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Occurrence of homobaric and heterobaric leaves in two forest types of southern Brazil

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ABSTRACT

In ombrophilous forests, light stratification provokes different adjustments by plants for better use of the environmental conditions of each stratum. Among the morphological traits that vary with strata, the presence of bundle sheath extensions (BSEs) is related to water transport, photosynthesis, and leaf mechanical support and classifies leaves as homobaric or heterobaric. This study analyzed the proportion of these types of leaves in a Lowland Ombrophilous Dense Forest (LLODF) and a Mixed Ombrophilous Forest (MOF), and among the strata of each forest type. The morphological leaf traits of 89 LLODF tree species and 57 MOF tree species were examined. The proportion of homobaric and heterobaric leaves did not differ between forests. However, in both forest types, the distribution of species with heterobaric or homobaric leaves depended on strata, with heterobaric species occurring mainly in higher strata, and homobaric species in lower strata. Thus, light stratification acts as an ecological filter on the composition of the vegetation of these forests, favoring heterobaric species in places with higher light intensity and temperature, such as the highest strata of canopy. On the other hand, homobaric species are more frequent in lower strata, where light is less available and humidity higher.

Keywords: bundle sheath extension, leaf morphological traits, light stratification, Lowland Ombrophilous Dense Forest, Mixed Ombrophilous Forest

Introduction

Environmental factors affect the growth and survival of plants (Valladares & Niinemets 2008) and influence their internal organization (Dickson 2000). In forests, variation in abiotic features along vertical stratification provokes different adjustments by plants for better use of the environmental conditions of each stratum (Valladares & Niinemets 2008; Niinemets 2010; Inoue *et al*. 2015). Such adjustments can be morphological, physiological, and/or phenological. Among such morphological traits, the presence of bundle sheath extensions (BSEs) is related to water transport (Zwieniecki *et al*. 2007), photosynthesis (Pieruschka *et al*. 2010), and leaf mechanical support (Turner 1994).

Bundle-sheath extensions (BSEs) are formed by parenchyma or sclerenchyma cells that extend from the vascular bundle to both sides of the leaf epidermis (Karabourniotis *et al.* 2000; Nikolopoulos *et al.* 2002). Leaves are classified as homobaric or heterobaric depending on the condition of the BSEs (Kenzo *et al.* 2007). The former lack, or have incomplete, BSEs and their mesophyll is

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more homogeneous. The latter have complete BSEs and their mesophyll is divided into several photosynthetic compartments (Karabourniotis *et al.* 2000; Nikolopoulos *et al.* 2002; Kenzo *et al.* 2007). Homobaric leaves have a continuous mesophyll (Terashima 1992).

Such structural differences are reflected in the functional proprieties of these leaf types (Kenzo *et al.* 2007; Pieruschka *et al.* 2010; Lynch *et al.* 2012; Inoue *et al.* 2015). The presence of BSEs can protect leaf lamina from hydric stress and increase light absorption and mechanical support (Terashima 1992; Karabourniotis 1998; Nikolopoulos *et al.* 2002; Rhizopoulou & Psaras 2003). Yet in homobaric leaves, gas diffusion in the mesophyll can be more efficient due the absence of BSEs (Pieruschka *et al.* 2006). Also, BSEs have been linked to light distribution within the mesophyll, allowing investments in thicker and, consequently, smaller leaves (Nikolopoulos *et al.* 2002).

Despite the light heterogeneity that characterizes Brazilian forests, the occurrence of heterobaric leaves in many plant formations of these biomes and their relation to light gradients is still poorly studied. This study reports on the morpho-anatomical traits of tree species from a Lowland Ombrophilous Dense Forest and a Mixed Ombrophilous Forest in order to investigate the presence and proportion of homobaric and heterobaric leaves in both forest types. Our hypotheses are: a) the frequency of heterobaric leaves is similar in the two studied forest types since both experience similar environmental conditions (annual precipitation and light stratification), independent of their floral composition; b) the distribution and frequency of homobaric and heterobaric leaves vary among different forest strata in response to light stratification, with heterobaric leaves occurring mainly in upper strata and c) leaf type is dependent upon micro-environmental features more so than taxonomic group, as represented by families and/or genus.

