FUNCTIONAL LEAF TRAITS

ANATOMICAL ADAPTATIONS TO DIFFERENT LIGHT ENVIRONMENTS AND FUNCTIONAL GROUPS IN A BOLIVIAN DRY FOREST

BY

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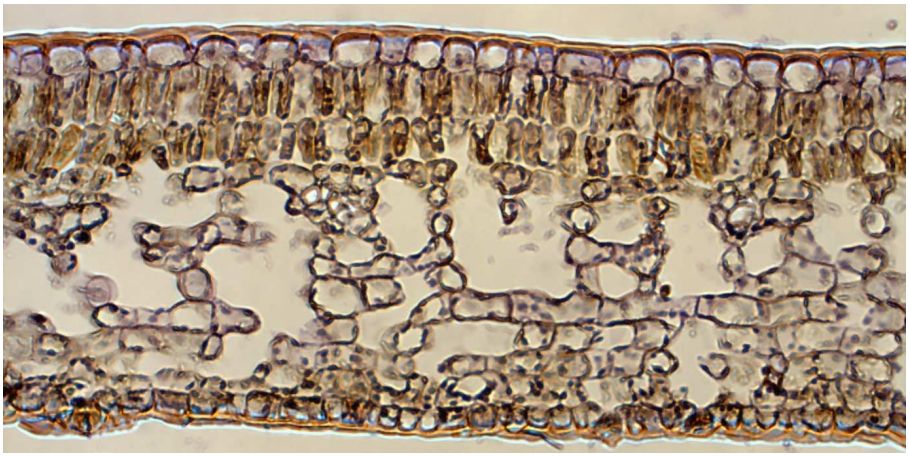

Jiciturichi colorada
Aspidosperma cylindrocarpon
Müll. Arg. (Apocinaceae)

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Summary

Niche-differentiation, with different species or groups of species specializing for different growth conditions, is possibly explanative to at least part of the high biological diversity found in many tropical forests. Still, while quite a bit is known from studies in tropical wet forests, and arid shrub vegetations, only little information on plant adaptive responses to different growth environments in tropical dry forests is available.

Cross-sections of leaf laminas and their primary vein were analyzed to examine leaf structural acclimation of 41 dry forest tree species to high and low light availability and to asses differences between functional groups of species associated to shade- and drought-tolerance.

Among species differences explain most of the variation in anatomical leaf traits. The leaf structural appearance of dry forest tree species thus differs substantially among species. Despite great differences among species, the average Chiquitano dry forest leaf is very thin compared to that of the average wet forest leaf. Carbon assimilation in the dry forests is probably limited by stomatal adaptations that reduce transpiration and CO_2 intake rates.

Sun- and shade-leaves differ substantially within species. Sun-leaves are thicker than shade-leaves and have a, in proportion to their leaf thickness, thicker cuticle, thicker palisade parenchyma tissue composed of more cell layers, a higher palisade to spongy parenchyma ratio and a thicker mesophyll tissue (tab. 3, fig. 4) Still the relative thickness of the spongy parenchyma in the mesophyll and the thickness of the lower epidermis are smaller in sun-leaves. The irradiance level does not seem to influence the relative thickness of the upper epidermis and hypodermis , nor the number of cell-layers in the hypodermis and the diameter or density of the xylem conduits in the primary vein. Leaf structural adjustments to differences in light availability thus emphasizes on acclimatizing the photosynthetic apparatus. Apart from the cuticle, adjustments that enhance water conservation are less pronounced.

A-priory defined functional groups related to shade-tolerance differ in the proportional thickness of their upper epidermis, palisade and spongy parenchyma and in their palisade to spongy parenchyma ratio, xylem conduit density and diameter. Plasticity in response to irradiance level does not differ between groups. Leaves of light-demanding species are characterized by a relatively thick epidermi, a large proportions of palisade parenchyma in the mesophyll tissue, high palisade to spongy parenchyma ratios and wide xylem conduits in small densities in the primary vein. These adaptations increase photosynthetic rates by efficient harvesting of directional light and protect the leaves against negative effects of high irradiance. Shade-tolerant species, do not differ from intermediate shade-tolerant species and are characterized by relatively thin epidermi, large proportions of spongy mesophyll, low palisade to spongy mesophyll ratios and narrow xylem conduits in greater densities in the primary vein. These adaptations increase photosynthetic efficiency by greater harvesting of diffuse light in the forest understory.

Functional groups related to drought-tolerance differed in mean leaf thickness, the proportional thickness of the upper epidermis and in both the absolute diameter and density of the xylem conduits, as their plasticity in response to irradiance level. Drought-intolerant species form thick leaves, probably because carbon assimilation in their preferred wet microhabitats is to a lesser extent limited by stomatal adaptations that reduce transpiration and CO_2 intake rates. Their leaves need less protection against high evaporation rates, as is emphasized by their thin upper epidermis.
The deciduous leaves of drought-avoiding species are characterized by being thin with thick upper erpidermi. Because these species have a limited time to photosynthesize, carbon assimilation is limited, which makes investing in thick leaves expensive. Thickening of the upper epidermis is a cheaper adaptation to minimize evaporation and may enhance photosynthesis by focusing the intercepted light. Drought-tolerant species do not differ much from drought-avoiding species in their leaf structure. Still their narrower xylem conduit diameters are better adapted to avoid cavitation and embolisms in the dry season.
Further study is needed to link adaptations of the xylem tissue to soil-water-plant relations, leaf water potential components and water-use-efficiency of the species studied here, in order to get a better understanding of its functional significance.

Introduction

The high biological diversity generally found in tropical forests has been suggested to result amongst others from different species specializing for different growth conditions, so-called niche-differentiation (Grubb, 1977). As all plants need light, water and nutrients. Species try to optimize morphological and physiological and anatomical characteristics that increase their competitive success (Bongers and Popma, 1988) and thus their chance of survival in habitats where the availability of one or more of these primary resources is limiting.

Leaves are fundamental for the functioning of trees and terrestrial ecosystems. Nitrogen uptake and carbon assimilation by plants and the decomposability of leaves drive biogeochemical cycles. Leaves can vary a great deal in their morphological, anatomical, chemical and physiological characteristics. Leaf properties are known to vary within individual trees at any given time, with age of a leaf, age of a tree, and among trees of the same species as a result of phenotypic acclimation (Turner, 2001). Variation in leaf characteristics due to environmental interactions are often strongly expressed in leaves. As such leaves are known to vary along environmental gradients, such as latitude, altitude, soil fertility, salinity, rainfall and light availability (see e.g. Chapin 1980; Givnish, 1984; Turner, 2001; Wright *et al.*, 2001; Wright *et al.*, 2002; Wright and Westoby, 2003, Markesteijn, 2004).

Especially light availability has extensively been reported to be an important environmental factor leading to leaf acclimation and adaptation. Still many tropical forest studies that examine the effect of light availability on leaf morphology and anatomy are being and have been conducted in ecosystems where water is initially not considered an important limiting factor (e.g. Bongers and Popma,1988; Chazdon and Kaufmann, 1993; Cornelissen, 1993; Field *et al*., 2001; Fisher, 1986; Givnish, 1988; McClendon and McMillen, 1982; Popma *et al*.,1992) or were carried out on plants that were grown in a controlled greenhouse environment eradicating possible variation due to water availability (e.g. Buisson and Lee, 1993; Sims and Pearcy, 1992; Lee *et al*., 2000). While the knowledge on leaf adaptation and acclimation of shrub species from vegetations, as the Mediterranean maquis (e.g. Gratani and Varone, 2004) and tropical arid vegetations as the Chaparral (e.g. Ackerley, 2004) is quite extensive, still little is known about tree species in tropical dry forests (Poorter, pers. comm.).

Tropical dry forest ecosystems are, although variable, characterized by a prolonged dry season (Bullock *et al*., 1995), in which the vegetation is subject to low soil water availability and a high vapour pressure deficit of the air. It is assumed that dry forests tree species acclimatize their leaf structure to enhance photosynthetic activity in different microclimates with respect to light availability within the dry forest ecosystem on the short term and that groups of species adapt their leaf structure to tolerate shade and drought on the long term.

Thus the objective of this study is to examine the leaf structural acclimation of 41 dry forest tree species to high and low light availability and to asses the role and value of leaf anatomy in the identification of functional groups of species associated to shade- and drought-tolerance. I aim to answer the questions; (i) whether species differ in their leaf structural appearance, (ii)

whether differences in light availability alter leaf structure, (iii) whether differences in leaf structure influences species shade- and drought-tolerance, and (iv) whether functional groups of species related to shade-and drought tolerance differ in their structural appearance .

It is hypothesized (i) that leaf anatomy differs most among species, (ii) that sun- and shade-leaves within species differ in leaf structure and (iii) that functional groups of species differing in shade- and drought tolerance have different anatomical adaptations that help to explain their existence. As functional groups of species may be expected not only to differ in their leaf structure but also in their acclimation response to different light environments, I further hypothesize that (iv) functional groups of species differ in their leaf structural plasticity.

(See table 1 and description below for more detailed hypotheses)

SUN- VERSUS SHADE-LEAVES

Sun-leaves are expected to be thicker than shade-leaves (Buisson and Lee, 1993; Gamage *et al*. 2003; Lee *et al*., 2000; Mendes *et al*. 2001), mainly due to an increased mesophyll thickness (Oguchi *et al*., 2003). Thicker leaves have a reduced light adsorption per unit biomass (Agusti *et al*., 1994), an increased photosynthesis per unit leaf area (Klich, 2000) and a reduced CO_2 exchange rate per unit biomass due to an increased CO2 diffusion pathway through intercellular air spaces (Givnish, 1988). This means that thick leaves are more efficient in minimizing overheating and transpirational water loss and in maximizing photosynthesis.

Palisade and spongy parenchyma both have a different function in light interception. Palisade parenchyma cells are long and tubular and help in channeling the light deep into the leaf and providing better access to the chloroplasts. The shape of spongy parenchyma cells is rather irregular and increases the light interception by scattering light (Evans, 1999; Vogelmann and Martin, 1993). The structure of leaves exposed to high irradiance, as opposed to shade-leaves, is generally characterized by an increased number of cell layers in the palisade parenchyma (Bongers and Popma, 1988; Cao and Booth; 2001 Gamage *et al*. 2003; Oguchi *et al*., 2003). The palisade parenchyma tissue in sun-leaves is expected to be thicker (Klich, 2000; Lee *et al*., 2000; Mendes *et al*., 2001), as is the spongy parenchyma tissue (Cao and Booth, 2001; Mendes *et al*; 2001). Still the palisade to spongy parenchyma thickness ratio is expected to be bigger in sun-leaves (Bongers and Popma, 1988; Mendes *et al*.; 2001). This implies relatively more palisade tissue in the leaf's mesophyll and that increases photosynthetic capacity by efficiently intercepting directional light (Evans, 1999; Vogelman and Martin, 1993).

A thick cuticle, upper and lower epidermis (Gamage *et al*., 2003, Mendes *et al*., 2001) and / or hypodermis (Paiva *et al*., 2003) protects the sun-leaf from water loss through evaporation and protects the photosynthetic tissue from excessive irradiance, by increasing the leaf's reflectance (Roth, 1984). The epidermis or hypodermis has further been suggested to have a function in focusing and concentrating the intercepted radiation, facilitating the penetration of light into the thicker sun-leaves (Vogelmann and Martin, 1993).

As sun-leaves spent more water then shade-leaves, because of their increased photosynthetic activity, it is expected that their xylem conduits are wider and occur in a lower density within the

leaf's primary vein. Wider conduits are suggested to be more efficient in transporting water to the leaf than narrow conduits (Zimmermann, 1983).

FUNCTIONAL GROUPS RELATED TO SHADE-TOLERANCE

Shade-tolerant species compete for light in the limited resource environment of the dry forest understory. Because carbon assimilation in this habitat is limited by the low light availability, often resulting in a negative carbon balance, leaves are costly to produce. Shade-tolerant species therefore tend to form well protected long-lived leaves that are able to pay back the high initial investments needed for their production (Niinemets, 2001; Wright and Westoby, 2003). The leaf structure of shade-tolerant species is thus expected to show adaptations that increase their chance of survival. As a protective measure leaves of shade-tolerant species are expected to be thick, as increased thickness is often related to increased tissue density and LMA (Niinemets, 2001; Wright and Westoby, 2003), making thick leaves less susceptible to damage by herbivory. Thicker leaves also have an increased photosynthesis per unit leaf area (Klich, 2000) allowing them to make more efficient use of the limited light available. Light in the forest understory is diffuse with only 1% of the photosynthetically active radiation reaching the forest floor (Chazdon, 1988). Spongy parenchyma cells are more efficient in intercepting diffuse light than palisade cells, that are more specialized to intercept directional irradiance (Evans, 1999; Vogelmann and Martin, 1993). To make efficient use of the limited light intercepted, leaves of shade-tolerant species will have a relatively thin layer of palisade parenchyma and a thick layer of spongy parenchyma.

Although shade-tolerant species are less in need of extra leaf surface protection from excessive radiation, the efficiency with which they produce their surface for adsorbing the limited radiation gives them an selective advantage in their energy-poor habitat (Lee and Graham, 1986). Instead of being protective, a thick upper epidermis might be present in leaves of shade-tolerant species to promote focusing of the intercepted light through the tick leaf tissue (Vogelmann and Martin, 1993). This may help to explain the higher efficiency of light absorbance found for shade-tolerant species (Lee and Graham, 1986; Poorter *et al*. 1995). As leaf level water demand is relatively low, because of the limited photosynthesis, shade-tolerant species are expected to have narrow xylem conduits that occur in a greater density in the bundle sheath. A with coming effect of an increased xylem conduit density is the greater structural support this lignified tissue offers to the leaf, which may add to the herbivory resistance of these costly leaves and prevent physical damage.

Light-demanding species at the other hand establish in the high resource environment of gaps and realize fast growth rates to compete with their neighbors and maintain a position in the top of the regrowing vegetation. They will establish high photosynthetic rates and try to optimize their carbon balance to be able to allocate more biomass to growth. To do so light-demanding species form short-lived leaves, with minimal carbon invested. Consequently leaves of light-demanders are thin, with, in relation to their spongy mesophyll, a lot of palisade parenchyma. A thick cuticle and upper epidermis can protect the light-demanding leaf from radiation impact

related damage and excessive transporational water loss (Roth, 1984). Species may exhibit a thickened hypodermis that may add to the leaf's capacity to reflect light of certain wavelengths and thus help to maintain the leaf temperature near the photosynthetic optimum (about 25 ºC). The high photosynthesis and high respiration rates imply that light-demanding species need wide transportation vessels to ensure a continuous flow of water and nutrients to the leaf. Their xylem vessels will occur in lower densities then in leaves of shade-tolerant species.

Finally, intermediate shade-tolerant species are those species that can establish and survive under shady conditions, but need a higher light availability to be able to reach their full adult stature. From this perspective intermediate shade-tolerant species are expected to be functionally situated between light-demanders and shade-tolerators along the shade-tolerance gradient. That is why their structural trait values of the group of species is also expected to mediate those of light-demanding and shade-tolerant species (tab. 1).

FUNCTIONAL GROUPS RELATED TO DROUGHT-TOLERANCE

Drought-tolerant species maintain their leaf cover during the dry season. Consequently these species need to conserve water by reducing excessive evaporation. To do so drought-tolerators will have thick leaves, with a thick cuticle and upper epidermis or hypodermis. These xeromorphic features are beneficial for species to withstand drought by reducing water loss, excessive irradiance and heat loads, and consequently reducing the species susceptibility to photoinhibition during the dry season (Cao, 2000). At the same time drought-tolerant species need to secure water and nutrient transport to the foliage to keep the leaves vital and functioning during the dry season. Drought-induced water deficit in the leaf tissue effects many physiological processes and influences a trees growth and survival. Among these processes, the loss of hydraulic conductivity in the xylem has been recognized as playing an important role in drought-tolerance (Tyree and Sperry, 1989). Loss of hydraulic conductivity evolves due to xylem cavitation, which is the breaking of the water column under negative xylem pressure (Zimmermann, 1983). If a xylem conduit cavitates it becomes air filled (embolized) and is no longer available for water transport (Tyree and Sperry, 1989). A trade-off between ´efficiency´ and ´safety´ of xylem conduits has been suggested (Zimmermann, 1983). Wide xylem conduits, may be more efficient water conductors than smaller ones, but at the same time they may also be more prone to dysfunction due to cavitation than small conduits (Zimmermann 1983).

Whilst the risk of cavitations and embolisms is greatest when a tree encounters drought stress, drought-tolerant species are expected to have narrow xylem conduits that provide a better structural support and reduce the risk of cavitation. Still, because of the trade-off between a xylem conduits structural support and its ability to maintain water conductivity, the density of conduits in the leaf's primary vein is expected to be high.

Drought-avoiding species have a deciduous leaf habit and shed their leaves in the dry season. Thus the carbon investment in the leaves will have to be minimal, reducing the leafs pay back time (Niinemets, 2001), while the photosynthetic apparatus will be adapted realize high photosynthetic rates to secure a high carbon assimilation. The latter is important given the short

time these species have to photosynthesize. This is expected to result in thin leaves, which are more cost efficient to produce (Niinemets, 2001), with relatively thick layers of palisade parenchyma that are better equipped to intercept directional radiation and establish high rates of photosynthesis (Vogelmann and Martin, 1993).

As the risk of drought-induced water deficit at the leaf level is less pronounced during the wet season (Tyree and Sperry, 1989), the chance cavitation of the xylem system is smaller. Drought-avoiding species are thus expected to sustain wider xylem conduits that are more efficient in transporting the greater quantities of water (Zimmermann, 1983) these species need to secure high rates of photosynthesis. Protective tissue layers as the cuticle, epidermis or hypodermis will be less expressed than in drought-tolerant species.

The last distinctive group of species is defined as drought-intolerant. Their topographical occurrence is limited to relatively wet habitats, near creeks and streams, within the dry forest habitat. Although drought-intolerant species maintain a permanent leaf cover during the dry season, most adaptations to conserve water will be less extensive than those of drought-tolerant and drought-avoiding species (tab. 1).

