α - β -carotene and the pool size of the violaxanthin-cycle pigments (VAZ) given on a Chl *a+b* basis. Symbols represent individual leaves or leaflets. Open symbols, sun leaves; closed symbols, shade leaves.

Fig. 8. Diel changes in contents of lutein epoxide (Lx, circles) and violaxanthin (V, triangles) in sun (open symbols, B) and shade leaves (closed symbols, C) of *Virola elongata*. The PAR incident on leaves (diamonds) is shown in (A). Error bars (shown when larger than symbols) indicate s.e. ($n=5$). Different sets of leaves were used at the four sampling times from morning to midday. The time courses from morning to midday are indicated for sun leaves by solid lines (PAR and Lx) or a dashed line (V). The hatched area indicates the nighttime. After measurements at 1240 hours, five sun leaves were detached and dark-adapted at ambient temperature. The grey symbols in (B) show the contents of pigments found in these detached sun leaves after 2 and 4.5 h of dark adaptation. The F_v/F_m (means \pm s.e.; $n=5$) measured in sun leaves was 0.767 ± 0.009 at 0705 hours (first morning), 0.700 ± 0.008 at 1240 hours, 0.735 ± 0.005 after 4.5 h dark adaptation of detached leaves and 0.785 ± 0.005 at 0650 hours (second morning *in situ*). The F_v/F_m values in shade leaves were 0.808 ± 0.001 at 0750 hours (first morning), 0.803 ± 0.003 at 1310 hours and 0.814 ± 0.001 at 0720 hours (second morning).





¹Cardini et al. 2006; ²Czeczuga 1986; ³Esteban et al. 2007; ⁴Matsubara et al. 2003; ⁵Matsubara et al. 2008; ⁶Schwickert JA, Matsubara S, unpublished; ⁷García-Plazaola et al. 2002; ⁸García-Plazaola et al. 2004; ⁹Llorens et al. 2002; ¹⁰Munné-Bosch et al. 2004; ¹¹Watson et al. 2004; ¹²Krause GH, Gallé A, Gademann R, Winter K, unpublished; ¹³Bungard et al. 1999; ¹⁴Rabinowitch et al. 1975.

Fig. 9. Occurrence of moderate to high Lx levels within the phylogenetic tree of vascular plants. The scheme has been adopted after the Missouri Botanical Garden phylogeny website (www.mobot.org). Blue dots mark plant orders, in which at least one species has been tested in the present study; when at least one species contained >5 mmol Lx mol Chl⁻¹ in shade leaves (or both shade and sun leaves), orders and families have been marked in red. Previous reports on significant amounts of Lx in leaves or other green tissues have also been considered by red marking (references given in the footnote).

Although high Chl *a+b* content and low LMA (Fig. 1*A, B*) are consistent with a primary demand of shade leaves for light harvesting, the economics of pigment accumulation has rarely been studied for carotenoids, presumably due to their small contribution to leaf dry mass (<0.3%, Fig. 2*B*). The results in Fig. 2 raise the question whether, as is the case with Chl, investment in carotenoids is more important for shade than for sun leaves. On a leaf area basis, total carotenoid content was very similar in sun and shade leaves (Fig. 1*C*). Based on leaf dry mass, sun-shade differences in N and LxL were highly significant, and those of VAZ and β -Car were found to be significant only on a leaf area or Chl basis (Fig. 3). Both N and L are integral components of LHCII, the major light-harvesting antenna of PSII (Kühlbrandt *et al.* 1994; Liu *et al.* 2004). Although N and L may fulfil photoprotective functions in LHCII (Niyogi *et al.* 1997; Pogson *et al.* 1998; Dall'Osto *et al.* 2007*a, b*; Ruban *et al.* 2007; Matsubara *et al.* 2008), selective mass-based accumulation of these pigments in shade leaves seems to be primarily coupled with accumulation of LHCII for light harvesting (Björkman 1981; Anderson *et al.* 1988), as was seen from enhanced accumulation of mass-based Chl and total carotenoids (Fig. 2) as well as generally lower values of Chl *a/b* in shade leaves compared with sun leaves of the same species (Table 2). For the sun-shade differences in VAZ and β -Car, in contrast, incident PAR flux and/or high-light stress caused by adverse environmental conditions are probably the major determinants because the variations were seen only on a leaf area or Chl basis (Fig. 3). The finding of a higher VAZ pool size (+50% based on Chl) in sun leaves of lianas compared with sun leaves of trees (Table 3) suggests that lianas possess a greater photoprotection capacity, possibly because they experience stronger high-light stress. This is in agreement with the observations by Santiago and Wright (2007) that outer-canopy sun leaves of lianas exhibit lower photosynthetic capacities than sun leaves of trees having similar LMA.

