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Ontogenetic changes in leaf traits of tropical rainforest trees differing in juvenile light requirement

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Abstract Relationships between leaf traits and the gap dependence for regeneration, and ontogenetic changes therein, were investigated in juvenile and adult tropical rainforest tree species. The juveniles of the 17 species included in the study were grown in high light, similar to the exposed crowns of the adult trees. The traits were structural, biomechanical, chemical and photosynthetic. With increasing species gap dependence, leaf mass per area (LMA) decreased only slightly in juveniles and remained constant in adults, whereas punch strength together with tissue density decreased, and photosynthetic capacity and chlorophyll increased. Contrary to what has been mostly found in evergreen tropical rainforest, the trade-off between investment in longevity and in productivity was evident at an essentially constant LMA. Of the traits pertaining to the chloroplast level, photosynthetic capacity per unit chlorophyll increased with gap dependence, but the chlorophyll *a/b* ratio showed no relationship. Adults had a twofold higher LMA, but leaf strength was on average only about 50% larger. Leaf tissue density, and chlorophyll and leaf N per area were also higher, whereas chlorophyll and leaf N per unit dry mass were lower. Ranking of the species, relationships between traits and with the gap dependence of the species were similar for juveniles and adults.

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However, the magnitudes of most ontogenetic changes were not clearly related to a species' gap dependence. The adaptive value of the leaf traits for juveniles and adults is discussed.

Keywords Gap dependence · LMA · Photosynthesis · Punch strength · Shade tolerance

Introduction

A closed evergreen rainforest is characterized by low light availability on the forest floor and a vertical gradient of increasing irradiance towards the top of the canopy. A disturbance such as that caused by tree falls, creates a gap in the canopy and introduces horizontal variation, which is the starting point of dynamical changes in microclimatic conditions (Chazdon et al. 1996). Most late-successional tropical rainforest trees are typically shade tolerant as juveniles, but are exposed to full daylight as adult trees when they have gained access to the canopy. Such trees cover a broad range of light conditions during their development. Typical early-successional species need canopy gaps or large disturbances throughout their life cycle. These shade avoiding species experience less change in light conditions during their development. Not all species can unequivocally be allocated to one of the above-mentioned groups. The light requirement for regeneration of species is thus preferably considered as a continuum (Popma and Bongers 1988; Osunkoya et al. 1994; Poorter et al. 2004; Coste et al. 2005).

Several studies have shown that functional leaf traits vary systematically with a species' juvenile light requirement (Valladares and Niinemets 2008). Evergreen species in moist tropical rainforest that are not or only to a limited

extent dependent on gaps generally have leaves with a higher mass per area (LMA) that are better defended against herbivory and damage in general, and have a longer lifespan than gap-dependent species (Bongers and Popma 1990; Coley and Barone 1996; Westoby et al. 2002; Poorter and Bongers 2006). They also have a lower photosynthetic capacity under otherwise similar conditions (Reich et al.1992; Raaimakers et al. 1995). This trade-off between investment in defense and in physiological functions, known as the growth–survival or the resource availability hypothesis, has not only been found in tropical rainforest but also in other environments (Mooney and Gulmon 1982; Coley et al. 1985).

Saplings acclimate to the variable light climate at the forest floor by adjusting leaf traits. Numerous studies indicate that at the leaf level plants growing in the shade compared to their counterparts at high irradiance have thinner leaves and a lower leaf LMA, which goes on the expense of photosynthetic capacity per unit leaf area (Lambers et al. 2008; Poorter et al. 2009). Also, leaf strength is reduced (Onoda et al. 2008). This change with shade acclimation is opposite to the increase in LMA with adaptation to shade that is mostly found in evergreen tropical trees (Veneklaas and Poorter 1998; Lusk et al. 2008). Leaf trait values change during ontogeny (Thomas and Winner 2002; Niinemets 2006). Adult trees typically have thicker leaves with higher LMA and dry matter density, and their leaves are tougher (Martinez-Garza and Howe 2005) with sometimes a higher photosynthetic capacity per unit area compared to sapling leaves (Pearcy 1987; Thomas and Bazzaz 1999; Rijkers et al. 2000; Kenzo et al. 2006). It is, however, not always clear from these studies to what extent the reported changes with ontogeny are developmentally determined or environmentally induced, because tree size varied not always independently of irradiance.

An important determinant of the longer lifespan of leaves of shade-tolerant trees is their greater toughness (Matsuki and Koike 2006; Alvarez-Clare and Kitajima 2007; Kitajima and Poorter 2010). This term is generally used to indicate the resistance of leaves against mechanical damage. However, it has recently been used to more specifically denote the energy required to fracture the leaf lamina per unit fracture area. The often used punch-and-die test, as also employed in this study, measures the force to punch a leaf, and is more appropriately called punch strength (Aranwela et al. 1999; Onoda et al. 2008). Leaf strength did not increase in proportion with LMA in sun leaves compared to the thinner shade leaves (Onoda et al. 2008; Lusk et al. 2010). Possibly, the higher LMA and the greater thickness of adult compared to juvenile tree leaves is also not associated with a proportional increase in strength.

Photosynthetic capacity relative to the amount of chlorophyll is an important parameter for the characterization of acclimation to irradiance at the chloroplast level (Anderson et al. 1995). In high light, photon absorption is not a limiting factor for photosynthesis and photosynthetic capacity is generally high relative to the amount of chlorophyll. However, in low light environments, photon absorption limits photosynthetic rates and shade-acclimated leaves typically have more chlorophyll relative to photosynthetic capacity (Evans 1989a; Hikosaka and Terashima 1996; Pons and Anten 2004). Evans (1989b) found a lower photosynthetic capacity per unit chlorophyll in evergreen trees compared to herbaceous plants when grown under identical light conditions, and Feng et al. (2004) for a few shade-tolerant compared to -intolerant tropical tree species. However, the trait is not unequivocally related to adaptation to shade in herbaceous plants (Murchie and Horton 1997). An important question thus remains to what extent photosynthetic capacity per chlorophyll is related to the juvenile light requirement in tropical rainforest trees when a larger range of species is investigated.