Structural trait		**Shade-tolerance**			**Drought-tolerance**		
	Differences between light environments	**Light-demanding species**	**Intermediate shade-tolerant species**	**Shade-tolerant species**	**Drought-avoiding species**	**Drought-intolerant species**	**Drought-tolerant species**
Leaf thickness (µm)	Sun > Shade	- -	-	+	- -	-	+
Relative thickness (µm µm^{-1}):							
Cuticle	Sun > Shade	+	-	- -	-	- -	+
Upper epidermis	Sun > Shade	+	-	- -	-	- -	+
Lower epidermis	Sun >= Shade	+	-	- -	- -	-	+
Mesophyll	Sun > Shade	- -	-	+	- -	-	+
Hypodermis	Sun > Shade	+	-	- -	-	- -	+
Palisade parenchyma	Sun > Shade	+	-	- -	+	-	- -
Spongy parenchyma	Sun > Shade	- -	-	+	- -	-	+
Palisade / Spongy parenchyma ratio (µm µm^{-1})	Sun > Shade	+	-	- -	+	-	- -
Number of hypodermis cell layers	Sun > Shade	+	-	- -	+	-	-
Number of palisade parenchyma cell layers	Sun > Shade	+	-	- -	+	-	- -
Xylem:							
Conduit density (µm^{-2})	Sun < Shade	- -	-	+	- -	-	+
Conduit diameter (µm)	Sun > Shade	+	-	- -	+	-	- -

Table 1. **Summary of hypotheses.** Within tolerance classes symbols represent; **+** = biggest trait value; **-** = smaller trait value; **- -** = smallest trait value.

Material and Methods

STUDY AREA

During 4 months of fieldwork I gathered data concerning leaf morphological and anatomical characteristics in a semi-deciduous Chiquitano dry forest approximately 40 kilometers east of the town of Concepción in the province Ñuflo de Chávez, department of Santa Cruz, eastern Bolivia (16°07'S, 61°43'W) (fig. 1). The altitude of the study area is approximately 458 m.

The Chiquitanía region is situated in the lowlands of Bolivia, in the transition zone between the most southern limit of Amazonian moist forest in the north and the xerofitic matorral of the Gran Chaco, with its thorn shrub vegetation, in the south (Killeen *et al*, 1998; Jardim *et al.* 2003). The

Chiquitano dry forest is characterized by deciduous and semi-deciduous vegetation types, of which the deciduous dry forest covers approximately 40% of the department Santa Cruz. Other important ecosystems in the region are cerrado savannas and pantanal wetlands at the border with Brazil.

Geomorphologically, the region is part of the Brazilian Shield. Low hills, composed of granite, gneiss and metamorphic rocks from Precambrian origin, dominate the landscape (Geobol, 1981). Soils are moderately acid (pH = 5,8 to 6,8 in the A horizon) and can be classified as inceptisols and alfisols (Killeen, 1997; Killeen *et al.*, 1998) and oxisols (Iporre, 1996). The study area is lacking main waterways, but on lower grounds there is evidence of seasonal creeks and streams (Schoonenberg *et al.*, 1999).

The region is characterized by a strong seasonality and the austral winter dry season occurs between April and September. Mean annual precipitation varies between 900 and 1200 mm, with a long-term average of 1100 mm per year. Precipitation peaks around 175 mm per month in the January and gets as low as 25 mm in August. This great yearly amplitude in rainfall results in a mean annual evapotranspiration that has been reported to be approximately 1300 mm, leading to a deficit of 100 to 400 mm on a yearly basis (Montes de Oca, 1989, but see Killeen *et al*, 1998). The mean annual temperature at Concepción is 24.3 ºC, ranging from 3 ºC in July and 31 ºC in October (fig. 1).

The data collection was carried out in a forest concession of approximately 30.000 ha, under exploitation of INPA Parket Ltda., in close cooperation with the Bolivian Forest Research Institute (IBIF). This organization maintains several permanent sample plots in the area, laid out in a nested design and studies amongst others the impact of silvicultural practices on forest growth and development in order to come to a sustainable forest management plan for the region.

Although the vegetation of the Chiquitanía region may be variable it is mainly dominated by *Acosmium cardenasii* H.S. Irwin & Arroyo (Fabaceae), *Anadenanthera macrocarpa* (Benth.) Brenan (Fabaceae), *Aspidosperma cylondrocarpon* Műll. Arg. (Apocynaceae), *Aspidosperma tomentosum* Mart. (Apocynaceae) and *Astronium urundeuva* (Allemão) Engl. (Anacardiaceae). Other abundant species are *Calycophyllum multiflorum* Griseb. (Rubiaceae), *Machaerium scleroxylum* Tul. (Fabaceae) and *Schinopsis brasiliensis* Engl. (Anacardiaceae) (Killeen *et al*, 1998; Jardim *et al*. 2003).

Commercially valuable timber species in the region are; *Cedrela fissilis* Vell. (Meliaceae), *Amburana cearensis* (Allemão) A.C. Smith (Fabaceae), *Machaerium scleroxylum* Tul. (Fabaceae), *Tabebuia impetiginosa* (Mart. Ex DC.) Standl. (Bignoniaceae), *Astronium urundeuva* (Allemão) Engl. (Anacardiaceae), *Centrolobium microchaete* (Mart. ex Benth.) Lima ex G. P. Lewis (Fabaceae), *Anadenanthera colubrine* (Vell.) Brenan (Fabaceae), *Aspidosperma cylondrocarpon* Műll. Arg. (Apocynaceae), *Cordia alliodora* (Ruiz & Pav.) Oken (Boraginaceae), *Guibourtia chodatiana* (Hassl.) J. Léonard (Fabaceae), *Schinopsis brasiliensis* Engl. (Anacardiaceae) and *Cariniana ianeirensis* R. Knuth (Lecythidiaceae).

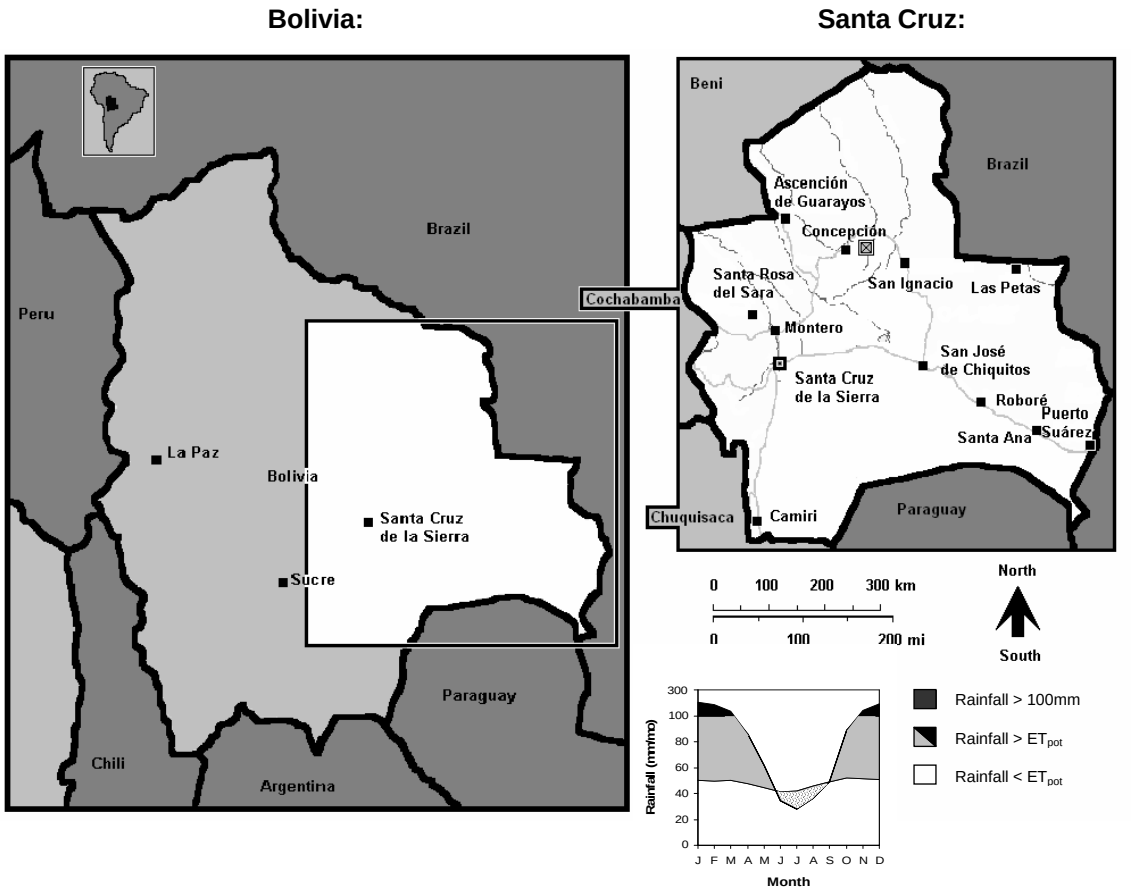

Figure 1. **The study area.** The map shows the approximate location of the study area (⊠) near the town of Concepción, in the eastern lowlands of Bolivia and a climatic diagram, that indicates mean monthly precipitation and potential evapotranspiration (Et_{pot}) at the study site. The dry season is shown in the dotted area

SPECIES

I studied the leaf structure of dry forest tree species varying greatly in phylogenetic origin, as the 41 species belong to 40 genera, 24 families and 19 orders. With six included species, Fabaceae is the biggest family in this study. This is in line with their dominance in the Chiquitano dry forest.

Species vary further in adult stature, leaf form, leaf habit, shade-tolerance and drought-tolerance. Three functional groups related to the level of shade-tolerance were distinguished. Light-demanding species are short- and long-lived pioneers that need full sunlight to establish and grow to their adult stature, intermediate shade-tolerant species are those that can establish under shady conditions, but need more light to grow and shade-tolerant species are species that can both establish and grow to adulthood in the shade. Drought-tolerance classifications were based on the species wilting index estimated for saplings during the dry season by dr. Poorter and the dominant occurrence of the species on either relatively dry elevated grounds (drought-tolerant species) or the more moist low areas, near creeks (drought-intolerant species) within the control plots. Species with a deciduous leaf habit were classified as drought-avoiding species. The expert opinion of forest engineers of the IBIF project, local field assistants and information from literature (Jardim *et al.*, 2003) added substantial information to these classifications.

Among the selected species are some of the most abundant species in this type of forest, as well as commercially valuable species (tab. 2). Especially *Tabebuia impetiginosa* is a highly valued timber species. Fruits of *Myrciaria cauliflora* and *Spondias mombin* are often for sale at local markets.

Species identification from the locally used species- and morpho-names, follow the identification presently used by IBIF and the former BOLFOR project. Identification was checked by an expert taxonomist and revised according to Jardim *et al.* (2003) and the nomenclature database of the Missouri Botanical Garden (W^3TROPICOS) where necessary.

Scientfic species name	Local name / Morphoname	Family	Adult stature (m)	Leaf form	Leaf habit	Shade-tolerance	Drought-tolerance	Use
Astronium uriundeuva	Cuchi	Anacardiaceae	27	C	D	LD	DA	Timber
Spondias mombin	Ocorocillo	Anacardiaceae	26	C	D	LD	DA	Edible fruit
Aspidosperma cylindrocarpon	Jichituriqui colorado	Apocynaceae	25	S	E	IS	DT	Timber
Aspidosperma tomentosum	Jichituriqui amarillo	Apocynaceae	23	S	D	IS	DA	Timber
Tabebuia impetiginosa	Tajibo negro	Bignoniaceae	30	C	D	LD	DA	Timber
Capparis prisca	Pacobillo	Capparaceae	15	S	E	ST	DI	Edible fruit
Jacaratia sp.	Chayote	Caricaceae	2	S	D	ST	DA	-
Combretum leprosum	Carne de toro	Combretaceae	19	S	E	ST	DT	-
Erithroxylum sp.	Coca don Israel	Erythroxylaceae	3	S	E	ST	DT	-
Actinostemon conceptionis	Don Concepcion	Euphorbiaceae	5	S	E	ST	DT	-
Manihot guaranitica subsp. *guaranitica*	Yucca	Euphorbiaceae	6	S	D	LD	DA	-
Phyllanthus sp. nov.	Maria pretina	Euphorbiaceae	4	S	E	ST	DT	-
Acosmium cardenasii	Tasaa	Fabaceae	24	C	E	IS	DT	-
Caesalpinia pluviosa	Momoqui	Fabaceae	29	C	E	LD	DT	Timber
Centrolobium microchaete	Tarara amarilla	Fabaceae	27	C	E	LD	DT	Timber
Guibourtia chodatiana	Sirari	Fabaceae	24	C	E	IS	DI	Timber
Platymiscium fragrans	Tarara colorada	Fabaceae	28	C	D	LD	DA	Timber
Sweetia fruticosa	Mani	Fabaceae	23	C	D	IS	DA	Timber
Casearia gossypiosperma	Cuse	Flacourtiaceae	18	S	D	IS	DA	-
Cariniana ianeirensis	Yesquero blanco	Lecythidaceae	31	S	D	IS	DA	Timber
Ceiba samauma	Mapajo	Malvaceae	32	C	D	LD	DA	-
Chorisia speciosa	Toborochi	Malvaceae	21	C	D	LD	DA	-
Eriotheca roseorum	Pequi blanco	Malvaceae	27	C	D	LD	DA	-
Trichilia elegans	Sama	Meliaceae	8	C	E	ST	DT	-
Myrciaria cauliflora	Guapuru	Myrtaceae	6	S	E	ST	DI	Edible fruit
Myrciaria floribunda	Sahuinto	Myrtaceae	26	S	E	IS	DT	-
Bougainvillea modesta	Comomosi	Nyctanginaceae	23	S	E	LD	DI	-
Neea hermafrodita	Mapabi	Nyctanginaceae	11	S	E	ST	DI	Timber
Gallesia integrifolia	Ajo ajo	Phytolaccaceae	22	S	E	IS	DI	-
Pogonopus tubulosus	Quina	Rubiaceae	10	S	D	ST	DA	-
Simira rubescens	Gabetillo blanco	Rubiaceae	16	S	D	ST	DA	-
Esenbeckia almawillia	Coca	Rutaceae	2	S	E	ST	DI	-
Galipea ciliata	Blanquillo falso	Rutaceae	12	C	E	ST	DT	-
Zanthoxylum monogynum	Naranjillo	Rutaceae	-	C	E	ST	DI	-
Talisia esculenta	Piton	Sapindaceae	16	C	E	ST	DT	-
Chrysophyllum gonocarpum	Aguai fruta chica	Sapotaceae	13	S	E	ST	DI	-
Pouteria gardneriana	Aguai fruta grande	Sapotaceae	-	S	E	ST	DI	-
Solanum cf. riparium	Tabacachi	Solanaceae	15	S	E	LD	DT	-
Ampelocera ruizii	Blanquillo	Ulmaceae	21	S	E	ST	DI	-
Phyllostylon rhamnoides	Cuta	Ulmaceae	26	S	E	IS	DI	-
Urera baccifera	Pica pica	Urticaceae	10	S	D	LD	DA	-

Table 2. **Species list.** The table shows taxonomical classification and common names of the 41 tree species from a Chiquitano dry forest in Santa Cruz, Bolivia. Shade tolerance (LD = light-demanding; IS = intermediate shade-tolerant; ST = shade-tolerant) and Drought-tolerance (DA = drought-avoiding; DI = drought-intolerant; DT = drought-tolerant) were determined per species prior to the study. The species adult stature, leaf form (S = simple; C = compound), leaf habit (E = evergreen; D = deciduous) and commercial value are given. Leaves of these species can be viewed in appendix III.

DATA COLLECTION

I sampled sun- and shade-leaves of these 41 species in the first half of the wet season from October 2003 to January 2004. Shade individuals were selected as much as possible in the

permanent sample control plots, situated within the forest concession, while light individuals were selected along logging roads and in tree fall gaps in the, due to silvicultural management, more open permanent sample plots.

I selected 5 trees per species growing in full sunlight and 5 trees growing in shaded conditions. Trees of comparable diameter and height (10 – 20 cm DBH, 10 – 20 m height) were sampled; only some individuals of the less common species exceed these ranges. Species like *Manihot guaranitica* subsp*. guaranitica* and *Jacaratia* sp. are small treelets and do not attain these sizes. Of every individually selected tree I estimated DBH, total height and the percentage of canopy openness. Furthermore I classified the canopy position of every tree with the Dawkins index, ranging from 1 to 5. 1 was appointed to a tree in the absolute undergrowth of the forest, receiving no direct sunlight during the course of the day at all, 2 was given to a tree above the undergrowth receiving no direct sunlight, 3 to a sub-canopy tree with some lateral illumination, 4 to a sub-canopy tree with full vertical light interception and 5 to an emergent tree fully illuminated for the entire day.

Per individual tree 5 leaves were collected halfway the outer leaf layer of the crown with an extendable pruner and transported to the field laboratory in plastic bags.

Four leaves per individual tree were included in a morphology study (Markesteijn, 2004), while a section of the remaining leaf was conserved in a 70% ethanol (EtOH) solution and stored to be included in this anatomy study. Of compound and lobed leaves a cross- section of an average-sized foliole or lobe was included and of leaves with tiny folioles or folioluls several were fixed to secure enough sampling material (fig. 2).