The striking similarity of shade and sun leaves with respect to mass-based VAZ and β -Car (Fig. 3*C*) is puzzling as it suggests that investment of leaves in these photoprotective pigments is little affected by growth light environment. This contrasts the investment into light-harvesting pigments (Chl, N, LxL and α -Car) which is strongly influenced by the light environment (Figs 2, 3*C*). Overall, however, the general trend of the relationship between mass-based photosynthetic pigments (Chl and carotenoids) and LMA across both sun and shade leaves (Fig. 2) suggests that trade-offs among these traits are under similar leaf functional constraints regardless of species and growth environment. The distinct sun-shade pattern of α -Car was independent of whether it is expressed on an area, dry mass, or Chl basis, a result which reflects the general preference of β -Car over α -Car under high irradiance.

Sun-shade acclimation and adaptation in carotenoid biosynthetic pathways

The balance shift between the β,ϵ - and β,β -branch of the carotenoid biosynthetic pathway (Fig. 4) seems to be a universal response in leaves of neotropical forest species to light environments. Based both on leaf area (Fig. 4*A*) and Chl (Fig. 4*B*), the data show a clear trend to higher β,β -carotenoids in

sun compared with shade leaves, whether it is adaptational or acclimatory. Although values of $\beta,\epsilon/\beta,\beta$ -carotenoids varied hugely among shade leaves of different species, it was rather uniform, mostly ≤ 1 , among sun leaves (Fig. 5). The same pattern was found for α/β -Car, i.e. the balance between the first products of the two branches, which correlates positively and linearly with the balance between the whole branches, i.e. $\beta,\epsilon/\beta,\beta$ -carotenoids (Fig. 5). A similar correlation between $\beta,\epsilon/\beta,\beta$ -carotenoids and α/β -Car has been observed in a previous study on sun and shade leaves of different *Inga* species (Matsubara *et al.* 2008). The confined data distribution of sun leaves in Fig. 5 suggests selection pressure by high light, constraining an increase in α/β -Car and $\beta,\epsilon/\beta,\beta$ -carotenoids. The fact that there was no single leaf having a large Chl-based pool of VAZ and high α/β -Car at the same time (Fig. 7) supports this notion. The absence of such stress under shade conditions may then allow the large interspecific variation of α/β -Car and $\beta,\epsilon/\beta,\beta$ -carotenoids (Fig. 5).

Furthermore, our data suggest that α - and β -Car substitute each other in most cases (Fig. 6), minimising variations in the sum of the two carotenes (see legend to Fig. 6). The substitution supposedly takes place in the core complexes of PSII and in the core and antennae of PSI (Matsubara *et al.* 2007). Accumulation of α -Car has been found in shade leaves of many different plants (e.g. Thayer and Björkman 1990; Demmig-Adams and Adams 1992; Siefermann-Harms 1994; Demmig-Adams 1998), including neotropical forest species (Königer *et al.* 1995; Krause *et al.* 2001, 2003, 2004; Matsubara *et al.* 2008; Table 2). Based on enhanced accumulation in shade environments, a possible function of α -Car in improving light harvesting has been proposed (Krause *et al.* 2001). Besides contributing to light harvesting, β -Car is thought to exert photoprotection in the core antennae by quenching of triplet-state Chl *a* and singlet-state dioxygen (Frank and Cogdell 1996; Scheer 2003) and in the PSII reaction centre by singlet-state dioxygen scavenging and mediating cyclic electron transfer (Telfer 2002). Differential functions of α - and β -Car in light harvesting and photoprotection need to be verified in the future.