A first question that was addressed is to what extent leaf traits at the leaf and chloroplast level are associated with the regeneration niche of tropical rainforest trees. To answer this question, tree species were selected that covered a wide range of requirement for canopy gaps for their regeneration. Trait values are plotted against an index of gap dependence, which is equivalent to the juvenile light requirement, which is equivalent to the inverse of juvenile shade tolerance. Since trait values vary not only with ontogeny but also with growth condition, leaves were sampled from saplings that were experimentally grown in a large gap and from adult trees, thus making light conditions for the leaves of the juveniles and adults as similar as possible. This also allows the addressing of the second question, to what extent the relationships with a species regeneration niche are consistent across ontogenetic stages. The change in leaf trait values with ontogeny is likely to be different between species, which raises a third question, to what extent these ontogenetic changes are associated with a species' juvenile light requirement. Finally, given the contrasting natural environment of the two ontogenetic stages, a fourth question is addressed regarding the functional significance of the leaf traits in juvenile and adult trees.

Materials and methods

Study site

The study was carried out in a largely undisturbed forest in Central Guyana at about 50 km south of the township

Mabura Hill in the West-Pibiri compartment of the local timber concession (5°02′N, 58°37′W). The dominant soil is a haplic Ferralsol, locally known as brown sandy soil. It is a highly weathered acidic soil that has a high iron, aluminum and manganese content, and is low in available nutrients (van Kekem et al. 1996). Average annual precipitation is around 2,800 mm (van Dam 2001).

Plant material

Young saplings and adult trees of 17 species that are common in the area were used for the study. The species differ in their requirement of canopy gaps for their regeneration. To what extent they depend on gaps was characterized for each species in a similar way as done by Osunkoya et al. (1994) and Mostacedo and Fredericksen (1999). The species were assigned to one of five categories by one of us (N.C. Houter), an experienced tree spotter (S. Roberts) and three ecologists with a long-standing working experience in the local forest (P. van der Hout, H. ter Steege and R.C. Zagt). The categories were based on the occurrence of seedlings and small saplings in canopy gaps, (1) mostly under a closed canopy and occasionally in small gaps, (2) mostly in small gaps and under a closed canopy, (3) mostly in gaps of different sizes and occasionally under a closed canopy, (4) exclusively in gaps of different sizes and not under a closed canopy of undisturbed forest, and (5) typically in large gaps and clearings. The results were averaged and the means were used as an index of association of a species as juveniles with canopy gaps, the gap association index (GAI). Species names and their gap association index are shown in Table 1. This index is thus representative for a species light requirement for regeneration and is inversely related to its shade tolerance. Species are further referred to with their genus names.

Experimental set up and leaf sampling

Changes in leaf trait values associated with ontogeny were studied by comparing juveniles growing in a large gap with adult trees growing in full daylight. Seedlings (10 per species) were planted in a large approximately circular gap of around 2,400 m^2. They were raised from seed in a nursery or alternatively collected in the surrounding forest. Trials had shown that it was difficult to grow shade-tolerant species in the high light environment of the large gap. By nursing the seedlings during a short period after planting, it was possible to also grow these species there in a common garden experiment. A plot of approximately 130 m^2 was prepared by excavating roots of surrounding trees. Litter from the surrounding forest was put on top of the soil and the plants received 5 g of slow release fertilizer (Osmocote

plus, release time 2–3 months) at planting. The plants, spaced 0.8 m apart, were shaded for 1 or 2 months until growth had resumed. Water stress was avoided by watering the plants during dry periods. At the time of sampling, cotyledons were fully exhausted, hence plants were beyond the seedling stage. Recently matured leaves from eight plants per species that had fully developed in the gap environment after the nursing period were sampled for the measurements. The plants had grown in the gap for about 5 months and had a height of 27 ± 13 cm (mean ± SD).

Adult sample trees were selected that had their crowns partly exposed, the upper part of their crowns fully exposed, or the crowns exposed on all sides, referring to the crown illumination indices 3, 4 and 5, respectively, of Clark and Clark (1992). The leaves were sampled from the exposed part of the crowns. In the case of the small shade-tolerant species *Oxandra*, the most exposed individuals were selected and leaves were taken from the upper part of the somewhat shaded crowns. Sampling was done by means of a shotgun on eight trees per species. Leaves were immediately put between moist filter paper until further processing. They were measured for thickness, area, fresh and dry mass, punch strength, chlorophyll, nitrogen and the light saturated rate of photosynthesis. Sampling was done between in November and December 1998 (adults) and March and April 2000 (juveniles), when daily irradiance was around 28 mol m^{-2} day^{-1} in a large clearing.

Photosynthetic parameters

Photosynthetic capacity of the young saplings was measured as the light-saturated rate of electron transport per unit leaf area (ETR$_{LA}$). A mini-PAM fluorometer (Walz, Effeltrich, Germany) was used with the leaf clip holder (2030-B) for measurement of the quantum yield of PSII in the light (ΦPSII $= \Delta F/F'_m$) and photon flux density (PFD). Observations in the gap were done in the morning shortly after direct sunlight hit the sample leaf (between 0700 and 0900 hours). When required, a halogen lamp was used to increase irradiance to saturating levels. The intensity of the measuring light and the irradiance of actinic light required for light saturation in each species were established in preliminary measurements. Irradiance was reduced when $\Delta F/F'_m$ was lower than 0.2. The saturating pulse was set at its maximum value (6 mmol m^{-2} s^{-1}). ETR$_{LA}$ was calculated according to Genty et al. (1989) as ETR = PFD $\times$ ΦPSII $\times$ α $\times$ β. The leaf absorptance (α) was derived from chlorophyll per unit area (chl; μmol m^{-2}) as α = chl/(chl + 76) (Evans and Poorter 2001), and an equal partitioning of photon absorption between the two photosystems was assumed (β = 0.5).