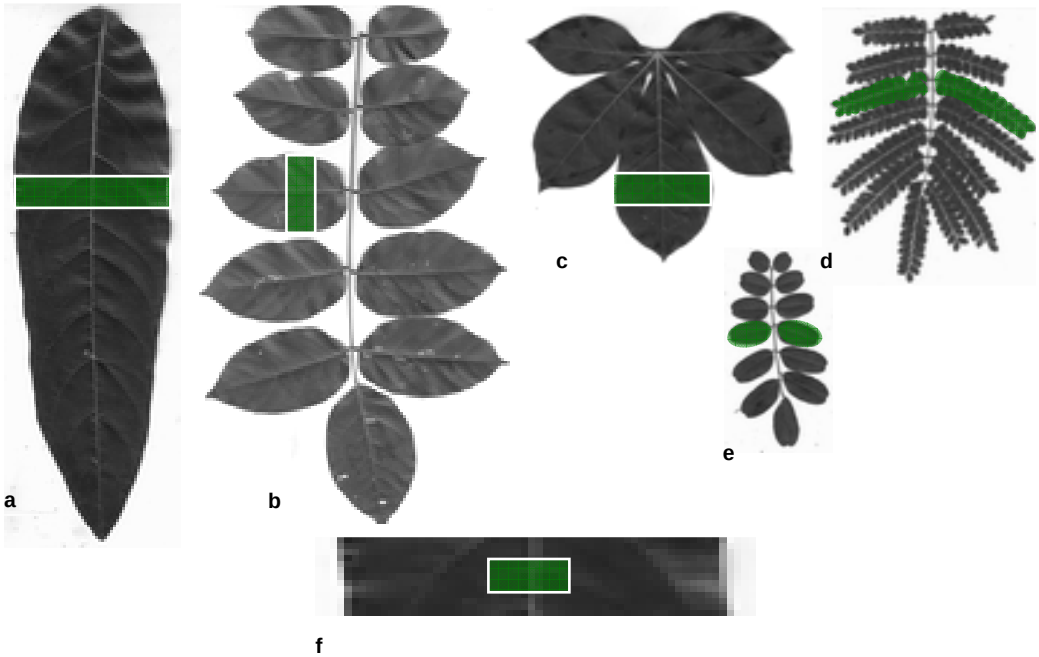

Figure 2. **Leaf samples.** Samples that were stored and included in this study were cut from leaf margin to leaf margin in a cross-sectional manner. Samples were, where possible, at least 2 cm wide. The green areas indicate the positions from where samples were cut in (a) simple leaf; (b) large compound leaf; (c) Lobed leaf; (d) bipinnately compound leaf and (e) small compound leaf. (f) A small sub-section of the samples, including a cross-section of the primary vein, was embedded in paraffin and analyzed in this study

In a laboratory at Wageningen University samples of the stored leaf tissue were selected (fig. 2f) dehydrated and embedded in paraffin (Paraplast® Plus). The embedded samples were then sectioned (16 µm thick) with a retraction-microtome (Microm HM350) in a cross-sectional manner and the tissue sections were mounted on a micro slide with glycerin-gelatin (Kaisers® glycerin-gelatin, Merck, Darmstadt, Germany). After de-waxing the paraffin from the tissue with tert-butyl alcohol (TBA) and EtOH series, I stained the tissue sections with Toluidine Blue. Per individual section images of the cross-sectional lamina and mid-rib were digitized using a microscope camera after which measurements were taken with the image processing and analysis program Image J (free download at http://rbs.info.nih.gov/ij/download.html). Embedding, sectioning and staining protocols, as used in this study, can be viewed in appendix II.

From the digital images the following traits were measured; leaf thickness (µm), cuticle thickness (µm), upper epidermis thickness (µm), hypodermis thickness (µm) (when present), palisade parenchyma thickness (µm), spongy parenchyma thickness (µm) and lower epidermis thickness (µm). I measured the diameter of 5 randomly selected xylem conduits (µm) in a perpendicular manner. Finally the number of palisade parenchyma cell layers in the mesophyll and the xylem conduit density in the mid-rib were determined. Additional observations were made on whether leaves displayed trichomes, whether stomata were sunken or not, whether cells contained crystals and whether extra or deviating tissue layers in the lamina were present.

From these data I calculated the palisade to spongy parenchyma ratio (palisade parenchyma thickness / spongy parenchyma thickness; µm µm^{-1}), and the relative thickness of the different cell layers to the total thickness of the leaf (thickness of cell layer X / leaf thickness; µm µm^{-1}).

Folioles were considered to be functionally equivalent to simple leaves and will be treaded as such in this study.

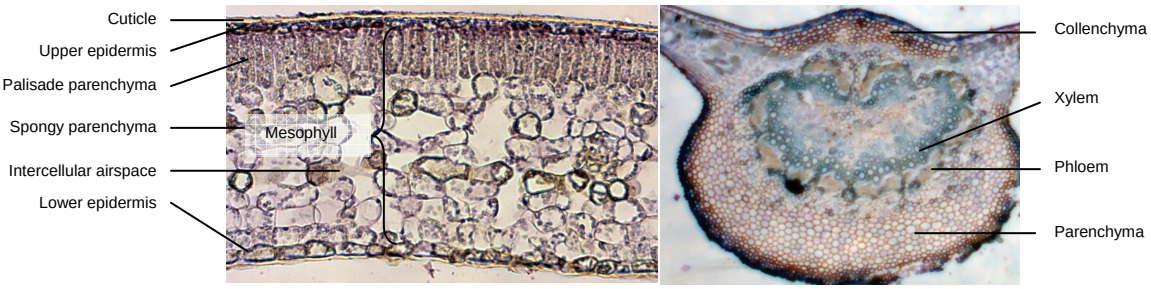

Figure 3. **Leaf cross-sections.** The figure shows the different components of an ´average´ dicot leaf including (**a**) the lamina (*Esenbeckia almawillia* (Rutaceae)); 200x) and (**b**) the primary vein (*Capparis prisca* (Capparaceae)); 20x). The different cell structures are indicated.

DATA ANALYSIS

To examine the variation in structural leaf traits among species and among light environments within species, I performed a two-way analysis of variance (ANOVA) with species and light environment within species included as fixed factors (independent variables) and the individual structural traits (log_{10}-transformed) as dependent variables. The factors included in the two-way ANOVA divide the measured values of the dependent variable into groups and the ANOVA

tests the null hypothesis that the mean values of the dependent trait are equal in a group; $H_{0, \text{group A}}$: $\mu_1 = \mu_2 = \ldots = \mu_A$, against its alternative that not all means the group are equal; $H_{1, \text{group A}}$: $\mu_1 \neq \mu_2 \neq \ldots \neq \mu_A$. If, in this study, the analysis outcome for the factor '*species'* is significant, it means that the mean values of the dependent variable are not all equal, so species differ. If the outcome for the factor '*light environment within species'* is significant, it means that the mean values of the dependent variable are not all equal, so sun- and shade-leaves differ per species. Eta-squared (η^2), which is analogous to r^2 in correlation analysis, was calculated per factor and per structural trait to estimate the effect-size. This effect-size of a factor expresses the proportion of the total variance that is explained by the effect of that factor. η^2 is calculated from the models sum of squares (SS) with the formula: $\eta^2 = SS_{effect} / SS_{total}$. The remaining unexplained variation was calculated with the same formula from the models error sum of squares (SS_{error} / SS_{total}).

To analyze the extent to which sun- and shade-leaves differ within species, shade-sun ratios (SHSU-ratios) for each structural leaf trait were tested for significant deviation from unity with a One-Sample T test. SHSU-ratios are defined as a species mean shade-value for a given trait divided by its mean sun-value and range from 0 to 1 when the sun-value bigger than the shade-value and from 1 to infinity when the shade-value is bigger than the sun-value. Because these ratios have a non-linear response range, they were linearized with an arctangent transformation. To explain this non-linear response, let's assume one has 5 sun-leaves and 5 shade-leaves of a given species A. The thickness of the mean sun-leaf of this species turns out to be 160 µm and the mean shade-leaf 80 µm. The SHSU-ratio of this species is 0,5 ,deviating 0,5 from 1. If a another species B has mean sun- and shade-leaf thicknesses that are exactly the other way around, so 80 µm in the sun and 160 µm in the shade, the ratio is 2, deviating 1 from 1. Still the absolute differences between the sun- and shade-leaves of species A and B should be equal. After the following transformation; *SHSU(x) = ARCTAN(shade-value(x) / sun-value(x)) – ARTAN(1)*, the former deviations of 0,5 and 2 are -0,77 and 0,78 respectively. As such the absolute deviation from unity remains the same whether the sun-value is *a* times bigger than the shade-value or the shade-value is *a* times bigger than the sun-value. Using these transformed SHSU-ratios per species the null hypothesis; H_0: *SHSU(x) = 0*, was tested against its alternative; H_1: *SHSU(x) ≠ 0*. If the outcome of the analysis is significant the null hypothesis can be rejected and the alternative hypothesis accepted. This means that the mean SHSU-ratio of the trait in question significantly deviates from unity. If the mean difference is between –1 and 0 the sun leaf has the greater value, if the mean difference lies between 0 and 1 it is the shade leaf with the greater value.

To evaluate the multivariate interspecific variation in the dataset I preformed a Bray Curtis Ordination (= Polar Ordination) and ranked the species in a three-dimentional variable space (along three ordination axes) on the bases of their multivariate dissimilarity in Euclidian distances. As such species ranking occurred along an ordination axis between two extracted end points, defined as the species that are least alike in their leaf structural appearance. Because the extraction of the ordination axis with the Bray Curtis method is highly susceptible

to outliers, all included structural traits were log_{10}-transformed to improve the normality of their distributions. Two species, *Ceiba samauma* (Malvaceae) and *Caesalpinia pluviosa* (Fabaceae), were excluded from the analysis because of missing values. The percentage of variance explained by the individual axes and cumulative variance explained by the ordination were calculated, based on the ratio of the sum of squares of the residual distance matrix (SSR) to the sum of squares of the original distance matrix (SST); *Cumulative variance explained, % = 100(1 - SSR/SST)* (McCune and Mefford. 1999). Pearson correlation coefficients of the structural traits with the extracted ordination axis were calculated to evaluate the biggest sources of variation in the dataset. Finally the species were plotted against the first two ordination axes to see whether 'natural' groups of species with clustered response along the axes could be identified. An overlay of the a-priory defined functional groups and their territories was included in the graphs to see whether functional groups showed any form of unimodality.

A Bray Curtis Ordination does not actually tell you 'why' groups of species cluster or not. I additionally chose to evaluate the differences between functional groups with a Canonical Correspondence Analysis (CDA). A CDA procedure for more than two groups, generates a set of discriminant functions, based on linear combinations of the predictor variables that provide the best discrimination between the groups. The functions are generated from a sample of cases (species) for which group membership is known (SPSS 11.0.0, 2001). As such a CDA forces the separation of the functional groups. I derived the separating power of the structural traits from their canonical correlation (Pearson coefficients) with the calculated canonical axis. Differences in structural leaf traits between functional groups were tested with a one-way ANOVA in combination with a post-hoc Duncan's Multiple Range test. Individual leaf trait-values per species (n = 39) were included in the analysis as dependent variables and functional groups as fixed factors (independent variables). An 0,05 criterion of statistical significance ($\alpha = 0,05$) was used for all tests. All statistical analyses and graphical display of data concerning the Analyses Of Variance, One-Sample T test and Canonical Correspondence Analyses were performed using the statistical package SPSS (version 11.0). The Bray Curtis Ordination was performed using PC-ORD (version 4.33), a statistical package for multivariate analysis of ecological data.

Results

AMONG SPECIES DIFFERENCES

The leaf of the average Chiquitano dry forest tree species is 87 µm thick and consists of a cuticle (1 µm), an upper epidermis (10 µm), 1 cell layer of palisade parenchyma (27 µm), spongy parenchyma (40 µm) and a lower epidermis (7 µm). Only 3 of the 41 species form a hypodermis, *Gallisia integrifolia* (Phytolaccaceae), *Tabebuia impetiginosa* (Bignoniaceae) and *Myrciaria floribunda* (Myrtaceae), that has an average thickness of 25 µm and consists of 2 cell layers, including the upper epidermis.

Overall *Neea hermafrodita* (Nyctanginaceae) has the thickest leaves (150,13 µm) and Actinostemon conceptionis (Euphorbiaceae) the thinnest (51 µm). In proportion to the total leaf thichness, *Actinostemon conceptionis*, has the thickest cuticle (2,4 %) and *Gallesia integrifolia* (Phytolaccaceae) (0,6%) the thinnest. *Chorisia speciosa* (Malvaceae) has the thickest upper epidermis (34%) and Pouteria gardneriana (Sapotaceae) the thinnest (5%). The relative thickness of the palisade parenchyma ranges from 16% to 43% in *Chrysophyllum gonocarpum* (Sapotaceae) *and Solanum cf. riparium* (Solanaceae) respectively. The thickness of the spongy parenchyma ranges from 26% to 68% in *Eriotheca roseorum* (Malvaceae) and *Esenbeckia almawillia* (Rutaceae) respectively. The greatest relative lower epidermis thickness is found in *Erithroxylum sp.* (Erithroxylaceae) (18 %) and smallest in *Pouteria gardneriana* (5%). The thickness of the hypodermis does not differ significantly among the three tree species mentioned earlier and occupies 21% to 26% of the total leaf thickness in these species. Still the number of cell layers that make up the hypodermis is greatest in *Tabebuia impetiginosa*. *Eriotheca roseorum* has the widest xylem conduits among species (24,56 µm) and Acosmium cardenasii (Fabaceae) the narrowest (5 µm), while the latter species has the highest xylem conduit density (23137 mm^{-2}) and Eriotheca roseorum the lowest (1610 mm^{-2}).

Leaves vary greatly in their structural appearance, differences among species explain 56% to 87% of the variation in leaf structural traits in general (tab. 3). The xylem conduit diameter and density show the greatest variability among species followed by the relative mesophyll thickness and total leaf thickness. Absolute values of sun- and shade-leaves per species can be viewed in appendix 1.

WITHIN SPECIES DIFFERENCES

Within species the effect of differences among light environments explains less of the total variation in structural traits than differences among species. Still the light environment effect is significantly explaining variation in 8 of the 13 included traits (3% - 14%) (tab. 3).

Dependent Variables	**Species**				**Light environment within species**				**Total variance explained**
	df	F	Sig.	η^2	df	F	Sig.	η^2	
Leaf thickness (µm)	40	27	****	0,73	40	2	***	0,06	79%
Relative thickness (µm μm^{-1}):									
Cuticle	39	18	****	0,66	39	2	**	0,07	73%
Upper epidermis	40	24	****	0,73	40	1	ns	0,04	77%
Lower epidermis	40	16	****	0,64	40	1	ns	0,05	69%
Mesophyll	40	27	****	0,74	40	2	****	0,06	80%
Hypodermis	2	0	ns	0,01	2	2	ns	0,24	24%
Palisade parenchyma	40	21	****	0,64	40	5	****	0,14	78%
Spongy parenchyma	40	26	****	0,70	40	4	****	0,10	80%
Palisade / Spongy parenchyma ratio (µm μm^{-1})	40	23	****	0,66	40	5	****	0,13	80%
Number of hypodermis cell layers	2	13	***	0,56	2	0	ns	0,02	58%
Number of palisade parenchyma cell layers	40	23	****	0,65	40	5	****	0,14	79%
Xylem:									
Conduit density (μm^{-2})	39	52	****	0,85	39	2	***	0,03	88%
Conduit diameter (µm)	39	63	****	0,87	39	1	ns	0,02	89%

Table 3. **Factor effects on leaf trait variation.** The table shows the results of a two-way ANOVA with species and light environment within species as fixed factors and the different log_{10}-transformed structural leaf traits as dependent variables. Degrees of freedom (df), F-values (F), and the proportion of explained variation (η^2) are given. Significance levels; **ns**; $p > 0,05$; * $p < 0,05$; **** $p < 0,01$; ***** $p < 0,001$; ****** $p < 0,0001$, are tested at $\alpha = 0,05$

The SHSU-ratios of these 8 traits significantly deviate from unity (fig. 4). Within species sun-leaves are thicker than shade leaves and have, relatively to thickness of the leaf, a thicker cuticle, more mesophyll and a thicker layer of palisade parenchyma. The number of cell layers increases with increase in light availability. The spongy parenchyma within the mesophyll of sun-leaves is relatively thin, as is the lower epidermis in proportion to the total leaf thickness. The ratio between the palisade and spongy parenchyma is bigger in sun-laves than in shade leaves. The latter again indicates that the proportion of palisade parenchyma in the mesophyll of sun-leaves is bigger than in shade-leaves. The diameter and density of the xylem conduits does not show a significant deviation from unity. Within the three species that exhibit a hypodermis, this tissue does not play a significant role in photosynthetic acclimation as neither their thickness nor the number of cell layers that form the hypodermis differ between sun- and shade-leaves.

Traits with a the biggest significant deviation from unity, are; the palisade to spongy parenchyma ratio (deviating 19%), the number of palisade parenchyma cell layers (16%), relative palisade parenchyma thickness (14%), leaf thickness (10%), relative cuticle thickness (10%), relative spongy parenchyma thickness (9%), and the relative lower epidermis thickness (8%). Within species these structural traits thus show the greatest plasticity in response to differences in light environment.

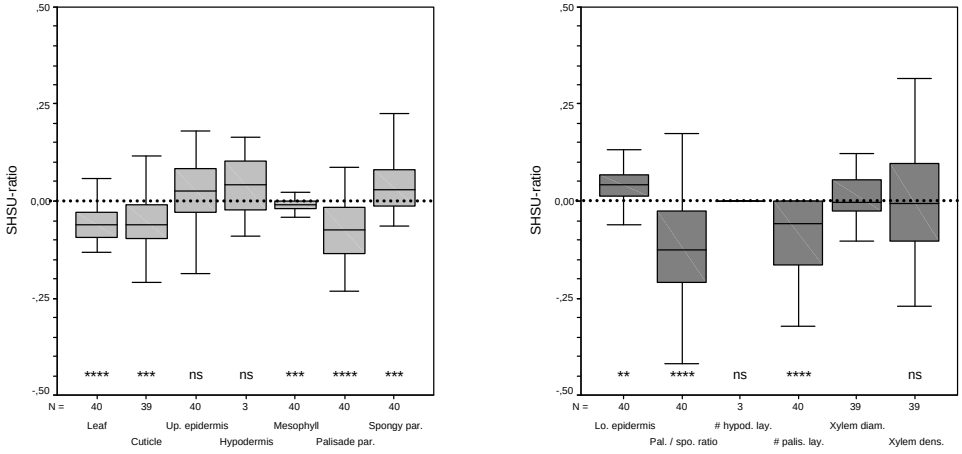

Figure 4. **Shade-sun ratios; deviation from unity.** The upper and lower limits of the boxes indicate the 25 and 75 percentile of the arctangent transformed shade – sun ratio values of 40 species (df = 39), with their median value. *Ceiba samauma* was excluded from the analysis, as only sun leaves of the species were collected. The error bars represent the total range of values. Significance of deviation from unity (0) is given per trait, ns; non-significant, *; $p < 0,05$; **; $p < 0,01$; ***; $p < 0,001$; and **** $p < 0,0001$. For the untransformed SHSU-ratios per species see appendix 1.