Several species, however, did exhibit low α -Car levels in shade leaves whereas sun leaves seemed to have a strict preference for β -Car (Figs 5–7; Table 2). This suggests that a high α/β -Car is not a general requirement for shade acclimation or adaptation. In those species, where α -Car is high in the shade, α -Car resembles Lx, which also accumulates to high levels under shaded environments, although the occurrence of Lx in tropical forest species is less frequent than of α -Car (Table 2). Since α -Car is a precursor of L, the most abundant and universal xanthophyll in leaves, all higher plants must have the enzymes necessary for α -Car synthesis. In *Arabidopsis thaliana*, which does not accumulate α -Car naturally, absence of the *lut5* gene product, a heme β -ring hydroxylase, leads to accumulation of α -Car (Kim and DellaPenna 2006; Fiore *et al.* 2006). It is possible that β -ring hydroxylation in the β,ϵ -branch is involved in the regulation of α/β -Car in shade and sun leaves of many higher plant species in response to growth irradiance. In addition, regulation of ϵ -ring cyclisation at the branching point of carotenoid biosynthesis (Pogson *et al.* 1996; Pogson and Rissler 2000) and β -ring hydroxylation in the β,β -branch (Davison *et al.* 2002) may

adjust β,ϵ - β -carotenoids and VAZ pool, respectively, to light environments.

Occurrence of Lx among vascular plant taxa

Among the 86 neotropical forest species collected in the present study, Lx was found in a broad range of rather unrelated plant taxa, as depicted in the scheme of the phylogenetic tree (Fig. 9), which also considers previous reports on occurrence of significant amounts of Lx. As Lx levels in leaves are subjected to both developmental and environmental control (García-Plazaola *et al.* 2007), the Lx values shown in Table 2 do not necessarily reflect the species capacity to accumulate this pigment in leaves. However, given the extended knowledge from the present and several previous studies (e.g. the references in Fig. 9), it is obvious that the ability to accumulate relatively high amounts of Lx has evolved independently among different plant taxa (García-Plazaola *et al.* 2004).

Our survey, focussed on neotropical forest species, indicates that occurrence of Lx is a rather common phenomenon in shade environments; 19 species (22%) had >5 mmol Lx mol Chl⁻¹, and eight species (9%) had >10 mmol Lx mol Chl⁻¹ in shade and/or sun leaves (Table 2). Analysing different life forms separately, four herbs, three lianas and 12 trees/shrubs contained >5 mmol Lx mol Chl⁻¹ in their leaves (22, 20 and 23% of the species for each life form, respectively). None of the herbs accumulated Lx to >10 mmol mol Chl⁻¹ whereas a liana and seven trees/shrubs did. Thus, Lx accumulation (>5 mmol mol Chl⁻¹) can be found rather widely and irregularly among different plant taxa and life forms, with a probability of $\sim 20\%$ for neotropical forest species, whereas higher Lx levels (>10 mmol mol Chl⁻¹) occur less frequently but still with a probability of $\sim 10\%$ for trees and shrubs in Panamanian forests. It is not known whether these numbers are higher or lower with respect to other habitats and vegetation types. Nevertheless, the wide distribution of Lx in tropical forest species implies functional significance in these plants.

Although the occurrence of Lx remains enigmatic both taxonomically and ecologically, extremely high Lx, so far, appears to be confined to tree species in a small number of orders. To these belong the two closely related magnoliid orders, Magnoliales and Laurales (Fig. 9), with high Lx contents in species of Myristicaceae and Lauraceae families (Table 2; Esteban *et al.* 2007, 2008; Matsubara *et al.* 2008), as well as Fabales in the clade of rosid I, with strong Lx accumulation in species of the Fabaceae-Mimosoideae family (Table 2; Matsubara *et al.* 2008). In the case of *Inga* species (Fabaceae-Mimosoideae), Lx is quickly de-epoxidised upon sunlight exposure but is restored very slowly in the shade (Matsubara *et al.* 2005, 2008), resulting in distinct sun-shade patterns of Lx levels, i.e. being much higher in shade than in sun leaves. Operation of this slowly reversible Lx cycle has been associated with shade-to-sun and sun-to-shade acclimation in long-lived leaves of *Inga* trees, wherein high Lx ensures efficient light harvesting in shade leaves and high L confers increased photoprotection in sun leaves (Matsubara *et al.* 2005, 2007, 2008), in addition to the well established function of the V cycle. Very high levels of Lx found in shade as well as sun leaves of *Virola* species (Table 2; Matsubara *et al.* 2008) represent