ETR measurements on attached leaves could not be performed on adult tree leaves. Instead, detached leaves

Table 1 The species used in the study with family name and gap association index (*GAI*)

Species	Family	GAI
Oxandra asbeckii (Pulle) R.E. Fr.	Annonaceae	1.0 ± 0.0
Mora gongrijpii (Kleinh.) Sandw.	Leguminosae (Caesalp.)	1.6 ± 0.9
Eschweileria sagotiana Miers	Lecythidiaceae	1.7 ± 0.6
Chlorocardium rodiei (Schomb.) Rohwer. Richter and v.d. Werff	Lauraceae	1.8 ± 0.8
Vouacapoua macropetala Sandw.	Leguminosae (Caesalp.)	1.8 ± 1.3
Catostemma fragrans Benth.	Bombacaceae	2.3 ± 0.7
Licania heteromorpha Benth. *var. perplexans* Sandw.	Chrysobalanaceae	2.3 ± 1.3
Ormosia coccinea (Aubl.) Jacks.	Leguminosae (Papil.)	2.5 ± 0.7
Hymenaea courbaril L. var. *courbaril*	Leguminosae (Caesalp.)	3.1 ± 1.3
Pentaclethra macroloba (Willd.) Kuntze	Mimosoideae	3.2 ± 0.4
Carapa guianensis Aublet	Meliaceae	3.3 ± 1.4
Peltogyne venosa (Vahl) Benth. subsp. *densiflora* (Spruce ex Benth.) M.F.Silva	Leguminosae (Caesalp.)	3.8 ± 1.0
Sclerolobium guianense Benth. Var. *Guianense*	Leguminosae (Caesalp.)	4.6 ± 0.4
Goupia glabra Aublet	Celastraceae	4.7 ± 0.4
Laetia procera (Poepp.) Eichler	Flacourtiaceae	5.0 ± 0.0
Jacaranda copaia (Aubl.) D.Don subsp. *Copaia*	Bignoniaceae	5.0 ± 0.0
Cecropia obtusa Tréc.	Moraceae	5.0 ± 0.0

The GAI ranges from 1 to 5, where low values indicate that regeneration from seed is predominantly found under a closed canopy and in small gaps and high numbers indicate predominant regeneration in large gaps and clearings. Values are the mean (±SD) of five estimates

were sampled in the morning and kept on moist filter paper in a box until measurement of photosynthetic capacity in the afternoon. Photosynthesis measurements were carried out with a leaf disc oxygen electrode (LD-II; Hansatech, Norfolk, UK) in 5% CO_2 in air. The electrode chamber was temperature controlled at 25°C. Lighting was with a 50-W halogen lamp and neutral density glass filters. Irradiance was increased until maximum O_2 evolution rates were found.

Structural, chemical and biomechanical parameters

Leaves used for the photosynthesis measurements were stored between moistened filter paper until further processing the same day. Leaf blades, or in case of large leaves such as *Cecropia* about 100 cm² sampled from the distal part of the blade, were measured for area with a leaf area meter (LI-1100; Licor, USA) and fresh mass. Dry mass was measured after 3 days at 70°C. Leaf mass per area (LMA) was calculated from the dry mass to area ratio. Thickness of the fresh leaf was measured with a thickness gauge (resolution 0.01 mm) excluding veins.

Chlorophyll was extracted in 5 ml dimethylformamide (DMF) from three leaf discs (1.9 cm²). After storage in darkness at room temperature for 4–10 days until extraction was complete, absorptance was measured with a spectrophotometer (UV/VIS 120-01; Shimadzu, Kyoto,

Japan) and chlorophyll *a* and *b* were calculated according to Inskeep and Bloom (1985). Total leaf nitrogen per unit dry mass was measured on ground leaf material using an elemental analyzer (Carlo Erba, Milan, Italy).

Leaf strength was measured on separate but similar leaves as used for the previous measurements according to Feeny (1970) and Choong et al. (1992). A leaf was put between two acrylic plates. The lower one had a hole of 5 mm diameter and the upper one of 2 mm. A freely moving metal rod (cross-section 2.5 mm²) with a beaker on top rested perpendicularly on the leaf. The force on the leaf was slowly increased by filling the beaker with water. The total weight when the rod penetrated the leaf was used to calculate the punch strength by dividing it through the cross-sectional area of the rod.

Calculations and statistical analyses

A two-way ANOVA was used to evaluate species specific differences in ontogenetic effects on leaf trait values. The relative change in a trait value per species associated with ontogeny (ontogenetic effect) was calculated as the difference between the means of the adults and juveniles divided by the juvenile value. Relationships of the species mean leaf trait values with the gap association index for the two developmental stages, and the ontogenetic effect, was evaluated with regression analysis. Maintenance of species

ranking between developmental stages was calculated by pairwise comparison of the species mean trait values. Significance was evaluated by calculating Kendall's correlation coefficient. Correlation between leaf traits was evaluated with the Spearman correlation coefficient (2-sided). The association between leaf traits of juveniles and adults was analyzed with a principal component analysis based on a correlation matrix. The statistical analyses were carried out using SPSS 18.

Results

The ANOVA revealed that large differences between species were evident for all leaf traits (Table 2). The effects of developmental stage were particularly strong. Species specific changes in leaf traits with developmental stage were also present as can be seen from the significant interactions. Regression analysis showed that several traits for juveniles and adults showed a relationships with the gap association index (GAI) (Table 3). This was only to a limited extent the case for the ontogenetic effect (Table 4). The results of a PCA analysis indicated that the relationship between traits were rather similar for juveniles and adults (Fig. 1). These aspects are described in further detail below.

Leaf mass per area and strength

A trend of a decreasing leaf mass per area (LMA) with the GAI was evident for juveniles (Table 3; Fig. 2), although the relation was not significant. Such a trend was absent for adults. LMA is made up of leaf thickness and tissue density (dry mass per volume). These traits were not related to the GAI in adults, but tissue density decreased significantly with GAI in juveniles (Table 3). The positive correlation between thickness and LMA indicates that much of the variation in LMA was the result of variation in thickness (Fig. 3). The smaller variation in density contributed less to variation in LMA. Some species showed combinations of thickness and density that deviated from the general trend such as juvenile *Cecropia* and adult *Ormosia* that had relatively thick leaves with low tissue density, and juvenile *Vouacapoua* and adult *Pentaclethra* with relatively thin leaves with high density (Fig. 3, Supplement Table 1).

LMA increased substantially with ontogenetic development (101%) (Table 3). This was on average the result of a stronger increase in thickness (54%) compared to density (33%) (Fig. 2, Table 4). A trend of an increasing ontogenetic effect with the GAI on LMA and density was evident, but the regressions were not significant (Table 4).