FUNCTIONAL GROUPS

The result of the Bray Curtis ordination are summarized in table 4 and figure 5. Figure 5 gives an graphical interpretation of the dissimilarity of the species ordered from minimum to maximum along the fist two derived axis (see app. I for abbreviations of species names). See table 4 for the percentages of explained variance.

The 3 extracted axis together explain 84,3 % of the dissimilarity between species. The first axis explains 67,7 % of the variation and ranks the species on the basis of their multivariate dissimilarity in leaf structure to *Eriotheca roseorum* (Malvaceae) (fig. 5; PEQ.B). The species that differs most from the latter is *Acosmium cardenasii* (Fabaceae) (fig. 5.; TAS). The density of the xylem conduits in the primary vein of the leaf (r = -0,971) and their mean diameter (r = 0,996) have the biggest explanative value for the species distribution along the 1st axis.

9,9% of the restating variance is explained by the second axis, which ranks the species based on their dissimilarity to *Neea hermafrodita* (Nyctanginaceae) (fig. 5; MAP). *Actinostemon conceptionis* (Euphorbiaceae) (fig. 5; DON.C) shows the greatest dissimilarity with this species. Species ranking along the 2nd axis occurs mainly due to the differences in leaf thickness (r = -0,962). The 3rd axis (tab. 4) has the lowest explanative value (6,8%) and ranks the species on their dissimilarity to *Centrolobium microchaete* (Fabaceae) (TAR.A), with *Jacaratia sp.* (Caricaceae) (COY) differing most. The palisade to spongy parenchyma ratio (r = 0,621) and its plasticity (r = -0,714) are strongly related to this axis.

Although the structure of the xylem in the primary veins, the leaf thickness and palisade to spongy parenchyma ratios amongst others explain much of the dissimilarity among species, ‘natural’ groups of species do not seem to arise. The spread of species within the two dimensional variable space (fig. 5) seems even and no strong clusters of species can be detected. The functional groups of species that were classified prior to the analysis are plotted in overlay with the species in the Bray Curtis Ordination figure (fig. 5). The group territories display a lot of overlap with each other, which means that the multivariate dissimilarity in leaf structure, that is so strongly explanative for differences among species, hardly seems to plays a significant role in explaining the functional group association of the species with respect to shade- and drought-tolerance.

Dry forest?

Not always.
With precipitation easily exceeding 100 mm per month during the wet season, moat-digging became a frequently observed activity amongst researchers

	Axis 1	2	3
End points (Species with greatest dissimilarity)	PEQ.B - TAS	MAP - DON.C	COY - TAR.A
% variance explained	67,7 %	9,9 %	6,8 %
Cumulative % variance explained	67,7 %	77,5 %	84,3 %
Structural traits			
Leaf thickness (µm)	-0,05	-0,96	-0,31
Relative thickness (µm µm^{-1}):			
Cuticle	0,04	0,23	-0,22
Upper epidermis	-0,49	0,38	0,38
Lower epidermis	0,15	0,38	0,17
Mesophyll	0,32	-0,26	-0,37
Palisade parenchyma	-0,46	0,06	0,47
Spongy parenchyma	0,58	-0,22	-0,61
Palisade / Spongy parenchyma ratio (µm µm^{-1})	-0,58	0,10	0,62
Number of palisade parenchyma cell layers	-0,24	-0,29	0,49
Xylem:			
Conduit density (µm^{-2})	-0,97	0,12	0,09
Conduit diameter (µm)	0,99	-0,16	-0,04
Plasticity in structural traits (SHSU-ratios)			
Leaf thickness (µm)	-0,10	0,08	0,01
Relative thickness (µm µm^{-1}):			
Cuticle	-0,04	-0,16	-0,48
Upper epidermis	-0,25	0,06	0,09
Lower epidermis	0,26	-0,14	-0,17
Mesophyll	-0,03	0,29	-0,65
Palisade parenchyma	-0,08	-0,17	0,73
Spongy parenchyma	0,01	0,06	0,23
Palisade / Spongy parenchyma ratio (µm µm^{-1})	0,003	0,31	-0,71
Number of palisade parenchyma cell layers	0,14	-0,26	-0,50
Xylem:			
Conduit density (µm^{-2})	-0,15	-0,07	0,23
Conduit diameter (µm)	0,21	-0,25	-0,49

Table 4. **Bray Curtis Ordination.** The table shows the Pearson correlation coefficients (r) of the structural traits and their plasticity with the three extracted ordination axis. The species with the greatest dissimilarity, the percentage and cumulative percentage of explained variance are given for each axis. 39 species were included in the analysis and to minimize the effect of outlying values, all traits were log_{10}-transformed.

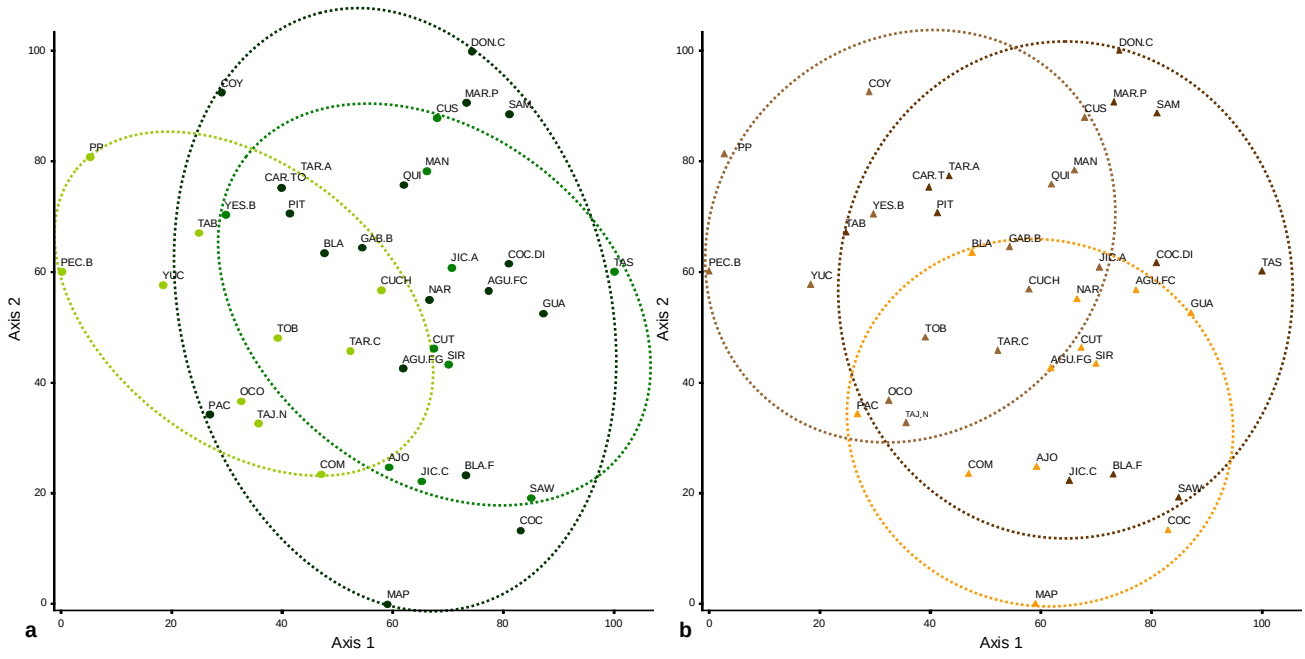

Figure 5.

Bray Curtis Ordination. The figures show the graphic interpretation of a Bray Curtis Ordination. 39 species were plotted and abbreviations of their local names were used to mark their location along the first two extracted axis. The functional group membership of the species is marked by the different colours of their symbols. (**a**) Symbols represent; ●, shade-tolerant species (n = 18); ●, intermediate shade-tolerant species (n = 10) and; ●, light-demanding species (n = 11). The circular shapes are rough interpretations of the group territories; ···· = shade-tolerant, ···· = intermediate shade-tolerant, and ···· = light-demanding species. (**b**); ▲, drought-tolerant species (n = 12); ▲, drought-avoiding species (n = 15) and; ▲, drought-intolerant species (n = 12). Group territoties; ···· = drought-tolerant, ···· = drought-avoiding, and ···· = drought-intolerant species

To provide more insight in the differences between the functional groups, as classified prior to the experiment, a Canonical Discriminant Analysis (CDA) was preformed on the same data of the Bray Curtis Ordination followed by a one-way ANOVA. A graphical interpretation of this analysis is provided in figure 6. Differences between functional groups are summarized in table 5. The palisade to spongy parenchyma ratio was excluded from the analysis for failing the tolerance test.

In line with the suggestion raised with the Bray Curtis Ordination above, shade-tolerance groups are not significantly separated along the two canonical axes (CA) (1^{st} CA; p = 0,148; 2^{nd} CA; p = 0,483). The 1^{st} CA explains 68,3% of the variation and mainly seperates species with high xylem conduit densities at the right side of the axis and species with wide conduits at the left. The 2^{nd} CA explains the restating 32,7% of the variation and separates species with a relatively thick upper epidermis (top) from the species with a higher number of palisade parenchyma cell layers (bottom). An extreme outlier species, *Myrciaria cauliflora* (Myrtaceae) is found that was initially classified as shade-tolerant. Still it shows greater resemblance with the intermediate shade-tolerant group. Other than that 94,9% of the species seems correctly classified.

Although shade-tolerance groups are not significantly discriminated on all included traits, several structural traits do differ between functional groups (tab. 5). Light-demanding species have a, in proportion to the total leaf thickness, thicker upper epidermis than shade-tolerant and intermediate shade-tolerant species and more palisade parenchyma tissue. Light-demanding species have the least amount of spongy parenchyma tissue and thus the highest palisade to spongy parenchyma ratio. The diameter of the xylem conduits is biggest in light demanding species, while the density of these vessels is lowest among functional groups. The shade-tolerant and intermediate shade-tolerant groups are much alike in their leaf structure and do not differ from each other in the traits described above (tab. 5). The relative thickness of the cuticle and the number of palisade parenchyma cell layers in the mesophyll of the leaves are marginally different between functional groups ($0,05 < p < 0,1$). Cuticles are marginally thicker in shade-tolerant than in light demanding species, both groups do not differ from intermediate shade-tolerant species. The number of palisade parenchyma cell layers is marginally bigger is shade-tolerants than in the other two groups. Functional groups related to shade-tolerance do not differ in plasticity of their leaf structural traits (tab. 5).

	Shade-tolerance groups (fig. 6a) Canonical axis		Drought-tolerance groups (fig. 6b) Canonical axis	
	1	2	1	2
Sig. of discrimination	0,148	0,483	0,015	0,355
% variance explained	68	32	76	24
Cumulative % variance explained	68	100	76	100
Structural traits				
Leaf thickness (µm)	-0,077	-0,075	-0,023	-0,441
Relative thickness (µm µm^{-1}):				
Cuticle	0,251	0,150	0,081	-0,082
Upper epidermis	-0,299	0,135	-0,154	0,362
Lower epidermis	0,100	0,197	0,135	0,184
Mesophyll	0,160	-0,074	0,055	-0,238
Palisade parenchyma	-0,392	0,048	-0,088	0,170
Spongy parenchyma	0,449	-0,104	0,110	-0,302
Palisade / Spongy parenchyma ratio (µm µm^{-1})	-0,536	0,084	-0,111	0,246
Number of palisade parenchyma cell layers	-0,192	-0,277	-0,096	-0,170
Xylem:				
Conduit density (µm^{-2})	0,472	-0,263	0,254	-0,219
Conduit diameter (µm)	-0,482	0,298	-0,221	0,146
Plasticity in structural traits (SHSU-ratios)				
Leaf thickness (µm)	-0,086	0,173	0,093	0,007
Relative thickness (µm µm^{-1}):				
Cuticle	-0,006	-0,132	-0,179	-0,089
Upper epidermis	-0,139	-0,025	-0,153	-0,064
Lower epidermis	-0,032	0,034	0,033	0,234
Mesophyll	0,181	-0,069	0,084	-0,184
Palisade parenchyma	0,147	-0,092	-0,027	0,062
Spongy parenchyma	-0,199	0,079	0,027	-0,009
Palisade / Spongy parenchyma ratio (µm µm^{-1})	0,196	-0,044	-0,031	0,062
Number of palisade parenchyma cell layers	0,064	-0,087	0,077	-0,164
Xylem:				
Conduit density (µm^{-2})	0,082	0,155	-0,070	-0,248
Conduit diameter (µm)	-0,072	-0,113	0,124	-0,013

Table 4. **Canonical Discriminant Analysis.** The table shows the Pearson correlation coefficients of the structural traits and their plasticity with the two canonical axis for functional groups related to shade-tolerance (fig. 6a) and drought-tolerance (fig. 6b).

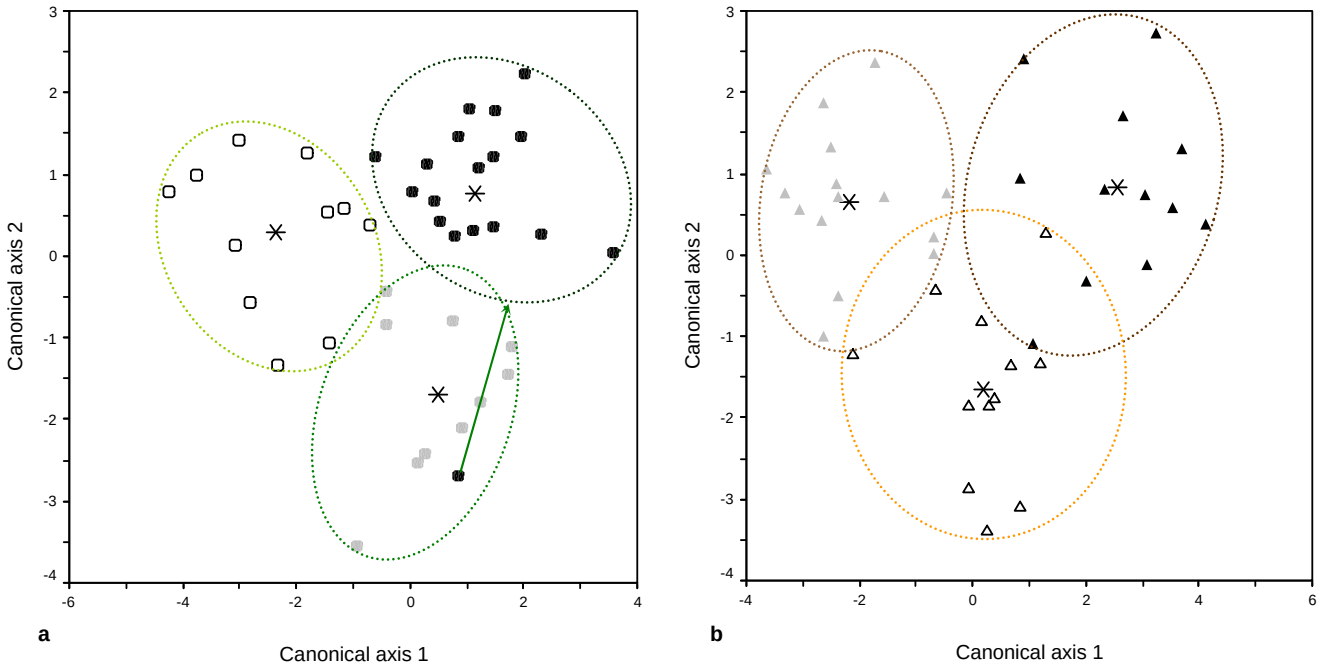

Figure 6. **Canonical Discriminant Analysis.** The figures show the graphic interpretation of a CDA. 39 species were plotted along two Canonical axis. The functional group membership of the species is marked by the different colours of their symbols. (**a**) Symbols represent; ●, shade-tolerant species (n = 18); ●, intermediate shade-tolerant species (n = 10) and; ○,

light-demanding species (n = 11). The circular shapes are rough interpretations of the group territories; ▪▪▪▪ = shade-tolerant, ▪▪▪▪ = intermediate shade-tolerant, and ▪▪▪▪ = light-demanding species. (**b**); ▲, drought-tolerant species (n = 12); ▲, drought-avoiding species (n = 15) and; ∆, drought-intolerant species (n = 12). Group territoties; ▪▪▪▪ = drought-tolerant, ▪▪▪▪ = drought-avoiding, and ▪▪▪▪ = drought-intolerant species.