a unique situation, in which daily sun exposure does not seem to limit Lx accumulation in sun leaves. The data indicate that occurrence of high Lx levels in different taxa (Fig. 9) do not necessarily signify the same regulation of the Lx-cycle operation.

Operation of the Lx cycle in *Virola elongata*

In species, in which kinetics of post-illumination Lx restoration have so far been investigated, Lx was restored generally more slowly than V. This applies, for example, to chloroplast-containing stems of *Cuscuta reflexa* (Convolvulaceae) (Bungard *et al.* 1999; Snyder *et al.* 2005) and leaves of mistletoes (Loranthaceae) (Matsubara *et al.* 2001), *Quercus robur* (Fagaceae) (García-Plazaola *et al.* 2002), *Persea americana* (Lauraceae) (Esteban *et al.* 2008) and *Inga* species (Fabaceae) (Matsubara *et al.* 2005, 2008). These observations led to the conclusion that sun-shade Lx patterns commonly found among Lx-cycle plants could result from slow L \rightarrow Lx epoxidation, with the extent of slowness differing between species but always limiting the Lx restoration when daily Lx \rightarrow L de-epoxidation exceeds the capacity of daily L \rightarrow Lx epoxidation.

The diel canopy experiment conducted on a *V. elongata* tree showed 60 and 70% de-epoxidation for Lx and V, respectively, in sun leaves in the strong daylight followed by full overnight restoration for both pigments (Fig. 8B). Hence, a large part of the Lx pool found in sun leaves of *V. elongata* is engaged in the xanthophyll cycling on a daily basis in parallel with V. Dark treatment of detached sun leaves in the afternoon revealed slower onset of restoration for Lx compared with V, with a delay of several hours (Fig. 8B). Similar situations have been reported for *Cuscuta reflexa* (Bungard *et al.* 1999) and mistletoe *Amyema miquelii* (Matsubara *et al.* 2001). The equally high Lx contents in sun and shade leaves of *Virola* plants, as opposed to marked sun-shade differences in Lx levels in other plants including mistletoes, may, therefore, suggest a large capacity of *Virola* sun leaves to restore Lx even though Lx restoration starts later than V restoration. At present, little is known about the regulation of epoxidation in the operation of the V cycle, and even less in the Lx cycle. Altered substrate specificity of the epoxidase enzyme to L has been suggested as a factor responsible for accumulation of Lx (Matsubara *et al.* 2003). Different binding affinities of light-harvesting complexes to L and Lx (Matsubara *et al.* 2007) could also play a role in distinct operation of the Lx cycle in different species. Clearly, more investigations are needed to elucidate the mechanism and physiological regulation of epoxidation in the Lx cycle. Species having extremely high levels of Lx with contrasting kinetics of Lx restoration, such as *Virola* and *Inga*, offer promising systems to address these questions. In particular, the possible function of the Lx cycle in leaves of *Virola* species, in which the levels of Lx and L are uncoupled from the marked sun-shade acclimation of VAZ and α - β -Car (Table 2; Fig. 7), needs to be explored in future studies.

Conclusion

Overall, our survey provided a new insight into the sun/shade balance between the various pigments of the two carotenoid biosynthesis branches and showed a wide distribution of α -

Car and Lx among taxa of tropical forest plants. The study of *Virola elongata* demonstrated for the first time a fully reversible diel operation of the Lx cycle in parallel with the V cycle in sun leaves containing comparable levels of Lx and V.

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