Table 2 The effect of species and ontogenetic stage and their interaction on leaf trait values

	Species F	Ontogeny F	Species × ontogeny F	r^2
LMA	21.19**	1,152.13**	9.66**	0.882
Thickness	43.49**	577.03**	14.55**	0.868
Density	8.78**	167.49**	4.15**	0.635
Punch strength$_{LA}$	35.83**	146.13**	4.21**	0.783
Punch strength$_{DM}$	22.92**	79.89**	2.97**	0.693
Chlorophyll$_{LA}$	9.47**	164.23**	3.90**	0.622
Chlorophyll$_{DM}$	20.57**	129.03**	8.71**	0.733
Chlorophyll$_N$	12.54**	54.57**	7.86**	0.637
Chlorophyll a/b	14.31**	19.62**	3.02**	0.567
N$_{DM}$	30.53**	89.30**	2.87**	0.736
N$_{LA}$	17.07**	690.64**	8.87**	0.832
A_{LA}	54.76**	–	–	–
A_{DM}	49.84**	–	–	–
A_{chl}	21.50**	–	–	–
A_N	37.87**	–	–	–

A two-way ANOVA was performed and F and r^2 values are shown. A conservative threshold P value of 0.01 was adopted because not all variables are fully independent. The ontogeny effect and the interaction was left out for the photosynthetic parameters because the stages could not be quantitatively compared

The level of significance: * $P < 0.01$, ** $P < 0.001$

LMA leaf mass per area, *A* rate of photosynthesis. Subscripts refer to the expression basis: leaf area (*LA*), dry mass (*DM*), chlorophyll (*chl*) and nitrogen (*N*).

Punch strength expressed per unit leaf area decreased significantly in juveniles with increasing gap dependence of the species (Table 3; Fig. 2). The trend was also apparent in adult trees, but the relation with the GAI was not significant (Table 3). Punch strength increased with ontogeny (32%), but the trend of an increasing ontogenetic effect with GAI was also not significant (Table 4; Fig. 2).

Within each developmental stage when comparing species, punch strength increased more than expected when strength would increase in proportion with LMA (Fig. 4). However, the large increase in LMA with ontogeny resulted in less increase in strength. Consequently, punch strength per unit dry mass decreased with ontogeny in most species (mean −20%) (Table 4), except in *Licania* and *Peltogyne*. Punch strength per mass decreased also with the GAI, but the change with ontogeny was not related to the GAI (Tables 3 and 4; Fig. 2). Ranking of the species for the two punch strength variables was maintained to a large extent between the two developmental stages (Table 4). Hence, gap-dependent species had weaker leaf tissue and the strength characteristics were to a considerable extent maintained with ontogenetic development.

Table 3 Overall means for leaf traits and relationships of leaf trait values with the gap association index (GAI) for juvenile and adult trees

	Juvenile trees				Adult trees				Comparison	
	Mean	a	b	r^2	Mean	a	b	r^2	a's	b's
LMA (g m^{-2})	64.5	76.9	−4.02	0.172^{ns}	127.0	133.3	−2.1	0.011^{ns}	***	ns
Thickness (mm)	0.202	0.193	0.003	0.004^{ns}	0.301	0.313	−0.004	0.005^{ns}	***	ns
Tissue density (kg m^{-3})	0.328	0.386	−0.019	0.279*	0.427	0.444	−0.006	0.014^{ns}	***	ns
Punch strength$_{LA}$ (N mm^{-2})	0.934	1.383	−0.145	0.269*	1.372	1.797	−0.137	0.143^{ns}	**	ns
Punch strength$_{DM}$ (N mg^{-1})	4.53	18.44	−1.51	0.306*	4.43	14.01	−1.04	0.260*	**	ns
Chlorophyll$_{LA}$ (μmol m^{-2})	0.385	0.215	0.055	0.564***	0.539	0.433	0.034	0.351*	***	ns
Chlorophyll$_{DM}$ (μmol g^{-1})	6.29	2.04	1.37	0.764***	4.53	3.64	0.29	0.100^{ns}	***	**
Chlorophyll$_N$ (mmol mol^{-1})	3.66	2.23	0.463	0.653***	3.08	2.95	0.042	0.007^{ns}	*	*
Chlorophyll a/b (mol mol^{-1})	3.42	3.34	0.028	0.008^{ns}	3.22	2.99	0.075	0.082^{ns}	ns	ns
N$_{DM}$ (mg g^{-1})	23.5	17.0	2.12	0.530***	20.5	17.4	1.01	0.140^{ns}	*	ns
N$_{LA}$ (mmol m^{-2})	106	97.9	2.56	0.033^{ns}	182.6	166.9	5.08	0.034^{ns}	***	ns
A$_{LA}$ (μmol m^{-2} s^{-1})^a	119	23.8	31.0	0.706***	13.99	−4.42	5.97	0.741***	–	–
A$_{DM}$ (nmol g^{-1} s^{-1})^a	1.98	0.123	0.683	0.841***	0.118	−0.050	0.054	0.770***	–	–
A$_{chl}$ (μmol mol^{-1} s^{-1})^a	308	190	38.5	0.462***	25.23	−3.90	9.44	0.762***	–	–
A$_N$ (μmol mol^{-1} s^{-1})^a	1129	273	278	0.792***	76.6	−14.4	29.5	0.786***	–	–

The results of regression analysis is shown where a denotes the intercept and b the slope. Comparison of regression lines was done by ANCOVA. For abbreviations of traits see Table 2. For species specific mean leaf trait values see Supplemental Table 1

Significant regressions and comparisons: ns, *, **, *** (respectively, non-significant, $P < 0.05$, $P < 0.01$, $P < 0.001$)

^{a} Photosynthetic units are in μmol electrons for juveniles and in μmol O$_2$ for adult trees

Nitrogen, pigments and photosynthetic capacity

Leaf N concentrations in dry matter (N$_{DM}$) of juveniles were somewhat higher compared to adult trees (13%) (Table 3). N$_{DM}$ increased with GAI in juveniles, but not clearly in adult trees. The decrease with ontogeny was thus stronger for gap-dependent species (Table 4). As a consequence of the large increase in LMA with ontogeny, N expressed per leaf area (N$_{LA}$) was much higher in adult trees compared to juveniles (75%) (Table 4).

Leaves of adult trees had a higher chlorophyll content per unit area compared to juveniles (47%) (Tables 3 and 4; Fig. 2). When chlorophyll was expressed per unit dry mass, values were lower for adult trees (−23%) as a result of their much higher LMA. Gap-dependent species had higher chlorophyll per area for both developmental stages, but the positive relationship with the GAI was only maintained in juveniles when chlorophyll was expressed per unit dry mass (Table 3). Relationships were similar for chlorophyll per N (Tables 3 and 4; Fig. 2). The chlorophyll a/b ratio was not associated with the species gap dependence and the values were rather similar for the two developmental stages (Tables 3 and 4).