	Light-demanding species	Intermediate shade-tolerant species	Shade-tolerant species	ANOVA			
	mean n = 11	mean n = 10	mean n = 18	df	F	Sig.	η^2
Structural traits							
Leaf thickness (µm)	88	86	81	2	0	ns	0,02
Relative thickness (µm µm^{-1}):							
Cuticle	0,01	0,01	0,02	2	3	ns	0,15
Upper epidermis	0,16 b	0,10 a	0,11 a	2	4	*	0,19
Lower epidermis	0,07	0,07	0,09	2	1	ns	0,06
Mesophyll	0,74	0,77	0,77	2	1	ns	0,06
Palisade parenchyma	0,36 b	0,29 a	0,28 a	2	7	**	0,27
Spongy parenchyma	0,37 a	0,49 b	0,49 b	2	9	***	0,33
Palisade / Spongy parenchyma ratio (µm µm^{-1})	1,05 b	0,63 a	0,61 a	2	12	****	0,40
Number of palisade parenchyma cell layers	1,60	1,60	1,25	2	3	ns	0,15
Xylem:							
Conduit density (µm^{-2})	3611 a	9337 b	7844 b	2	11	***	0,38
Conduit diameter (µm)	14,33 b	7,72 a	8,85 a	2	12	***	0,40
Plasticity in structural traits (SHSU-ratios)							
Leaf thickness (µm)	0,93	0,86	0,90	2	1	ns	0,05
Relative thickness (µm µm^{-1}):							
Cuticle	0,85	0,91	0,83	2	0	ns	0,02
Upper epidermis	1,10	1,03	1,01	2	1	ns	0,05
Lower epidermis	1,08	1,06	1,07	2	0	ns	0,00
Mesophyll	0,96	0,98	0,98	2	2	ns	0,08
Palisade parenchyma	0,80	0,88	0,86	2	1	ns	0,06
Spongy parenchyma	1,17	1,06	1,07	2	2	ns	0,09
Palisade / Spongy parenchyma ratio (µm µm^{-1})	0,69	0,84	0,84	2	2	ns	0,09
Number of palisade parenchyma cell layers	0,80	0,86	0,83	2	0	ns	0,02
Xylem:							
Conduit density (µm^{-2})	0,96	0,94	1,06	2	1	ns	0,04
Conduit diameter (µm)	1,03	1,04	1,00	2	0	ns	0,03

Table 5. **Differences among functional groups; Shade-tolerance.** The table shows mean structural trait values and their plasticity per functional group. Differences in means were tested with a one-way ANOVA with a post-hoc Duncan's Multiple Range test. Degrees of freedom, F-values, significance levels and explained variance (η^2) are given. Means that share the same letter are not significantly different at the 5% level. All traits were log10-transformed.

The 2nd Canonical Discriminant Analysis, performed on the a-priory defined drought-tolerance groups significantly separates the functional groups along the 1st CA (p = 0,015). The discrimination along the 2nd CA is not significant (p = 0,355). The 1st CA explains 76% of the total variance and mainly separates drought-tolerant species from drought-avoiding species. The palisade to spongy parenchyma ratio was excluded from the analysis, again for not passing the tolerance test.

Species at the right side of the 1st CA are mainly characterized by the high density of xylem conduits in their primary veins and at the left side by their wide xylem conduits. The 2nd CA explains 24% of the variance and mainly distinguishes between species with a relatively thick upper epidermis at the top of the axis and species with thick leaves at the bottom (fig. 6). Overall 92,3% of the species seems correctly classified. Significant differences among drought-tolerance groups can be viewed in table 6.

	Drought-intolerant species	Drought-avoiding species	Drought-tolerant species	ANOVA			
	mean n = 12	mean n = 15	mean n = 12	df	F	Sig.	η^2
Structural traits							
Leaf thickness (µm)	101 b	79 a	76 a	2	5	*	0,20
Relative thickness (µm µm^{-1}):							
Cuticle	0,01	0,01	0,01	2	1	ns	0,03
Upper epidermis	0,09 a	0,15 b	0,12 ab	2	5	*	0,21
Lower epidermis	0,07	0,08	0,09	2	2	ns	0,11
Mesophyll	0,79	0,74	0,76	2	2	ns	0,08
Palisade parenchyma	0,28	0,33	0,30	2	1	ns	0,07
Spongy parenchyma	0,50	0,42	0,46	2	3	ns	0,14
Palisade / Spongy parenchyma ratio (µm µm^{-1})	0,62	0,84	0,70	2	2	ns	0,11
Number of palisade parenchyma cell layers	1,55	1,48	1,26	2	1	ns	0,07
Xylem:							
Conduit density (µm^{-2})	8281 b	4456 a	8556 b	2	6	**	0,25
Conduit diameter (µm)	8,85 a	12,11 b	8,33 a	2	4	*	0,19
Plasticity in structural traits (SHSU-ratios)							
Leaf thickness (µm)	0,90	0,88	0,93	2	1	ns	0,03
Relative thickness (µm µm^{-1}):				2	3	ns	0,12
Cuticle	0,88	0,94	0,74				
Upper epidermis	1,05	1,09	0,96	2	2	ns	0,09
Lower epidermis	1,02	1,08	1,11	2	1	ns	0,07
Mesophyll	0,99	0,96	0,98	2	1	ns	0,07
Palisade parenchyma	0,83	0,87	0,85	2	0	ns	0,01
Spongy parenchyma	1,10	1,08	1,10	2	0	ns	0,00
Palisade / Spongy parenchyma ratio (µm µm^{-1})	0,77	0,82	0,79	2	0	ns	0,01
Number of palisade parenchyma cell layers	0,88	0,78	0,84	2	1	ns	0,06
Xylem:							
Conduit density (µm^{-2})	1,10 b	0,99 ab	0,90 a	2	6	**	0,25
Conduit diameter (µm)	1,02 ab	0,99 a	1,05 b	2	4	*	0,19

Table 6. **Differences among functional groups; Drought-tolerance.** The table shows mean structural trait values and their plasticity per functional group. Differences in means were tested with a one-way ANOVA with a post-hoc Duncan's Multiple Range test. Degrees of freedom, F-values, significance levels and explained variance (η^2) are given. Means that share the same letter are not significantly different at the 5% level. All traits were log10-transformed.

Surprisingly, drought-intolerant species have thicker leaves than both drought-tolerant and drought-avoiding species. Drought-intolerant species have relatively the thinnest upper epidermis and drought-avoiding species the thickest. Drought-tolerant species do not differ from the latter groups in the thickness of the upper epidermis. Drought-avoiding species have the widest xylem conduits that occur within the leaf's primary vein in the lowest densities. Drought-tolerant and intolerant species do not differ in their xylem diameter and density. The plasticity of the xylem conduit diameter and density in respect to light availability further show differences among groups. The diameter of the conduits in drought-intolerant species decreases between shade- and sun-leaves, while in drought-tolerant species it's the other way around, their xylem conduits become wider. Drought-avoiding species hardly show any plasticity in this trait and does not differ from the other groups. Drought-avoiding species show the lowest plasticity in their xylem conduit density and drought-tolerant species the greatest. Drought-intolerant species do not differ from the other groups in these characteristics.

Discussion

LEAF THICKNESS

The thickness of the mean Chiquitano dry forest leaf was found to be remarkably low, only 87 µm (se = 3,8, n = 41). The mean leaf thickness of species from a lowland tropical rainforest in Mexico was 208 µm (n = 60) (Bongers and Popma, 1990). Intuitive one would expect leaves of dry forest tree species to be thicker than leaves of wet forest tree species. In general leaf thickness is positively related to LMA (Niinemets, 2001, Wright *et al*., 2004), as is also the case for the leaves of Chiquitano dry forest tree species (Markesteijn, 2004). With the concept of leaf pay back time (PT) Niinemets (2001) explains why leaves in arid environments are not necessarily thick. Leaf pay back time can be defined as; $PT = C_c \times LMA / A_{net}$ (equ. 1), where C_c is leaf construction cost per unit leaf dry mass (g glucose g^{-1}), *LMA* is leaf mass per unit area (g m^{-2}) and A_{net} is the net rate of carbon assimilation per day (g glucose m^{-2} d^{-1}). The result of this equitation defines PT as the number of days a leaf needs to photosynthesize in order to re-assimilate (pay back) the initial amount of carbon invested in its formation.

Niinemets (2001) argues that drought stressed plants will reduce evaporation by decreasing stomatal openness (Schultze, 1986) and although thick leaves have a potentially high A_{net}, stomatal closure, besides reducing water loss from the leaf's intercellular airspace, also limits the CO_2 entry into the leaf, reducing the actual A_{net}. As a consequence, increases in leaf thickness may not result in an actual high A_{net}, but bring about greater leaf construction costs per unit leaf area. According to equitation 1, this results in a greater leaf pay back time in dry ecosystems compared to wet ecosystems (Niinemets, 2001). Increasing leaf thickness to increase drought resistance of dry forest tree species may thus be limited by decreased carbon assimilation. In the near future examination of stomatal prints of the 41 species included in this study may reveal more insight on this matter.

Another explanation could be that many dry forest tree species have a deciduous leaf habit. Leaves of deciduous species are deployed during the wet season, which could imply that adaptations that minimize drought stress are less needed and that they emphasize on maximizing assimilation given their limited leaf life span.

DIFFERENCES BETWEEN SUN- AND SHADE-LEAVES

The leaf structural appearance of dry forest tree species differed substantially both among species and between different light environments within species. While differences among species explain most of the variation in all structural components of the leaves (on average 65%), several traits are also strongly influenced by the amount of irradiance the leaves experience (tab. 3).

The results of differences in leaf structure between sun- and shade-leaves are overall in line with the hypotheses. Sun-leaves are thicker than shade-leaves and have a, in proportion to their leaf thickness, thicker cuticle, thicker palisade parenchyma tissue composed of more cell layers, a higher palisade to spongy parenchyma ratio and a thicker mesophyll tissue (tab. 3, fig. 4) Still

in contrast to the hypotheses, the relative thickness of the spongy parenchyma in the mesophyll and the thickness of the lower epidermis are smaller in sun-leaves. The irradiance level does not seem to influence the relative thickness of the upper epidermis and hypodermis, nor the number of celllayers in the hypodermis and the diameter or density of the xylem conduits in the primary vein (fig. 4).

Increasing leaf thickness is a commonly found adjustment to high irradiance. Thicker sun- than shade-leaves have been found within individual trees (Klich, 2000; Wylie, 1951; Yáñez-Espinosa *et al*., 2003) and for trees growing in different light environments (Bongers and Popma, 1988; Buisson and Lee, 1993; Cao, 2000; Chazdon and Kaufmann, 1993; Field *et al*., 2001; Fisher, 1986; McClendon and McMillen, 1980).

The greater thickness of sun-leaves is related to an increase in the proportional thickness of the mesophyll tissue (tab. 3). Both traits are suggested to be structural mechanisms that increase photosynthesis per unit leaf area and enable a greater water-use efficiency (Klich, 2000). Still the mesophyll tissue consists of two distinct components, the palisade parenchyma and the spongy parenchyma, that play a significantly different role in the profile of light capture through the leaf. Palisade parenchyma enables a better light penetration to the chloroplasts, while spongy parenchyma enhances the light capture by scattering light (Evans, 1999). Both tissues are found to have a different response to increased irradiance (fig. 4). With a greater proportion of palisade tissue, sun-leaves have a higher photosynthesis, because the intercepted vertical light can penetrate deeper into the leaf with better access to the chloroplasts. The proportionally thinner spongy parenchyma found in sun-leaves, may suggest that enhancement of light interception through backscattering is less important under high irradiance. The higher palisade to spongy parenchyma ratios found for sun leaves underline this suggestion. Differences between sun- and shade-leaves in the proportion of palisade to spongy tissue are often found and well documented (e.g. Bongers and Popma, 1988; Cao, 2000; Mendes *et al*., 2001)

The upper epidermis thickness and the thickness of the hypodermis, in the three species that form this extra tissue, did not differ significantly between sun- and shade-leaves (fig. 4) Generally upper epidermi are found to be thicker in sun-leaves (Bongers and Popma, 1988; Gamage *et al*., 2003; Mendes *et al*. 2001; Sims and Pearcy, 1992) still other studies showed no differences for shrub or herbaceous species (Chazdon and Kaufmann, 1993; Paiva *et al*, 2000). I expected that the epidermis and hypodermis would have a protective function as they minimize the damaging effect of high irradiance by reflecting the light (Cao, 2000) and minimize leaf surface evaporation. It is also possible that instead these tissues are functioning in focusing or concentrating the intercepted light. The focusing of light by lens shaped epidermal cells concentrates the light and facilitates the penetration of the light into the leaves (Vogelmann, 1996; Vogelmann and Martin, 1993).

The diameter and density of the xylem conduits in the primary vein did not differ between sun- and shade-leaves. Still, Klich (2000) found a proportional increase of the vein density in the petioles of upper sun-leaves in the crowns of *Eleaegnus angustifolia*. Increased conduit density is found to be positively correlated with water stress in the high light habitat (Pyykkö, 1966). As

no such differences were found in this study, differences in water availability between shade- and sun-environments are either not that pronounced or leaves have other means of dealing with water stress in full sun light.

FUNCTIONAL GROUPS; SHADE-TOLERANCE

Functional groups related to shade-tolerance were found to differ in their anatomical traits, with light demanding species having a higher proportional thickness of their upper epidermis, palisade parenchyma, spongy parenchyma, and palisade to spongy parenchyma ratio. Furthermore the light demanding species had a higher mean xylem conduit diameter but a lower conduit density. The other traits were not significantly different among groups nor was the plasticity of the traits in response to irradiance level (tab. 5). The found differences are in line with my prior expectations, still I expected that differences in the other leaf structural traits among groups would be more pronounced, so overall the results are not in line with my hypotheses (tab. 1).

Light-demanding species are short- and long-lived pioneers that need full sunlight to establish and grow to their adult stature. These species realize fast growth rates to compete with their neighbors to maintain their position in the top of the regrowing vegetation. To do so light-demanding species aim to realize high photosynthetic rates and maximize carbon assimilation.

Light-demanding species have the thickest upper epidermis among groups (tab. 5) A thicker upper epidermis may be helpful in reflecting excessive irradiance in the high light environment (Cao, 2000; Roth, 1984), where these species tend to occur. Increased reflectance can be beneficial to a leaf as it helps to reduce the heat load and transpiration. A leaf can thus maintain its temperature near the photosynthetic optimum (Givnish, 1984). Light-demanding species (sun species) have actually been found to have a bigger reflectance than shade-tolerant species (Lee and Graham, 1986), still cloud forest pioneers and shade-tolerants did not show such differences (Poorter et al., 2000). The cuticle may also be responsible for reflecting light (Vogelmann and Martin, 1993), but because no differences were found among groups, reflectance does not seem to be influenced by cuticle thickness (tab. 5). A thick epidermis might also help to focus light of certain wave lengths. It has been observed that focusing by epidermal cells occurs when leaves are irradiated with directional light, but not with diffuse light (Vogelmann, 1996). This might explain the difference in thickness of the epidermal layer between light-demanding species, that are adapted to high-light environments with directional irradiance, and shade-tolerant and shade-intolerant species, that tend to occur in low-light environments with more diffuse irradiance (tab. 5). A survey of 47 species collected from a wide variety of habitats indicated that many plants have leaf epidermal cells with lens properties (Vogelmann, 1996). Thicker cells enhance the focusing of larger quantities and imply a stronger concentration of the intercepted light, enabling it to penetrate deeper into the leaves, with better access to the chloroplasts (Evans, 1999; Vogelmann and Martin, 1993).

Light-demanding species further enhance photosynthetic rates with a thicker layer of palisade tissue (tab. 5), known for supporting light channeling of directional irradiance and enabling a

better penetration to the chloroplasts through their central vacuole (Evans, 1999; Vogelmann and Martin, 1993).

In line with prior expectations light-demanding species had the widest xylem conduits among groups (tab. 5). Wider xylem conduits are more efficient water conductors (Zimmermann, 1983) and may thus reflect the greater need for water and nutrients at the leaf level. Still the question is whether wider xylem conduits result from a higher water demand because of high rates of photosynthesis and high transpiration rates or whether leaf level evaporation may be explanative. Leaf mass per unit leaf area (LMA) is generally a good predictor for photosynthetic rate. Still the mean xylem conduit diameter is not significantly related to LMA (r^2 = 0,01; p = 0,49), but instead strongly related to leaf area (r^2 = 0,51; p < 0,0001) (app. III). This suggests that the greater hydraulic conductivity of the wider conduits is most dependent on leaf level water demand resulting from evaporational water loss, then resulting from transpirational water loss.

Shade-tolerant species can complete their entire life cycle in the low-light environment of the forest understory. To do so shade-tolerant species need to make efficient use of the limited light that is available. In line with the hypothesis these species had a thick layer of spongy parenchyma tissue (tab. 5). This tissue layer is better adapted to intercept diffuse light in the forest understory, because it enhances back scattering of light in the leaf's mesophyll, that increases light interception by the chloroplasts (Evans, 1999; Vogelmann and Martin, 1999).

Functional groups related to shade-tolerance did not differ in amount of plasticity of any of the examined structural traits in response to irradiance level (tab. 5). This is not consistent with the specialization – plasticity hypothesis (Lortie and Aarssen, 1996) that predicts that specialization in favorable environments increases plasticity, whereas specialization in less favorable environments decreases the plasticity. My results are also in contradiction with actual findings of other authors. Shade-tolerant species have been reported to lack flexibility in their leaf structure, while light-demanding species show a greater plasticity in response to irradiance (Cao and Booth, 2001, Chazdon et al., 1996). Still the ANOVA of table 5 does not show whether sun and shade-leaves within functional groups actually differ in their mean anatomical traits. One sample T-tests analyzing significance of deviation from unity of the SHSU-ratios within functional groups reveal some interesting patterns (app. III).

Sun- and shade-leaves within all three functional groups related to shade-tolerance differ in leaf thickness and the number of palisade parenchyma cell layers. Trait adjustments in response to irradiance seem least plastic in intermediate shade-tolerant species, with eight of the eleven SHSU-ratios of traits not differing significantly from unity. Light-demanding and shade-tolerant species are much alike in this perspective with both 5 of 11 SHSU-ratios not significantly differing from unity, of which they have 4 in common. Sun-leaves of shade-tolerant species have a significantly thicker proportion of mesophyll tissue compared to shade-leaves within the group, while cuticles do not differ. Sun-leaves of light-demanding species have a significantly thicker cuticle, compared to shade-leaves within the group, but do not differ in the proportional thickness of their mesophyll. From this perspective intermediate shade-tolerant species tend to

have the smallest plasticity among groups, while light-demanding and shade-tolerant species tend to have a greater plasticity and are more alike. It is important to note that many of the non-significant differences between sun- and shade-leaves in intermediate shade-tolerant are marginally so ($p < 0,1$).