Materials and methods

This study was based on leaf morphological data collected during previous studies in two forest sites: a Lowland Ombrophilous Dense Forest (LLODF), located at Volta Velha Reserve (26º04'S, 48º38'W), within the city of Itapoá, SC (a detailed description can be found in Boeger *et al.* 2004); and a Mixed Ombrophilous Forest (MOF), located in the Botanical Garden Francisca Maria Garfunkel Rischbieter (25º23'10"S, 49º12'58''W), within the boundaries of the city of Curitiba, PR (for more details, see Silveira *et al.* 2015). The environmental characteristics of each forest type are summarized in Tab. 1. All species included in this study were selected according to two criteria: 1) higher values of importance based on a previous phytosociological survey and 2) the presence of at least three individuals in the forest type. All specimens of collected from LLODF were deposited in UPCB (Herbarium of Department de Botany, UFPR, Curitiba, PR) and specimens from MOF were deposited in MBM (Herbarium of Municipal Botanical Museum, Curitiba, PR).

Morphological data, such as leaf area, leaf thickness, and the presence of homobaric and heterobaric leaves, were collected from 89 LLODF (Tab. 2) and 57 MOF tree species (Tab. 3). Leaves with complete BSEs were classified as heterobaric while leaves with incomplete and/or no BSEs were classified as homobaric leaves, according to Kenzo *et al.* (2007). The nomenclature and taxonomic classification of each species were checked against International Plant Names Index (www.ipni.org).

All species were classified according to their occurrence in four strata. Species of LLODF were classified into: Stratum 1, <5 m; Stratum 2, 5 - 9.99 m; Stratum 3, 10 - 14.99 m and Stratum 4, >15 m. Species of MOF were classified into: Stratum 1, <7 m; Stratum 2, 7 - 14.99 m; Stratum 3, 15 - 26 m and Stratum 4, >26 m. In MOF, photosynthetically active radiation (PAR) was 28.2 ± 3.46 μmol $s^{-1}m^{-2}$ (2.3% irradiance) in Stratum 1; 36.2 ± 24.3 μmol $s^{-1}m^{-2}$ (3.8% irradiance) in Stratum 2; 73.6 ± 22.8 μmol $s^{-1}m^{-2}$ (10% irradiance) in Stratum 3 and 744.3 ± 68.4 μmol $s^{-1}m^{-2}$ (100% irradiance) in Stratum 4 (canopy). In LLODF, PAR was 55.2 ± 25.8 μmol $s^{-1}m^{-2}$ (4.3% irradiance) in Stratum 1; 297.4 ± 56.1 μmol $s^{-1}m^{-2}$ (23% irradiance) in Stratum 2; 480.5 ± 91.1 μmol $s^{-1}m^{-2}$ (37.3% irradiance) in Stratum 3 and 1286.8 ± 79.9 μmol $s^{-1}m^{-2}$ (100% irradiance) in Stratum 4 (canopy).

The mean values for the distinct strata of each forest type were compared through *One-way* ANOVA followed by Tukey test. Leaf area and leaf thickness of homobaric and

Table 1. Environmental features of studied sites. Legend: LLODF - Lowland Ombrophilous Dense Forest; MOF: Mixed Ombrophilous Forest. Climate type according to Köppen classification.

Feature	LLODF	MOF
Average moisture (%)	85	81
Annual precipitation (mm)	2170	1662
Soil type	Spodosol no hidromorphic. with moderate sandy texture and low concentrations of exchangeable cations.	Cambisoil humic aluminic and gleisolic typical clay soil.
Altitude (m)	9	947
Mean temperature (°C)	20.3	17.9
Climate type	Cfb	Cfb

Table 2. Presence and absence of bundle sheath extension (BSE) on tree species from Lowland Ombrophilous Dense Forest, by stratum.