Photosynthetic capacity was measured as the light saturated electron transport rate with chlorophyll fluorescence on attached leaves of juveniles. Values were expressed per leaf area (A$_{LA}$), dry mass (A$_{DM}$), chlorophyll (A$_{chl}$) and per leaf N (A$_N$). All these photosynthetic parameters increased strongly with the GAI (Table 3; Fig. 2). Since chlorophyll fluorescence measurements could not be done on attached leaves of adult trees, photosynthetic capacity was measured on detached leaves as O$_2$ production at CO$_2$ saturation. Clear positive relationships of this measure of photosynthetic capacity with the GAI for all four expression bases were also observed among the 14 species adult trees that were included (Table 3; Fig. 2).

Discussion

Trait values of juveniles in high light conditions

Chlorophyll on all expression bases showed strong positive correlations with a species' gap dependence (Table 3). The large gap where the juveniles were growing is the natural environment of the gap-dependent species, but irradiance was high compared to where shade-tolerant saplings normally occur. The shade-tolerant juveniles were growing well and chlorophyll content was not particularly low in the gap. However, the same species growing in shade had similar chlorophyll contents compared to gap dependent species (results not shown). It is thus likely that the shade-tolerant juveniles have suffered some light stress (Houter and Pons 2005) resulting in somewhat reduced chlorophyll

Table 4 Relative ontogenetic changes

| | Mean | Regression on GAI | | | | Rank maintenance |
	%	a	b	r^2	P	%
LMA	101.2	66.9	11.1	0.125	ns	61[ns]
Thickness	53.5	51.3	0.7	0.001	ns	76**
Tissue density	32.5	11.1	6.9	0.135	ns	68*
Punch strength$_{LA}$	54.3	15.9	12.4	0.219	ns	85***
Punch strength$_{DM}$	−20.2	−23.3	1.0	0.010	ns	80***
Chlorophyll$_{LA}$	47.0	86.8	−12.8	0.225	ns	55[ns]
Chlorophyll$_{DM}$	−23.0	16.1	−12.6	0.389	**	72*
Chlorophyll$_N$	−12.8	20.8	−10.8	0.300	*	60[ns]
Chlorophyll a/b	−5.3	−8.8	1.1	0.025	ns	70*
N$_{DM}$	−12.6	−2.0	−3.4	0.258	*	83***
N$_{LA}$	75.3	65.5	3.2	0.013	ns	63*
A_{LA}	–	–	–	–	–	85***
A_{DM}	–	–	–	–	–	81**
A_{chl}	–	–	–	–	–	74*
A_N	–	–	–	–	–	77*

The change was calculated per species as the percentage difference between the two [(adult − juvenile)/juvenile]. Presented are the mean change and a regression analysis of the relationship between the change and the GAI, where a denotes the intercept and b the slope. The right column depicts the percentage maintenance of ranking in a pairwise comparison of species between juveniles and adults. For abbreviations of traits see Table 2. For species specific ontogenetic changes see Supplemental Table 2

Significant regressions and rank maintenance: ns, *, **, *** (respectively non-significant, $P < 0.05$, $P < 0.01$, $P < 0.001$)

Fig. 1 Results of a principal component analysis (PCA). Factor loadings are shown on the axes for leaf traits of juvenile (*open squares*) and adult trees (*closed triangles*), and the gap association index (*diamond*). Traits expressed per leaf area were excluded from the analysis except LMA. Trait *labels* are abbreviated versions of the names mentioned in Table 2. The dry mass expression basis is not explicitly mentioned

contents. Although less pronounced, adult shade-tolerant trees showed a similar trend of reduced chlorophyll per area with the GAI, whereas the high irradiance in the upper part of the canopy is the normal environment for these leaves. The similar trends of chlorophyll with juvenile light requirement in the two ontogenetic stages growing in high light are thus likely to represent an inherent one.

Acclimation of plants to canopy shade is generally accompanied by a shift from investment at the chloroplast level from more in photosynthetic capacity to more in light harvesting (Anderson et al. 1995). This is evident from a decrease in photosynthetic capacity per unit chlorophyll (A_{chl}). The decrease of A_{chl} with increasing shade tolerance at constant irradiance (Table 3) suggests that such a shift from investment in capacity of the photosynthetic apparatus to light harvesting may also be a form of shade adaptation. However, Murchie and Horton (1997) did not find a relationship of A_{chl} with shade adaptation in a comparative study of western European herbaceous plants. A similar shift was found for inherently slow-growing species that showed more investment in pigment protein complexes relative to photochemistry in comparison with inherently fast-growing species (Poorter and Evans 1998; Pons and
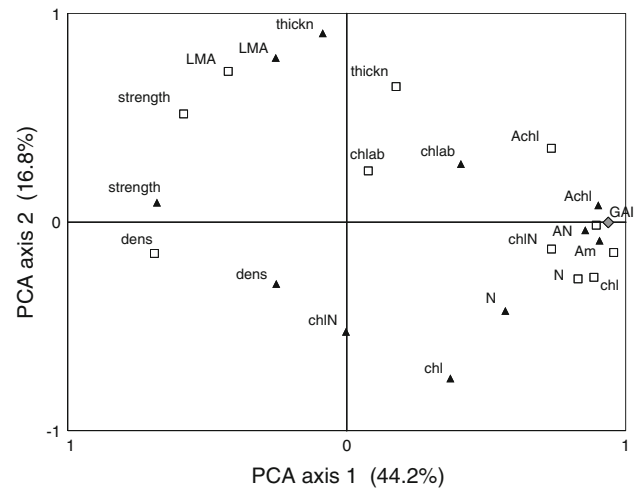
Westbeek 2004) and slow-growing tropical trees compared to fast-growing herbaceous species (Evans 1989b). Gap-independent tropical tree species have lower inherent growth rates compared to gap-dependent species (Veneklaas and Poorter 1998). Feng et al. (2004) found indeed a higher A_{chl} for two light-requiring species compared to two shade-tolerant ones. A low photosynthetic capacity per unit chlorophyll seems to be of general occurrence in slow-growing species, but is not necessarily related to shade adaptation. The chlorophyll a/b ratio is also a component of acclimation to shade at the chloroplast level (Anderson et al. 1995). However, contrary to A_{chl}, this trait, measured at the high irradiance of the gap, was not related to a species' gap dependence at all (Table 3).