FUNCTIONAL GROUPS; DROUGHT-TOLERANCE

Functional groups related to drought-tolerance differed in mean leaf thickness, the proportional thickness of the upper epidermis and in both the absolute diameter and density of the xylem conduits, as their plasticity in response to irradiance level. Still most traits did not differ among groups, which makes that the overall results are in contradiction with the hypotheses (tab. 1).

Surprisingly drought-intolerant species had the thickest leaves, while no differences were found between drought-tolerant and drought-avoiding species. This is not in line with the hypothesis as I expected drought tolerant-species to have the thickest leaves.

The concept of leaf pay-back time (*PT*), that I used to provide an explanation of the small thickness of the average Chiquitano leaf (above), can also be useful in explaining why drought-intolerant species have thicker leaves than drought-avoiding and drought-tolerant species. According to Niinemets (2001) drought stressed plants reduce evaporation by decreasing stomatal openness (Schultze, 1986). Thick leaves have a potentially high net assimilation rates, but stomatal closure limits the CO_2 entry into the leaf, which reduces actual net assimilation rates. As a consequence, increases in leaf thickness may bring about greater construction costs per unit leaf area. This results in a greater *PT* in dry compared to wet ecosystems (Niinemets, 2001). If this is true it may also be applicable to differences in water availability between habitats within the forest. Drought-tolerant species are generally found on dry elevated grounds, whereas drought-intolerant species occur on wet lower grounds near creeks and streams. It may be true that leaves of drought-tolerant species have a greater *PT*, because carbon assimilation is limited by stomatal closure and decreased CO_2 intake during the dry season. In contrast drought-intolerant species need to worry less about decreasing their transpiration and can maintain a relatively high stomatal openness, that secures a continued intake of CO_2. This in turn decreases the PT of drought-intolerant species, which makes their leaves less expensive to produce (Niinemets, 2001). Drought-avoiding species have a deciduous leaf habit. Their leaves are deployed during the wet season and as such carbon assimilation is limited by the shorter time these species have to photosynthesize. Drought-avoiding species will optimize their carbon investment, given their short leaf life span. Drought-stress is less of a problem for these species, so increasing leaf thickness out of a water conservation perspective is an unneeded and more expensive strategy.

A better and cheaper adaptation that helps to control leaf level evaporation in drought-avoiding species is the thicker upper epidermis found for these species (tab. 6). Still a thick upper epidermis may be favorable to deciduous leaves in more than one way. It can minimize evaporation from the leaf surface (Roth, 1984), it may protect against excessive irradiance and high temperatures by increasing the leaf's reflectance (Roth, 1984; Vogelmann and Martin,

1993) and it may help in establishing high photosynthetic rates by focusing the intercepted light (Vogelmann, 1996).

Diameters of the xylem conduits in leaves of drought-tolerant and drought-intolerant species were much alike and were significantly narrower than those of drought-avoiding species. This suggests that drought-tolerant and drought-intolerant species have adapted their xylem tissue to minimize the chance of drought-induced cavitation (Zimmermann, 1983). This is feasible, as both groups have an evergreen leaf habit and thus maintain their foliage during the dry season, which makes them more susceptible to cavitation. Similar trends in xylem diameters have been described for drought-tolerant shrub and tree species from other arid ecosystems (Lo Gullo and Salleo, 1988; Dong and Zhang, 2001). Still drought-intolerant species occur in relatively wet microhabitats near creeks and streams within the dry forest, so one could also hypothesize that drought-intolerant species are less exposed to soil water deficits than drought-tolerant species during the dry season and thus less in need of narrow xylem conduits. Although the plasticity of the xylem conduit diameter and xylem conduit density in response to irradiance differs among groups (tab. 6), sun- and shade-leaves did not significantly differ within functional groups (app. III).

More extensive studies on soil-water-plant relations, leaf water potential components and water-use-efficiency of the species studied here could be useful to get a better impression of the functional significance of xylem tissue adaptations.

Conclusions

- *Do species differ in their leaf structural appearance?*

  Interspecific differences explain most and irradiance explains little of the variation in anatomical leaf traits. The leaf structural appearance of dry forest tree species thus differs substantially among species. Despite great differences among species, the average Chiquitano dry forest leaf is very thin compared to that of the average wet forest leaf. Limited carbon assimilation or the deciciduous leaf habit of many dry forest tree species is probably explanative. Stomatal adaptations that reduce transpiration limit CO_2 intake rates and increase leaf construction costs. The formation of thin leaves reduces leaf pay back time.

- *Do differences in light availability alter the leaf structure of sun- and shade-leaves?*

  Sun- and shade-leaves differ substantially within species. Sun-leaves are thicker than shade-leaves and have a, in proportion to their leaf thickness, thicker cuticle, thicker palisade parenchyma tissue composed of more cell layers, a higher palisade to spongy parenchyma ratio and a thicker mesophyll tissue (tab. 3, fig. 4) Still the relative thickness of the spongy parenchyma in the mesophyll and the thickness of the lower epidermis are smaller in sun-leaves. The irradiance level does not seem to influence the relative thickness of the upper epidermis and hypodermis, nor the number of cell-layers in the hypodermis and

the diameter or density of the xylem conduits in the primary vein. Leaf structural adjustments to differences in light availability thus emphasizes on acclimatizing the photosynthetic apparatus. Apart from the cuticle, adjustments that enhance water conservation are less pronounced.

- *Do differences in leaf structure influence species shade- and drought-tolerance?*

No strong natural groups of species, sharing clear suites of traits that differ from suites of traits of other groups, seem to arise. Among species dissimilarity in leaf structure does not strongly distinguish between a-priory defined functional groups of species related to shade- or drought-tolerance. Rather than belonging to well defined groups, species shade- and drought-tolerance could alternatively be examined as continuous gradients. It could also be that morphological and physiological adaptations are more important than leaf structural adaptations.

- *Do functional groups of species related to shade-and drought tolerance differ in their leaf structural appearance?*

A-priory defined functional groups related to shade-tolerance differ in the proportional thickness of their upper epidermis, palisade and spongy parenchyma and in their palisade to spongy parenchyma ratio, xylem conduit density and diameter. Plasticity in response to irradiance level does not differ between groups.

Leaves of light-demanding species are characterized by a relatively thick epidermi, a large proportions of palisade parenchyma in the mesophyll tissue, high palisade to spongy parenchyma ratios and wide xylem conduits in small densities in the primary vein. These adaptations increase photosynthetic rates by efficient harvesting of directional light and protect the leaves against negative effects of high irradiance.

Shade-tolerant species, do not differ from intermediate shade-tolerant species and are characterized by relatively thin epidermi, large proportions of spongy mesophyll, low palisade to spongy mesophyll ratios and narrow xylem conduits in greater densities in the primary vein. These adaptations increase photosynthetic efficiency by greater harvesting of diffuse light in the forest understory.

Functional groups related to drought-tolerance were in mean leaf thickness, the proportional thickness of the upper epidermis and in both the absolute diameter and density of the xylem conduits, as their plasticity in response to irradiance level.

Drought-intolerant species form thick leaves, probably because carbon assimilation in their preferred wet microhabitats is to a lesser extend limited by stomatal adaptations that reduce transpiration and CO_2 intake rates. Their leaves need less protection against high evaporation rates, as is emphasized by their thin upper epidermis.

The deciduous leaves of drought-avoiding species are characterized by being thin with thick upper erpidermi. Because these species have a limited time to photosynthesize, carbon assimilation is limited, which makes investing in thick leaves expensive. Thickening of the

upper epidermis is a cheaper adaptation to minimize evaporation and may enhance photosynthesis by focusing the intercepted light.

Drought-tolerant species do not differ much from drought-avoiding species in their leaf structure. Still their narrower xylem conduit diameters are better adapted to avoid cavitation and embolisms in the dry season.

More studies are needed that link adaptations of the xylem tissue to soil-water-plant relations, leaf water potential components and water-use-efficiency of the species studied here, in order to get a better understanding of it's functional significance.

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Appendix I

Code	Local name	Scientific name	value	a	b	c	d	e	f	g	h	i	j	k	l	m
			Shade	85,64 ± 4,09	1,86 ± 0,15	5,37 ± 0,75		71,37 ± 2,98	12,49 ± 1,93	58,88 ± 2,68	3,66 ± 0,22	0,21 ± 0,04		1,00 ± 0,00	8,27 ± 0,64	14290 ± 2437
AGU.FC	Aguai fruta chica	*Chrysophyllum gonocarpum*	Sun	70,78 ± 1,78	1,75 ± 0,10	6,91 ± 0,98		56,46 ± 2,33	12,47 ± 0,98	43,99 ± 2,91	4,55 ± 0,48	0,29 ± 0,04		1,00 ± 0,00	9,60 ± 0,83	11163 ± 425
			SHSU-ratio	1,21	1,06	0,78		1,26	1,00	1,34	0,80	0,73		1,00	0,86	1,28
			Shade	93,23	1,60	4,81		79,81	22,50	57,31	4,59	0,39		1,00	10,30	12252
AGU.FG	Aguai fruta grande	*Pouteria gardneriana*	Sun	99,01 ± 8,00	2,11 ± 0,00	4,53 ± 1,74		86,18 ± 3,92	29,27 ± 3,86	56,91 ± 0,06	4,42 ± 0,39	0,51 ± 0,07		1,00 ± 0,00	10,71 ± 0,89	6230 ± 118
			SHSU-ratio	0,94	0,76	1,06		0,93	0,77	1,01	1,04	0,76		1,00	0,96	1,97
			Shade	101,58 ± 1,61	0,57 ± 0,07	9,56 ± 0,41	22,71 ± 1,00	69,00 ± 1,83	20,58 ± 2,58	48,42 ± 2,28	7,81 ± 0,36	0,44 ± 0,08	2,00 ± 0,00	1,80 ± 0,20	11,01 ± 0,90	6141 ± 1001
AJO	Ajo ajo	*Gallesia integrifolia*	Sun	125,09 ± 10,13	0,80 ± 0,03	9,76 ± 1,07	25,65 ± 2,03	87,51 ± 8,01	37,16 ± 7,94	50,35 ± 3,92	10,20 ± 1,08	0,77 ± 0,18	2,00 ± 0,00	2,00 ± 0,00	9,80 ± 0,63	10325 ± 829
			SHSU-ratio	0,81	0,71	0,98	0,89	0,79	0,55	0,96	0,77	0,57	1,00	0,90	1,12	0,59
			Shade	78,19 ± 4,50	1,16 ± 0,10	11,13 ± 1,12		56,02 ± 4,46	23,13 ± 2,05	32,89 ± 3,61	7,71 ± 0,21	0,73 ± 0,09		1,40 ± 0,24	13,59 ± 1,41	5875 ± 1470
BLA	Blanquillo	*Ampelocera ruizii*	Sun	77,16 ± 3,32	1,53 ± 0,23	11,65 ± 0,94		54,11 ± 3,53	26,07 ± 2,24	28,04 ± 1,70	7,74 ± 0,68	0,93 ± 0,07		2,00 ± 0,32	11,74 ± 1,13	5708 ± 368
			SHSU-ratio	1,01	0,76	0,96		1,04	0,89	1,17	1,00	0,78		0,70	1,16	1,03
			Shade	119,14 ± 12,55	0,65 ± 0,04	9,13 ± 0,94		95,55 ± 11,09	35,92 ± 3,88	59,63 ± 9,51	11,91 ± 0,83	0,64 ± 0,09		1,00 ± 0,00	7,88 ± 0,20	12845 ± 2024
BLA.F	Blanquillo falso	*Galipea ciliata*	Sun	125,39 ± 3,82	0,85 ± 0,05	11,85 ± 1,52		101,43 ± 3,25	46,55 ± 2,85	54,88 ± 4,75	9,46 ± 0,99	0,89 ± 0,12		1,00 ± 0,00	8,01 ± 0,73	10108 ± 1180
			SHSU-ratio	0,95	0,77	0,77		0,94	0,77	1,09	1,26	0,72		1,00	0,98	1,27
			Shade	66,54 ± 2,37	0,77 ± 0,05	9,01 ± 0,98		50,21 ± 2,33	22,71 ± 1,16	27,50 ± 1,40	4,40 ± 0,42	0,83 ± 0,03		1,25 ± 0,25	15,43 ± 0,71	4026 ± 457
CAR.T	Carne de toro	*Combretum leprosum*	Sun	80,68 ± 4,40	1,30 ± 0,08	7,58 ± 0,41		64,12 ± 3,99	26,43 ± 1,19	37,69 ± 2,81	6,41 ± 0,31	0,71 ± 0,02		2,00 ± 0,00	12,88 ± 0,83	5022 ± 371
			SHSU-ratio	0,82	0,59	1,19		0,78	0,86	0,73	0,69	1,17		0,63	1,20	0,80
			Shade	129,69 ± 2,21	1,87 ± 0,13	7,10 ± 0,51		109,45 ± 2,62	19,74 ± 1,30	89,71 ± 2,34	7,98 ± 1,00	0,22 ± 0,02		1,00 ± 0,00	6,27 ± 0,32	16327 ± 1361
COC	Coca	*Esenbeckia almawillia*	Sun	140,94 ± 3,59	3,35 ± 0,14	6,73 ± 0,82		120,38 ± 3,23	26,38 ± 1,91	94,00 ± 1,46	8,15 ± 0,45	0,28 ± 0,02		1,00 ± 0,00	6,17 ± 0,13	11802 ± 1636
			SHSU-ratio	0,92	0,56	1,05		0,91	0,75	0,95	0,98	0,79		1,00	1,01	1,38
			Shade	73,31 ± 1,72	0,84 ± 0,02	10,37 ± 0,62		46,33 ± 1,42	14,41 ± 1,04	31,92 ± 0,96	14,52 ± 0,93	0,45 ± 0,04		1,00 ± 0,00	5,63 ± 0,14	15222 ± 2262
COC.DI	Coca don Israel	*Erithroxylum sp.*	Sun	82,70 ± 3,34	1,13 ± 0,07	12,52 ± 1,39		54,76 ± 1,40	17,93 ± 0,53	36,82 ± 1,59	14,04 ± 1,04	0,49 ± 0,03		1,00 ± 0,00	6,88 ± 0,42	10658 ± 1432
			SHSU-ratio	0,89	0,75	0,83		0,85	0,80	0,87	1,03	0,92		1,00	0,82	1,43
			Shade	112,25 ± 7,01	0,91 ± 0,11	12,12 ± 1,03		86,01 ± 5,16	36,09 ± 3,60	49,92 ± 4,82	11,12 ± 1,76	0,77 ± 0,15		2,00 ± 0,00	11,88 ± 0,93	5948 ± 1046
COM	Comomosi	*Bougainvillea modesta*	Sun	132,70 ± 17,89	1,21 ± 0,11	16,06 ± 2,20		101,93 ± 15,71	51,96 ± 9,22	49,97 ± 9,21	10,90 ± 1,42	1,12 ± 0,19		2,20 ± 0,20	10,62 ± 0,29	5289 ± 282
			SHSU-ratio	0,85	0,75	0,75		0,84	0,69	1,00	1,02	0,69		0,91	1,12	1,12
			Shade	58,55 ± 5,78	0,78 ± 0,07	8,91 ± 1,27		41,81 ± 4,53	14,51 ± 1,32	27,30 ± 4,11	5,68 ± 0,90	0,59 ± 0,11		1,00 ± 0,00	12,66 ± 0,95	3186 ± 270
COY	Chayote	*Jacaratia sp.*	Sun	70,73 ± 4,37	0,75 ± 0,07	10,44 ± 1,16		51,90 ± 3,14	15,04 ± 1,41	36,85 ± 2,60	6,33 ± 0,37	0,41 ± 0,05		1,00 ± 0,00	15,61 ± 1,32	2557 ± 297
			SHSU-ratio	0,83	1,04	0,85		0,81	0,96	0,74	0,90	1,43		1,00	0,81	1,25
			Shade	85,37 ± 3,76	1,12 ± 0,12	11,51 ± 1,06		66,84 ± 2,74	34,91 ± 0,90	31,93 ± 2,34	5,12 ± 0,40	1,11 ± 0,08		1,00 ± 0,00	8,82 ± 1,01	6981 ± 976
CUCH	Cuchi	*Astronium uriundeuva*	Sun	93,89 ± 4,68	1,40 ± 0,19	10,83 ± 0,74		74,74 ± 4,74	37,28 ± 3,52	37,46 ± 1,76	6,30 ± 0,39	0,99 ± 0,08		1,00 ± 0,00	8,90 ± 0,94	6663 ± 794
			SHSU-ratio	0,91	0,80	1,06		0,89	0,94	0,85	0,81	1,12		1,00	0,99	1,05
			Shade	54,01 ± 3,84	0,79 ± 0,07	6,51 ± 0,55		39,37 ± 2,39	10,58 ± 1,76	28,79 ± 1,20	5,98 ± 1,13	0,37 ± 0,06		1,00 ± 0,00	8,59 ± 0,06	9301 ± 510
CUS	Cuse	*Casearia gossypiosperma*	Sun	61,05 ± 3,74	0,95 ± 0,06	9,83 ± 1,19		42,89 ± 2,11	12,12 ± 1,75	30,77 ± 1,17	5,42 ± 0,52	0,40 ± 0,06		1,00 ± 0,00	8,63 ± 0,56	8800 ± 679
			SHSU-ratio	0,88	0,83	0,66		0,92	0,87	0,94	1,10	0,93		1,00	1,00	1,06
			Shade	81,78 ± 6,57	0,87 ± 0,08	9,92 ± 0,80		62,05 ± 6,38	28,34 ± 2,62	33,71 ± 4,04	7,53 ± 0,73	0,86 ± 0,06		2,50 ± 0,29	6,62 ± 0,50	9975 ± 683
CUT	Cuta	*Phyllostylon rhamnoides*	Sun	101,60 ± 11,81	1,07 ± 0,10	9,59 ± 0,27		79,81 ± 11,80	34,86 ± 3,97	44,94 ± 9,39	8,61 ± 0,54	0,87 ± 0,15		2,60 ± 0,24	6,73 ± 0,29	8058 ± 175
			SHSU-ratio	0,80	0,81	1,03		0,78	0,81	0,75	0,88	0,98		0,96	0,98	1,24
			Shade	47,41 ± 4,23	1,09 ± 0,07	6,12 ± 0,49		34,81 ± 3,67	11,58 ± 0,77	23,23 ± 3,18	5,37 ± 0,37	0,52 ± 0,05		1,00 ± 0,00	6,85 ± 0,30	10085 ± 686
DON.C	Don Concepcion	*Actinostemon conceptionis*	Sun	54,86 ± 5,46	1,39 ± 0,09	7,39 ± 0,85		41,81 ± 4,32	13,06 ± 1,16	28,74 ± 3,24	5,21 ± 0,82	0,46 ± 0,03		1,40 ± 0,24	7,31 ± 0,42	10753 ± 1187
			SHSU-ratio	0,86	0,79	0,83		0,83	0,89	0,81	1,03	1,13		0,71	0,94	0,94
			Shade	72,53 ± 3,15	0,90 ± 0,09	9,46 ± 0,78		53,34 ± 3,38	26,39 ± 1,70	26,95 ± 2,10	7,33 ± 0,48	0,99 ± 0,07		1,20 ± 0,20	9,28 ± 0,22	5404 ± 686
GAB.B	Gabetillo blanco	*Simira rubescens*	Sun	85,97 ± 1,59	1,07 ± 0,11	10,41 ± 0,53		65,26 ± 1,26	32,93 ± 1,48	32,34 ± 0,75	7,68 ± 0,26	1,02 ± 0,06		2,00 ± 0,00	8,07 ± 0,63	6848 ± 816
			SHSU-ratio	0,84	0,84	0,91		0,82	0,80	0,83	0,95	0,97		0,60	1,15	0,79
			Shade	78,71 ± 2,77	0,88 ± 0,03	4,09 ± 0,56		68,18 ± 2,41	22,07 ± 1,15	46,11 ± 2,62	3,85 ± 0,43	0,49 ± 0,05		1,00 ± 0,00	6,06 ± 0,72	15342 ± 4925
GUA	Guapuru	*Myrciaria cauliflora*	Sun	88,14 ± 4,64	0,78 ± 0,07	4,61 ± 0,40		76,69 ± 4,71	25,77 ± 2,83	50,92 ± 5,40	4,50 ± 0,37	0,53 ± 0,10		1,25 ± 0,25	5,27 ± 0,33	15159 ± 2818
			SHSU-ratio	0,89	1,13	0,89		0,89	0,86	0,91	0,86	0,92		0,80	1,15	1,01
			Shade	68,84 ± 2,25	0,87 ± 0,02	6,97 ± 0,98		54,96 ± 2,31	22,46 ± 1,79	32,50 ± 2,50	4,94 ± 0,65	0,70 ± 0,09		2,00 ± 0,00	7,24 ± 0,73	9039 ± 860
JIC.A	Jichituriqui amarillo	*Aspidosperma tomentosum*	Sun	80,48 ± 4,05	1,23 ± 0,13	7,71 ± 0,44		65,84 ± 3,83	29,37 ± 2,53	36,47 ± 2,08	4,97 ± 0,38	0,81 ± 0,07		2,00 ± 0,00	7,49 ± 0,47	10890 ± 1284
			SHSU-ratio	0,86	0,71	0,90		0,83	0,76	0,89	0,99	0,87		1,00	0,97	0,83
			Shade	133,09 ± 4,06	2,14 ± 0,07	9,49 ± 1,22		110,85 ± 2,91	34,84 ± 2,06	76,01 ± 2,51	6,89 ± 0,59	0,46 ± 0,03		2,00 ± 0,00	7,90 ± 0,34	9243 ± 966
JIC.C	Jichituriqui colorado	*Aspidosperma cylindrocarpon*	Sun	134,60 ± 4,49	2,19 ± 0,20	9,89 ± 0,75		112,64 ± 4,10	31,92 ± 1,18	80,72 ± 3,93	6,22 ± 0,17	0,40 ± 0,02		2,00 ± 0,00	7,40 ± 0,47	8132 ± 642
			SHSU-ratio	0,99	0,98	0,96		0,98	1,09	0,94	1,11	1,16		1,00	1,07	1,14
			Shade	60,68 ± 5,44	0,81 ± 0,06	6,18 ± 0,79		48,59 ± 5,26	19,86 ± 2,08	28,73 ± 4,01	4,22 ± 0,49	0,73 ± 0,09		1,00 ± 0,00	7,62 ± 0,25	6778 ± 254
MAN	Mani	*Sweetia fruticosa*	Sun	73,13 ± 1,03	1,05 ± 0,06	6,13 ± 0,44		59,93 ± 1,19	23,47 ± 0,88	36,46 ± 1,59	4,85 ± 0,23	0,65 ± 0,05		1,80 ± 0,20	6,58 ± 0,43	9520 ± 1505
			SHSU-ratio	0,83	0,77	1,01		0,81	0,85	0,79	0,87	1,12		0,56	1,16	0,71
			Shade	135,30 ± 7,77	1,17 ± 0,04	11,67 ± 0,76		108,68 ± 7,27	27,43 ± 3,29	81,25 ± 7,51	10,67 ± 0,51	0,35 ± 0,05		1,40 ± 0,24	8,61 ± 0,77	7029 ± 803
MAP	Mapabi	*Neea hermafrodita*	Sun	164,95 ± 20,16	1,39 ± 0,11	13,33 ± 0,46		134,60 ± 19,28	56,15 ± 12,06	78,44 ± 7,71	12,14 ± 1,03	0,69 ± 0,09		1,60 ± 0,40	8,09 ± 0,48	7366 ± 146
			SHSU-ratio	0,82	0,84	0,88		0,81	0,49	1,04	0,88	0,51		0,88	1,06	0,95
MAPA	Mapajo	*Ceiba samauma*	Sun	83,77 ± 7,02	1,71 ± 0,02	14,05 ± 2,00		58,42 ± 5,76	32,15 ± 4,14	26,28 ± 4,16	7,66 ± 0,97	1,32 ± 0,21		2,00 ± 0,00	16,46 ± 0,45	3118 ± 306
				-	-	-		-	-	-	-	-		-	-	-