Chlorophyll per unit leaf N (chl$_N$) is used as an indirect measure to describe relative investment of photosynthetic N in light harvesting (Niinemets 1997; Kull and Niinemets 1998; Rozendaal et al. 2006). A_{chl}, a more direct measure for the same purpose, should thus be negatively related to chl$_N$. However, the two were not clearly related (Fig. 1; Supplemental Table 3) and both decreased with decreasing species' gap dependence (Table 3; Fig. 2). The reason is that the proportion of total leaf N that is involved in photosynthesis increased with a species gap dependence as evident from the increasing photosynthetic nitrogen use efficiency (A_N) (Table 3; Fig. 2). This is also a well-known phenomenon among tropical trees (Reich et al. 1992; Raaimakers et al. 1995; Coste et al. 2005). Photosynthetic capacity was thus not proportional to leaf N (Fig. 5), and consequently chl$_N$ is not a good measure of the investment
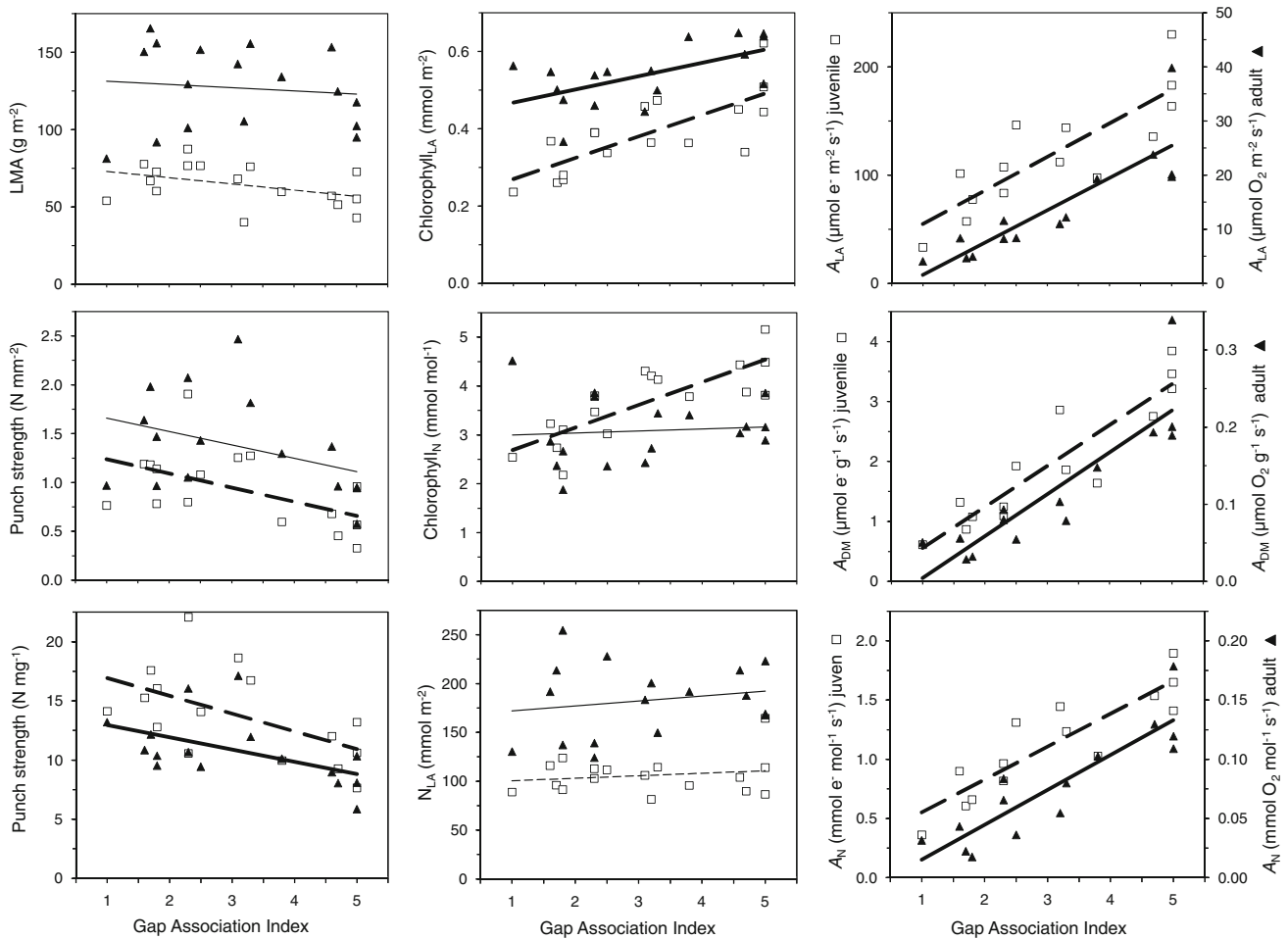
Fig. 2 Leaf traits of the 17 species involved in the study plotted against their gap association index (GAI). Trait values were measured on leaves from juvenile and adult trees (*open squares* and *closed triangles*, respectively). *Bold regression lines* are significant, *thin lines* are not ($P < 0.05$); *dashed lines* denote juveniles and continuous lines adults. For abbreviation of traits and regression parameter values see Tables 2 and 3. For species specific mean ($\pm$SE) trait values see Supplemental Table 1

Fig. 3 Leaf thickness (**a**) and leaf tissue density (leaf dry mass per volume) (**b**) plotted against leaf mass per area (LMA). Significant correlations are depicted by *thick trend lines*, non-significant ones by *thin lines*; correlation coefficients are shown next to the lines (**$P < 0.01$; ***$P < 0.001$). Juveniles: *open squares* and *dashed lines*; adults: *closed triangles* and *continuous lines*
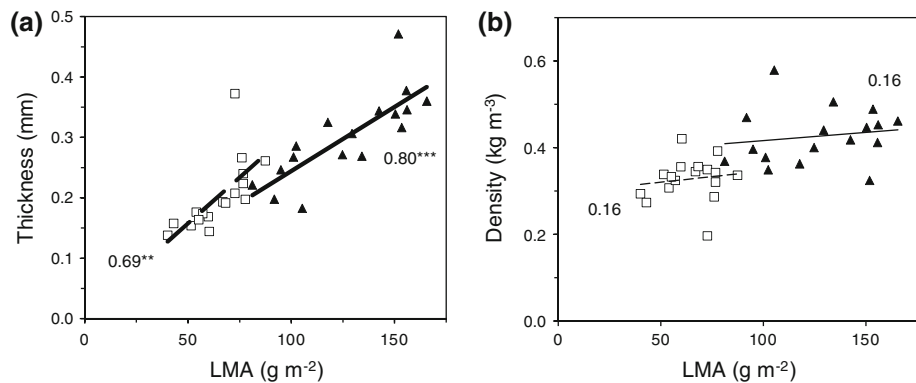
in light harvesting relative to photosynthetic capacity in a comparison of tropical tree species differing in shade tolerance.

The character of the leaf tissue changed with gap dependence of the species. Tissue density and punch strength per dry mass increased with increasing shade tolerance, whereas nitrogen concentration (N_{DM}) and photosynthetic capacity (A_{DM}) decreased (Table 3; Fig. 2). Increasing leaf strength with increasing shade tolerance was thus at the expense of the photosynthetic function of
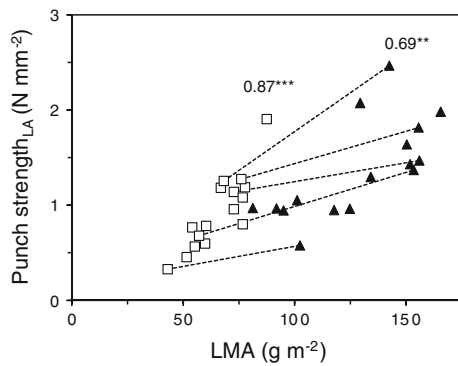
Fig. 4 Punch strength per area plotted against LMA. Correlation coefficients for juveniles (*open squares*) and adults (*closed triangles*) are shown above each series (**$P < 0.01$; ***$P < 0.001$). For five species, values for juveniles and adults are connected with *dashed lines*. The species are from high to low punch strength, *Hymenaea*, *Carapa*, *Chlorocardium*, *Sclerolobium*, *Jacaranda*

the leaf as illustrated by the negative relationship between photosynthetic capacity (A_{DM}) and punch strength (Fig. 5). The stronger leaf tissue of gap-independent species protects them against herbivory and other damage and consequently enhances leaf longevity (Coley and Barone 1996). It is likely the result of a larger proportion of fibers (van Arendonk and Poorter 1994; Alvarez-Clare and Kitajima 2007), epidermis (Onoda et al. 2008) or cell wall in general (Lusk et al. 2010). This in turn should increase tissue density, although the relationship with leaf strength was not strong (Fig. 1; Supplemental Table 3). The result is a steep, more than proportional, increase in strength with LMA (Fig. 4). The higher A_{DM} of gap-dependent species indicates that their leaf tissue contained more mesophyll relative to other tissue (Terashima et al. 2006). The larger content of photosynthetic proteins was reflected in the higher N_{DM}. The results are consistent with the concept of

a shift in the trade-off between the maximization of survival in shade of shade-tolerant evergreen tropical trees as opposed to light-requiring pioneers that maximize growth on the expense of longevity (Coley and Barone 1996; Poorter and Bongers 2006).

A smaller fraction of leaf N involved in photosynthesis in the shade-tolerant species could be the result of more N that is involved in structural reinforcement, such as cell walls (Onoda et al. 2004), and other defense functions in the stronger and longer-living leaves. Moreover, the possibly thicker mesophyll cell walls would result in a lower mesophyll conductance for CO_2 diffusion (Terashima et al. 2006), and consequently in a lower efficiency of the photosynthetic proteins (Hikosaka et al. 1998; Pons and Westbeek 2004; Niinemets et al. 2009). These factors would contribute to a negative intercept of the A_{DM}–N_{DM} relationship (Fig. 5) and a decrease of A_N with shade tolerance (Fig. 2).

In evergreen tropical trees, a decrease in LMA with decreasing shade tolerance is often found (Kitajima 1994; Veneklaas and Poorter 1998; Lusk and Warton 2007; Lusk et al. 2008). However, only a weak negative trend was evident for the juveniles in our study (Table 3; Fig. 2). In a literature review, Veneklaas and Poorter (1998) found that the decrease of LMA with gap dependence is strongest for plants growing in low light. Since pioneers show generally a stronger increase of LMA at high irradiance (Strauss-Debenedetti and Bazzaz 1996; Veneklaas and Poorter 1998), the relationship becomes less clear for high light-grown plants. Our juveniles were grown in a large gap, which is the likely explanation for the absence of a clear relationship of LMA with a species' gap dependence. The change in character of the leaf tissue referred to above occurred thus at a rather constant LMA. Apparently, the trade-off between leaf traits that enhance defense on the
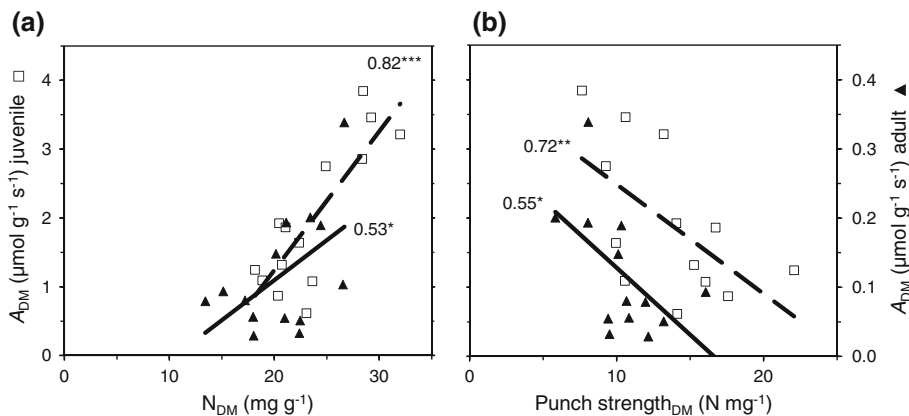

Fig. 5 Photosynthetic capacity per unit dry mass (A_{DM}) plotted against leaf N concentration (N_{DM}) (**a**) and against punch strength per dry mass (**b**). Significant correlations are depicted by *thick regression lines*. Juveniles are plotted on the left *Y axis* (electron transport rates): *open squares* and *dashed lines*. Adults are plotted on the right *Y axis* (O_2 evolution rates): *closed triangles* and *continuous lines*. Correlation coefficients are shown next to the regression lines (*$P < 0.05$; ***$P < 0.001$)

one hand and productivity on the other can also operate at an essentially constant LMA across a range of species' gap dependence as found for our gap-grown saplings (Figs. 1, 2 and 5). The character of the leaf tissue is thus a better predictor of shade tolerance than LMA in high light-grown tropical evergreens.