Data summary. The appendix shows mean structural trait-values for sun- and shade-leaves with standard errors and (untransformed)SHSU-ratios per species. Leaf traits; **a**, leaf thickness (µm); **b**, cuticle thickness (µm); **c**, Upper epidermis thickness (µm); **d**, hypodermis thickness (µm)**; e**, mesophyll thickness (µm) ; **f**, palisade parenchyma thickness (µm); **g**, Spongy parenchyma thickness (µm); **h**, lower epidermis thickness (µm); **i**, palisade to spongy parenchyma ratio (µm µm^{-1})**; j**, number of hypodermis cell layers; **k**, number of palisade parenchyma cell layers; **l**, xylem conduit diameter (µm); and **m**, xylem conduit density (µm^{-2}).

Appendix I continued

Code	Local name	Scientific name	value	a	b	c	d	e	f	g	h	i	j	k	l	m
			Shade	57,01 ± 5,37	0,97 ± 0,09	6,03 ± 0,60		42,65 ± 4,46	13,33 ± 1,75	29,32 ± 6,07	5,58 ± 0,34	0,51 ± 0,14		1,00 ± 0,00	7,04 ± 0,59	9688 ± 1405
MAR.P	Maria pretina	*Phyllanthus sp. nov.*	Sun	50,82 ± 2,33	1,01 ± 0,06	6,05 ± 0,41		38,01 ± 2,18	13,88 ± 1,06	24,13 ± 1,53	4,84 ± 0,27	0,58 ± 0,04		1,00 ± 0,00	6,64 ± 0,20	9784 ± 741
			SHSU-ratio	1,12	0,96	1,00		1,12	0,96	1,22	1,15	0,88		1,00	1,06	0,99
			Shade	55,60 ± 2,48	0,68 ± 0,07	6,94 ± 0,28		40,13 ± 2,39	19,42 ± 0,88	20,71 ± 2,26	6,58 ± 0,29	0,99 ± 0,12		1,00 ± 0,00		
MOM	Momoqui	*Caesalpinia pluviosa*	Sun	71,13 ± 4,08	0,86 ± 0,07	6,87 ± 0,43		55,79 ± 3,55	28,64 ± 3,01	27,16 ± 1,57	6,07 ± 0,31	1,06 ± 0,12		1,00 ± 0,00		
			SHSU-ratio	0,78	0,80	1,01		0,72	0,68	0,76	1,08	0,93		1,00		
			Shade	76,81 ± 4,52	1,04 ±	12,00 ± 1,62		54,52 ± 2,47	19,34 ± 5,72	35,18 ± 8,19	8,13 ± 0,06	0,62 ± 0,31		1,00 ±	6,87 ±	7293 ±
NAR	Naranjillo	*Zanthoxylum monogynum*	Sun	95,31 ± 8,06	1,15 ± 0,11	10,58 ± 1,58		73,79 ± 6,64	25,69 ± 2,18	48,10 ± 4,93	8,92 ± 0,63	0,54 ± 0,03		1,20 ± 0,20	7,70 ± 0,49	8766 ± 966
			SHSU-ratio	0,81	0,90	1,13		0,74	0,75	0,73	0,91	1,15		0,83	0,89	0,83
			Shade	105,34 ± 2,29	1,04 ± 0,06	12,44 ± 0,58		85,58 ± 2,60	30,03 ± 1,30	55,55 ± 3,45	5,09 ± 0,36	0,55 ± 0,06		1,00 ± 0,00	13,77 ± 0,57	3955 ± 470
OCO	Ocorocillo	*Spondias mombin*	Sun	117,25 ± 8,40	1,20 ± 0,09	13,50 ± 0,47		94,88 ± 8,14	40,16 ± 5,64	54,72 ± 2,82	6,10 ± 0,40	0,72 ± 0,07		1,40 ± 0,24	14,18 ± 0,49	2961 ± 183
			SHSU-ratio	0,90	0,87	0,92		0,90	0,75	1,02	0,83	0,76		0,71	0,97	1,34
			Shade	103,95 ± 2,48	1,66 ± 0,13	9,83 ± 0,84		77,86 ± 1,28	36,22 ± 3,96	41,64 ± 3,14	6,71 ± 0,56	0,92 ± 0,17		2,00 ± 0,00	13,74 ± 0,94	2965 ± 418
PAC	Pacobillo	*Capparis prisca*	Sun	120,35 ± 10,04	2,18 ± 0,27	12,00 ± 0,75		90,68 ± 10,08	51,63 ± 7,34	39,05 ± 2,83	7,38 ± 0,24	1,30 ± 0,12		2,50 ± 0,29	14,52 ± 0,83	3227 ± 164
			SHSU-ratio	0,86	0,76	0,82		0,86	0,70	1,07	0,91	0,71		0,80	0,95	0,92
			Shade	88,00 ± 7,70	1,25 ± 0,03	21,49 ± 2,00		55,32 ± 5,26	29,84 ± 4,56	25,48 ± 2,47	7,28 ± 0,86	1,21 ± 0,21		2,00 ± 0,00	25,00 ± 1,54	1388 ± 129
PEC.B	Pequi blanco	*Eriotheca roseorum*	Sun	83,24 ± 3,85	1,70 ± 0,10	21,29 ± 1,00		54,67 ± 3,24	35,35 ± 1,15	19,32 ± 2,62	6,20 ± 0,64	1,94 ± 0,22		2,80 ± 0,20	24,11 ± 2,04	1813 ± 307
			SHSU-ratio	1,06	0,73	1,01		1,01	0,84	1,32	1,17	0,63		0,71	1,04	0,77
			Shade	71,33 ± 3,75	1,10 ± 0,05	9,18 ± 0,77		53,79 ± 3,19	22,14 ± 2,25	31,65 ± 1,56	5,60 ± 0,47	0,70 ± 0,07		1,00 ± 0,00	14,28 ± 0,59	3426 ± 304
PIT	Piton	*Talisia esculenta*	Sun	73,62 ± 0,92	1,76 ± 0,13	11,14 ± 0,40		54,34 ± 1,02	26,00 ± 0,59	28,35 ± 1,16	5,49 ± 0,30	0,92 ± 0,05		1,00 ± 0,00	11,14 ± 0,45	5978 ± 391
			SHSU-ratio	0,97	0,63	0,82		0,99	0,85	1,12	1,02	0,76		1,00	1,28	0,57
			Shade	65,86 ± 4,35	0,72 ± 0,08	23,04 ± 3,70		35,67 ± 1,95	15,62 ± 0,98	20,05 ± 1,30	5,52 ± 0,72	0,79 ± 0,05		1,00 ± 0,00	21,87 ± 1,07	1564 ± 170
PP	Pica pica	*Urera baccifera*	Sun	68,62 ± 5,16	0,83 ± 0,07	16,17 ± 1,61		47,05 ± 3,83	24,80 ± 3,19	22,25 ± 2,20	5,13 ± 0,86	1,16 ± 0,20		2,00 ± 0,00	20,66 ± 0,94	1675 ± 94
			SHSU-ratio	0,96	0,87	1,42		0,76	0,63	0,90	1,08	0,68		0,50	1,06	0,93
			Shade	56,73 ± 5,81	0,59 ± 0,05	7,12 ± 0,69		43,98 ± 5,20	14,24 ± 0,72	29,74 ± 5,20	4,99 ± 0,45	0,56 ± 0,13		1,00 ± 0,00	6,90 ± 0,27	7945 ± 264
QUI	Quina	*Pogonopus tubulosus*	Sun	61,01 ± 3,10	0,66 ± 0,07	7,84 ± 0,44		47,98 ± 2,63	24,85 ± 1,42	23,14 ± 1,30	4,17 ± 0,12	1,08 ± 0,03		2,00 ± 0,00	8,12 ± 0,33	6243 ± 1100
			SHSU-ratio	0,93	0,89	0,91		0,92	0,57	1,29	1,20	0,52		0,50	0,85	1,27
			Shade	40,43 ± 2,74		5,66 ± 0,27		29,13 ± 1,92	8,11 ± 0,69	21,02 ± 1,30	4,19 ± 0,20	0,38 ± 0,02		1,00 ± 0,00	6,52 ± 0,23	11588 ± 1231
SAM	Sama	*Trichilia elegans*	Sun	63,52 ± 5,26		8,34 ± 0,37		47,62 ± 4,33	19,89 ± 1,83	27,73 ± 2,53	5,99 ± 0,50	0,72 ± 0,02		1,50 ± 0,29	7,22 ± 0,79	15772 ± 3449
			SHSU-ratio	0,64		0,68		0,61	0,41	0,76	0,70	0,54		0,67	0,90	0,73
			Shade	105,25 ± 1,01	0,79 ± 0,03	7,12 ± 1,11	20,36 ± 1,11	77,66 ± 1,54	14,44 ± 1,20	63,22 ± 1,36	5,55 ± 0,71	0,23 ± 0,02	2,00 ± 0,00	1,00 ± 0,00	5,41 ± 0,32	12551 ± 982
SAW	Sahuinto	*Myrciaria floribunda*	Sun	125,93 ± 8,85	0,83 ± 0,06	12,03 ± 2,36	28,46 ± 3,42	95,73 ± 7,75	26,71 ± 4,94	69,02 ± 7,90	6,32 ± 0,98	0,42 ± 0,12	2,00 ± 0,00	1,40 ± 0,24	4,92 ± 0,11	15235 ± 1209
			SHSU-ratio	0,84	0,95	0,59	0,72	0,81	0,54	0,92	0,88	0,55	1,00	0,71	1,10	0,82
			Shade	87,64 ± 6,49	1,37 ± 0,26	7,68 ± 0,38		71,46 ± 5,46	27,26 ± 2,09	44,20 ± 5,01	6,12 ± 0,38	0,65 ± 0,09		1,80 ± 0,20	7,71 ± 0,44	11058 ± 993
SIR	Sirari	*Guibourtia chodatiana*	Sun	96,91 ± 4,78	1,89 ± 0,16	6,82 ± 0,73		81,02 ± 5,56	37,41 ± 4,88	43,61 ± 3,97	6,08 ± 0,45	0,88 ± 0,16		2,25 ± 0,25	7,46 ± 0,25	9215 ± 351
			SHSU-ratio	0,90	0,72	1,13		0,88	0,73	1,01	1,01	0,74		0,80	1,03	1,20
			Shade	86,02 ± 2,90	0,60 ± 0,06	8,74 ± 0,91		69,17 ± 3,22	36,45 ± 2,06	32,72 ± 1,34	7,13 ± 0,30	1,11 ± 0,04		1,00 ± 0,00	16,85 ± 0,44	2004 ± 89
TAB	Tabacachi	*Solanum cf. riparium*	Sun	77,90 ± 3,66	0,75 ± 0,06	8,39 ± 0,59		62,27 ± 3,42	33,50 ± 1,01	28,77 ± 3,02	5,88 ± 0,50	1,21 ± 0,12		1,00 ± 0,00	13,21 ± 1,27	3539 ± 833
			SHSU-ratio	1,10	0,80	1,04		1,11	1,09	1,14	1,21	0,92		1,00	1,28	0,57
			Shade	106,29 ± 15,51	0,98 ± 0,09	7,59 ± 0,77	32,50 ± 4,63	74,69 ± 8,39	34,42 ± 3,25	40,27 ± 5,42	6,89 ± 0,61	0,89 ± 0,07	2,20 ± 0,58	2,40 ± 0,24	13,50 ± 1,06	4716 ± 591
TAJ.N	Tajibo negro	*Tabebuia impetiginosa*	Sun	128,88 ± 20,49	1,41 ± 0,16	7,18 ± 1,35	28,33 ± 2,88	98,34 ± 16,01	46,81 ± 6,51	51,54 ± 10,62	7,23 ± 0,64	1,03 ± 0,17	2,20 ± 0,37	2,60 ± 0,24	16,21 ± 1,32	4006 ± 571
			SHSU-ratio	0,82	0,70	1,06	1,15	0,76	0,74	0,78	0,95	0,86	1,00	0,92	0,83	1,18
			Shade	58,62 ± 5,65	0,56 ± 0,04	7,78 ± 0,83		44,61 ± 5,17	17,51 ± 1,33	27,10 ± 4,06	5,69 ± 0,39	0,68 ± 0,06		1,40 ± 0,24	12,73 ± 0,73	4063 ± 614
TAR.A	Tarara amarilla	*Centrolobium microchaete*	Sun	56,01 ± 4,67	0,89 ± 0,07	6,92 ± 0,34		43,77 ± 4,71	27,65 ± 3,00	16,11 ± 2,27	4,49 ± 0,48	1,79 ± 0,19		2,00 ± 0,00	11,50 ± 0,60	5645 ± 368
			SHSU-ratio	1,05	0,62	1,12		1,02	0,63	1,68	1,27	0,38		0,70	1,11	0,72
			Shade	81,49 ± 5,15	0,73 ± 0,03	12,36 ± 2,34		61,99 ± 3,85	23,14 ± 2,71	38,86 ± 1,32	6,39 ± 0,39	0,59 ± 0,06		2,00 ± 0,00	10,23 ± 1,46	6351 ± 1137
TAR.C	Tarara colorada	*Platymiscium fragrans*	Sun	87,70 ± 8,36	0,96 ± 0,06	10,07 ± 0,75		68,46 ± 8,44	37,67 ± 6,65	30,79 ± 3,11	7,28 ± 0,51	1,24 ± 0,21		2,40 ± 0,24	11,14 ± 0,75	6415 ± 366
			SHSU-ratio	0,93	0,77	1,23		0,91	0,61	1,26	0,88	0,48		0,83	0,92	0,99
			Shade	64,94 ± 4,58	0,94 ± 0,07	8,28 ± 0,43		47,67 ± 3,87	20,93 ± 2,30	26,75 ± 2,08	6,14 ± 0,48	0,79 ± 0,07		1,20 ± 0,20	4,73 ± 0,47	23244 ± 3955
TAS	Tasaa	*Acosmium cardenasii*	Sun	69,85 ± 5,40	1,06 ± 0,08	8,81 ± 1,09		53,23 ± 5,15	30,52 ± 3,15	22,71 ± 2,84	6,43 ± 0,53	1,40 ± 0,17		1,60 ± 0,24	4,83 ± 0,19	22995 ± 841
			SHSU-ratio	0,93	0,88	0,94		0,90	0,69	1,18	0,96	0,56		0,75	0,98	1,01
			Shade	75,80 ± 8,27	0,93 ± 0,04	28,80 ± 4,87		40,94 ± 3,70	17,40 ± 1,92	23,53 ± 2,23	6,09 ± 0,40	0,75 ± 0,07		1,20 ± 0,20	14,29 ± 1,21	3910 ± 420
TOB	Toborochi	*Chorisia speciosa*	Sun	98,74 ± 8,04	1,02 ± 0,05	29,35 ± 3,27		61,09 ± 7,52	36,96 ± 3,76	24,13 ± 4,23	6,83 ± 0,62	1,61 ± 0,14		2,00 ± 0,00	12,44 ± 0,61	5056 ± 491
			SHSU-ratio	0,77	0,91	0,98		0,67	0,47	0,98	0,89	0,46		0,60	1,15	0,77
			Shade	69,96 ± 5,26	0,93 ± 0,08	8,52 ± 0,60		56,91 ± 4,61	18,67 ± 1,86	38,24 ± 3,98	4,08 ± 0,62	0,51 ± 0,08		1,00 ± 0,00	15,52 ± 0,47	3019 ± 239
YES.B	Yesquero blanco	*Cariniana ianeirensis*	Sun	87,03 ± 7,78	1,40 ± 0,15	10,31 ± 1,73		70,04 ± 5,95	22,93 ± 1,74	47,12 ± 4,94	5,63 ± 0,78	0,50 ± 0,04		1,00 ± 0,00	15,96 ± 1,13	3386 ± 169
			SHSU-ratio	0,80	0,66	0,83		0,81	0,81	0,81	0,72	1,03		1,00	0,97	0,89
			Shade	82,32 ± 2,99	0,76 ± 0,05	7,88 ± 0,78		68,21 ± 2,88	32,39 ± 1,36	35,82 ± 2,28	5,57 ± 0,22	0,92 ± 0,06		1,00 ± 0,00	17,89 ± 1,70	2600 ± 581
YUC	Yucca	*Manihot guaranitica*	Sun	94,33 ± 8,10	0,93 ± 0,05	8,42 ± 0,96		79,60 ± 7,79	43,49 ± 3,19	36,11 ± 4,69	5,93 ± 0,51	1,23 ± 0,08		1,00 ± 0,00	18,66 ± 1,13	2065 ± 172
			SHSU-ratio	0,87	0,82	0,94		0,86	0,74	0,99	0,94	0,74		1,00	0,96	1,26