Ontogenetic changes

Several leaf traits changed substantially with ontogeny (Table 3) and the magnitude of the changes were different per species (Table 2; Supplemental Table 2). Shade-tolerant species cover a larger range of irradiance during growth to the canopy as compared to gap-dependent species. It could be hypothesized that they exhibit larger inherent changes during ontogeny. However, most relative changes in trait values were not correlated with the GAI, except for chlorophyll and nitrogen concentration (Table 4). These were also the traits that showed different associations in the PCA analysis between juveniles and adults.

An increase of LMA with tree size is universally found (Thomas and Winner 2002), including evergreen tropical rainforest trees (Thomas and Bazzaz 1999; Rijkers et al. 2000; Kitajima and Poorter 2010; Caveleri et al. 2010). We found on average a doubling of LMA when comparing exposed leaves of adult trees with small saplings growing in a large gap. The increase was somewhat stronger for gap-dependent species, resulting in complete absence of a trend of LMA with the GAI in adult trees (Table 3; Fig. 2). Mostly, an increase of LMA with shade tolerance is found in evergreen tropical trees, but these results are mostly based on studies with juveniles (Lusk and Warton 2007). Apparently, the relationship is not unequivocal in adults.

Increased LMA generally increases leaf strength (Wright and Cannon 2001; Onoda et al. 2008), which was also true for the ontogenetic change in our study (Fig. 2). However, contrary to the relationship with the interspecific variation in LMA, the large ontogenetic increase in LMA was associated with only a relatively small increase in punch strength (Fig. 4). This is strikingly similar to the small increase in punch strength with increasing LMA as a result of higher growth irradiance (Onoda et al. 2008, 2011; Lusk et al. 2010). Although tissue density increased with ontogeny (Fig. 3), it did not result in an increase in mass specific strength (Fig. 2). This contrasts with the study of Kitajima and Poorter (2010) who found a larger mass-specific punch strength in adult tropical trees compared to saplings in Bolivia.

Electron transport is typically about five times higher than O_2 evolution (Seaton and Walker 1990), but there may be species-specific variation in this factor. The different methods of photosynthesis measurement used for juveniles and adults thus prohibit a full quantitative comparison of the two ontogenetic stages. Additionally, a possible residual effect of the fertilizer may have increased A_{LA} and A_{DM} in juveniles somewhat. Although the results suggest a lower A_{LA} in particularly the shade-tolerant adult trees, no conclusions can be drawn on the ontogenetic changes, since differences were not large. However, the much larger differences between the ontogenetic stages leave little doubt that A_{DM} was lower in adults mainly as a result of their higher LMA. The possible residual fertilizer effect was probably not important for A_{chl} and A_N, because photosynthetic capacity and chlorophyll tend to increase proportionally with leaf N. The large differences thus suggest that A_{chl} and A_N tend to be lower in adults (Table 3; Fig. 2).

The lower photosynthetic nitrogen use efficiency (A_N) of adult trees compared to juveniles follows from a roughly similar A_{LA} at a much higher N_{LA} (Table 3; Fig. 2). Increased LMA is often accompanied by a lower mesophyll conductance for CO_2 diffusion (Flexas et al. 2008; Hassiotou et al. 2009; Niinemets et al. 2009). The reduced A_N of adult tree leaves would then partly be the result of reduced efficiency of the photosynthetic proteins and partly of the larger N investment in reinforcement structures in the form of cell wall proteins, although the latter may not be as straightforward as previously thought (Harrison et al. 2009). The stronger leaves in large trees are likely to be functional in the exposed parts of the adult tree crowns. Their structural reinforcement is important to cope with the abrasive action of branch and leaf movements in strong winds, and possibly larger water potential gradients. If so, the required increase in strength takes a substantial investment in dry matter, and goes on the expense of the efficiency of the use of carbon and nitrogen for photosynthesis (A_{DM} and A_N).

Developmental constraint and adaptation

Leaf traits showed similar associations for juveniles and adults (Fig. 1). The ranking of the species for several leaf traits was also similar for the two developmental stages (Table 4). This was particularly evident for leaf strength and photosynthetic parameters. It suggests that plasticity in the traits concerned is developmentally constrained (Thomas and Bazzaz 1999). In juveniles, chlorophyll, leaf strength, and photosynthetic capacity on all expression bases showed significant relationships with the GAI (Table 3). The conserved ranking means that leaf traits of adult trees are also related to the GAI, although, apart from the photosynthetic parameters, not as strong as for the juveniles (Table 3).

This raises the question regarding the cause of the correlation of adult leaf trait values with the regeneration niche. Poorter (2007) argued that these traits are selected in

the juvenile phase and conserved in the adult phase as a result of developmental constraints. However, Thomas and Bazzaz (1999) showed a correlation of sapling photosynthetic trait values in late successional trees with adult stature, and thus light availability for the adult tree. They concluded that these traits are selected in the adult phase. Most of our high statured species are gap dependent and did not show a trend similar to the phenomenon described by Thomas and Bazzaz (1999) (results not shown). Also, Poorter (2007) did not find evidence for a relationship of leaf traits with adult stature. Martinez-Garza and Howe (2005) found greater changes in LMA and leaf size with ontogeny for tall species compared to short ones, which was interpreted as a reflection of the larger change in irradiance during growth.

Successional status is strongly correlated with leaf longevity (Reich et al. 1995; Poorter et al. 2004). Long-lived leaves improve long-term performance in shade because of the inherently long pay-back times of invested carbon (Poorter 1994; Westoby et al. 2002; Poorter and Bongers 2006; Selaya et al. 2008). However, long-lived leaves are also functional under other stress conditions such as low nutrient availability (Westoby et al. 2002). This increases the residence time of nutrients in the plant (Berendse and Aerts 1987; Aerts and Chapin 2000). The forest in Central Guyana where the study was carried out has a low nutrient status (van Kekem et al. 1996; Hammond 2005; Pons et al. 2007). However, the soil is transiently enriched after a larger disturbance (ter Steege et al. 1995). Hence, the gradient in light availability coincides to some extent with a gradient in nutrient availability. Leaves built for high productivity that are consequently short-lived are important for rapid growth (Lambers and Poorter 1992) at the transiently high nutrient availability shortly after a disturbance. This type of leaf is also functional in a canopy that is rapidly growing in height accompanied by fast leaf-turnover (Selaya and Anten 2010). At the other end of the light requirement spectrum, the suite of traits that enhances leaf longevity of juveniles in shade could also be functional in the adult phase for maintaining a position in the canopy of evergreen forest on nutrient poor soils.

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