Data summary. The appendix shows mean structural trait-values for sun- and shade-leaves with standard errors and (untransformed)SHSU-ratios per species. Leaf traits; **a**, leaf thickness (µm); **b**, cuticle thickness (µm); **c**, Upper epidermis thickness (µm); **d**, hypodermis thickness (µm)**; e**, mesophyll thickness (µm) ; **f**, palisade parenchyma thickness (µm); **g**, Spongy parenchyma thickness (µm); **h**, lower epidermis thickness (µm); **i**, palisade to spongy parenchyma ratio (µm µm^{-1})**; j**, number of hypodermis cell layers; **k**, number of palisade parenchyma cell layers; **l**, xylem conduit diameter (µm); and **m**, xylem conduit density (µm^{-2}).

Appendix II

STANDARDIZED PROTOCOLS

1. Embedding in paraffin:

- Tissue selection

- Fixation

- Dehydration of the selected tissue with ethanol; (EtOH) – H_2O series

10, 30, 50, 70, 80, 90, 100 and 100% EtOH.	-	20 minutes each

- Replacement of EtOH in the tissue with intermediary medium (solving agent) Tert-Butyl Alcohol (TBA) ($C_4H_{10}O$) or dimethylbenzene (Xylene / Xylol) ($C_6H_4(CH_3)_2$) series.

EtOH : TBA / Xylol =	3 : 1	-	30 minutes
	1 : 1	-	30 minutes
	1 : 3	-	30 minutes
	100% TBA / Xylol	-	30 minutes
	100% TBA	-	30 minutes

- Infiltration of the tissue with paraffin (Paraplast Plus ®) in series

Saturated paraffin in TBA at 30 ºC	-	60 minutes
Saturated paraffin in TBA at 42 ºC	-	60 minutes
100% metded paraffin at 60 ºC	-	2 x 48 hours

- Embedding in paraffin at room temperature in embedding mould.

- Leave the paraffin to harden at room temperature.

2. Sectioning of the embedded tissue:

- (if necessary) Cut the embedded paraffin cubes that contain the tissue in to the desired proportions

- Mount the sample on a holder to fit the microtome.

- Label the micro-slides

- Rinse the micro-slide and degrease with EtOH.

- Coat the micro-slides with a layer of Kaisers glycerin-gelatin (the thinner the better).

- Adjust the microtome to the desired standards, mind the inclination of the knife.

- Section the tissue with a desired thickness

- After sectioning leave the ribbon with sectioned tissue to stretch in a water bath (45 ºC max.) or in a drop of water on the micro-slide on a heating plate with the smooth surface of the ribbon facing down.

- When the sectioned ribbons are fully stretched, fish them out of the water bath and mount them on the appropriately labeled micro-slide (again with the smooth surface facing the glass).

- Bake the micro-slides with the positioned tissue until all the water has evaporated.

- The micro-slides with the mounted tissue can now be stored until further treatments

3. *Dewaxing of the sectioned tissue (immediately before staining):*

- Make sure you have an appropriate stock of staining solution at hand before dewaxing the tissue.

- Dewax (remove the paraffin from) the micro-slides containing the mounted tissue by incubating the slides in a xylene series (xylene works more efficiently than TBA).

100% xylene	-	5 minutes
EtOH : xylene	-	5 minutes
100% EtOH	-	5 minutes

- If the selected staining procedure involves the stain to be dissolved in H_2O, rehydrate the sections in an EtOH – H_2O series.

100, 90, 70, 50, 30, 10% EtOH and 100% H_2O	-	2 minutes each

(for soft or thin tissues 100% and 50% EtOH and 100% water (2x) is also sufficient)

If the selected stain is dissolved in EtOH, no rehydration in needed.

- Do NOT let the dewaxed tissue allow to dry in before staining.

4. Staining with 0,1% Toluidin Blue ($C_{15}H_{16}N_3SCl$):

- Prepare (before dewaxing) by making a stock of staining solution (if a relatively large amount of tissue needs to be stained).

- To make a 0,1% Toluidine Blue solution carefully dissolve 1 mg of Toluidine Blue in 100 ml demi-H_2O, use a stirring plate to enhance the dissolving.

- Stain the tissue by dipping the dewaxed micro-slides into the solution for 1 to 5 minutes

- Rinse the micro-slides with abundant water (If the water tap is used to rinse, do NOT let the jet of water directly impact the tissue, it is better to let the water flow gently over the tissue by turning the micro-slide upside-down under the jet of water).

- Check the result of the staining under a microscope.

- If the staining is to strong one can differentiate by dipping the slides with the tissue in EtOH, which will gradually discolor the tissue again.

- Check the discolored tissue again and if satisfied allow the tissue to dry on a heater (40 ºC)

- One can directly analyze the tissue samples and discard them afterwards or make the slides permanent by adding a drop of DePex (a neutral solution of polystyrene and plasticizers in xylene), covering the tissue with a coverslip and letting the mounting medium harden.

- The permanent tissue samples can be stored for further use and digitalization.

NOTE: In this study I used a 0,01% Toluidine Blue solution in demi-water because that immediately provided a satisfying differentiation of the leaf tissue. It allowed me to skip the step of the differentiating by discolouring the tissue with EtOH and saved a lot of time. 0,01% Toluidine Blue may not work well for all types of tissue, but it is worth trying to find a good working solution in advance.

Appendix III

THE RELATION BETWEEN XYLEM CONDUIT DIAMETER, LMA AND LEAF AREA

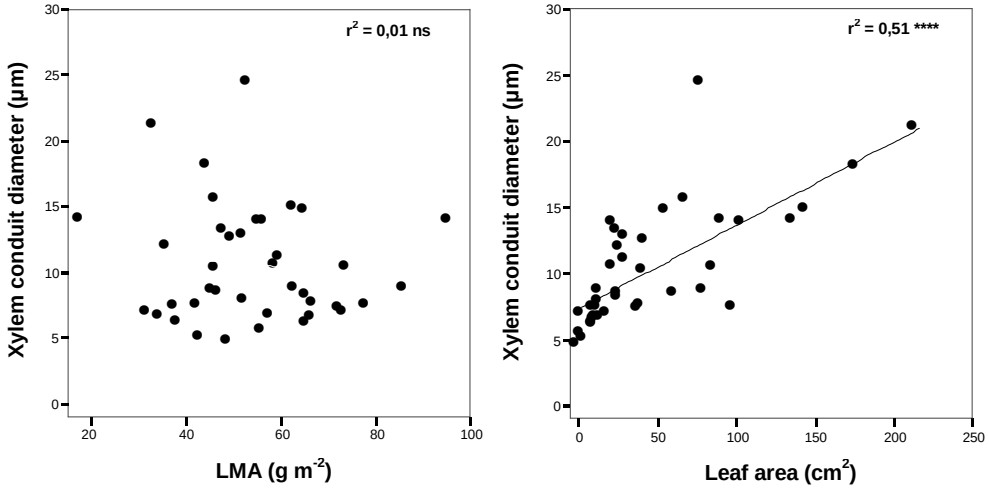

Figure A. **Relation between xylem conduit diameter and LMA and leaf area.** The figures show linear regressions between the xylem conduit diameter and LMA and leaf area per species. r^2 is given per graph, as is the level of significance (****; $p < 0,0001$) at $\alpha = 0,05$

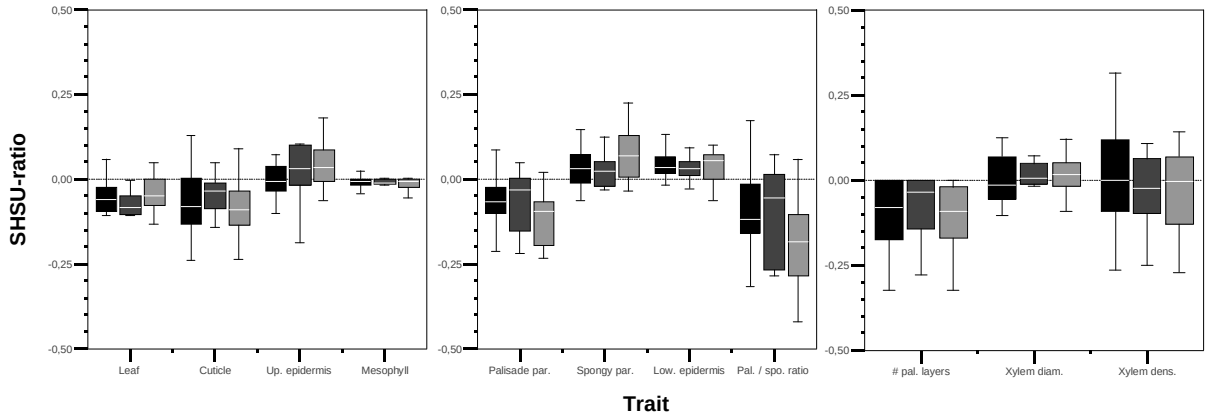

Figure B. **Shade-sun ratios; deviation from unity.** The upper and lower limits of the boxes indicate the 25 and 75 percentile of the arctangent transformed shade–sun ratio values per functional group related to shade-tolerance. The error bars represent the total range of values; ■ = shade-tolerant species, ■ =intermediate shade-tolerant species, and ■ = light-demanding species

	Light-demanding species	Intermediate shade-tolerant species	Shade-tolerant species
Leaf thickness (µm)	0,04	0,0001	0,004
Relative thickness (µm µm-1):			
Cuticle	0,01	0,03	0,05
Upper epidermis	0,06	0,71	0,98
Lower epidermis	0,06	0,09	0,13
Mesophyll	0,06	0,06	0,03
Palisade parenchyma	0,001	0,05	0,001
Spongy parenchyma	0,01	0,11	0,05
Palisade / Spongy parenchyma ratio (µm µm-1)	0,001	0,06	0,01
Number of palisade parenchyma cell layers	0,006	0,03	0,001
Xylem:			
Conduit density (µm-2)	0,49	0,34	0,62
Conduit diameter (µm)	0,40	0,13	0,83

Table A. **Shade-sun ratios; deviation from unity.** The table shows the significance levels ($\alpha = 0,05$) of the SHSU-ratio deviation from unity (0) within functional groups related to shade-tolerance.

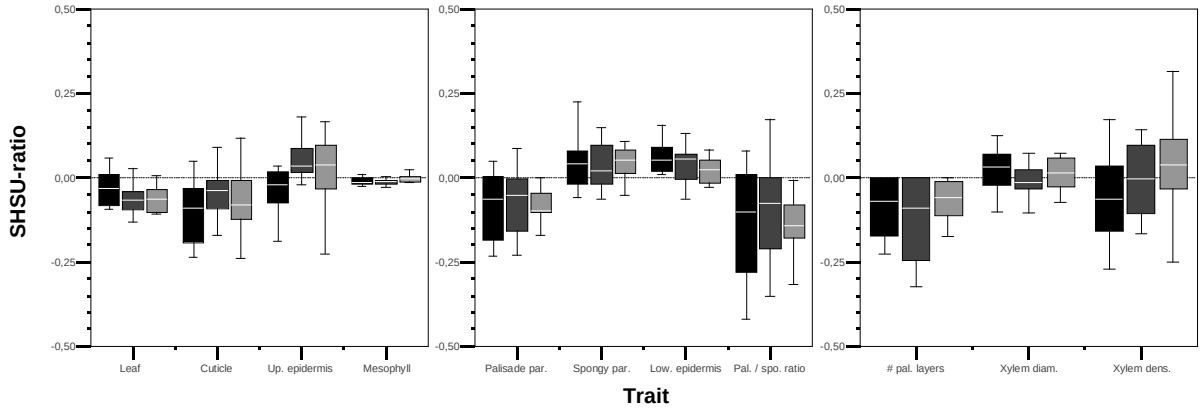

Figure C. **Shade-sun ratios; deviation from unity.** The upper and lower limits of the boxes indicate the 25 and 75 percentile of the arctangent transformed shade–sun ratio values per functional group related to drought-tolerance. The error bars represent the total range of values; ■ = drought-tolerant species, ■ = drought-avoiding species, and ■ = drought-intolerant species.

	Drought-intolerant species	Drought-avoiding species	Drought-tolerant species
Leaf thickness (µm)	0,009	0,00002	0,09
Relative thickness (µm µm-1):			
Cuticle	0,04	0,09	0,03
Upper epidermis	0,49	0,04	0,38
Lower epidermis	0,74	0,04	0,02
Mesophyll	0,27	0,03	0,008
Palisade parenchyma	0,0004	0,009	0,01
Spongy parenchyma	0,01	0,07	0,07
Palisade / Spongy parenchyma ratio (µm µm-1)	0,001	0,02	0,02
Number of palisade parenchyma cell layers	0,002	0,002	0,008
Xylem:			
Conduit density (µm-2)	0,36	0,78	0,16
Conduit diameter (µm)	0,48	0,55	0,24

Table B. **Shade-sun ratios; deviation from unity.** The table shows the significance levels (α = 0,05) of the SHSU-ratio deviation from unity (0) within functional groups related to drought-tolerance.