Family	Species	Stratum	BSE
Anacardiaceae	*Tapirira guianensis* Aubl.	3	Absent
Annonaceae	*Annona cacans* E. Warming	4	Absent
	Guatteria australis A. St. Hil.	1	Absent
	Xylopia brasiliensis Spreng.	3	Present
Aquifoliaceae	*Ilex dumosa* Reissek	2	Absent
	Ilex integerrima Reissek	2	Absent
	Ilex pseudobuxus Reissek	2	Absent
	Ilex theezans Mart.	2	Absent
Araliaceae	*Oreopanax capitatus* Decne. & Planch.	2	Absent
Burseraceae	*Protium kleinii* Cuatrec.	2	Present
Calophyllaceae	*Calophyllum brasiliense* Cambess.	1	Absent
Celastraceae	*Maytenus robusta* Reissek	2	Absent
Clethraceae	*Clethra scabra* Pers.	3	Present
Clusiaceae	*Clusia parviflora* Engl.	2	Absent
	Garcinia gardneriana Planch. & Triana	1	Absent
Cunoniaceae	*Weinmannia paulliniifolia* Pohl ex Ser.	3	Absent
Elaeocarpaceae	*Sloanea guianensis* Benth.	2	Present
Erytroxilaceae	*Erythroxylum vaccinifolium* Mart.	1	Absent
Euphorbiaceae	*Alchornea triplinervia* Müll. Arg.	2	Present
	Aparisthmium cordatum (A. Juss.) Baill.	2	Absent
	Maprounea guianensis Aubl.	2	Absent
	Pera glabrata ex Baill.	2	Absent
Fabaceae	*Andira anthelminthica* Benth.	2	Present
	Copaifera trapezifolia Hayne	3	Absent
	Ormosia arborea Harms	1	Absent
	Pithecellobium langsdorffii Benth.	2	Absent
Lauraceae	*Aiouea saligna* Meisn.	3	Present
	Aniba firmula (Nees & Mart.) Mez	2	Present
	Endlicheria paniculata (Spreng.) J.F.Macbr.	1	Absent
	Nectandra grandiflora Nees & Mart.	2	Present
	Nectandra megapotamica Mez	3	Present
	Nectandra oppositifolia Nees & Mart.	4	Present
	Ocotea aciphylla Mez	2	Present
	Ocotea dispersa (Nees & Mart.) Mez	2	Present
	Ocotea elegans Mez	2	Present
	Ocotea glaziovii Mez	2	Present
	Ocotea odorifera (Vell.) Rohwer	2	Present
	Ocotea pulchella Mart.	2	Present
	Ocotea pulchra (Ekman & Schmidt) Alain	4	Present
Malpighiaceae	*Byrsonima ligustrifolia* A. Juss.	2	Absent
Melastomataceae	*Miconia cabucu* Hoehne	3	Absent
	Miconia cubatanensis Hoehne	1	Absent
	Miconia hymenonervia (Raddi) Cogn.	1	Absent
	Miconia sellowiana Naudin	1	Absent
	Mouriri chamissoana Cogn.	2	Absent
Meliaceae	*Cabralea canjerana* (Vell.) Mart.	1	Absent
Meliaceae	*Guarea macrophylla* Vahl	2	Absent
Monimiaceae	*Mollinedia uleana* Perkins	1	Absent
Myristicaceae	*Virola oleifera* (Schott) A.C.Sm.	2	Absent
Myrtaceae	*Blepharocalyx salicifolius* (Kunth.) O.Berg	2	Absent
	Calyptranthes concinna DC.	2	Absent
	Calyptranthes lucida Mart. ex DC.	1	Absent
	Campomanesia guaviroba (DC.) Kiaersk.	3	Present
	Eugenia cerasiflora Kurz.	2	Absent
	Eugenia obovata Wall. ex Duthie	2	Absent
	Eugenia subavenia O.Berg	3	Absent

Table 2. Cont.

Family	Species	Stratum	BSE
Myrtaceae	*Eugenia tristis* D.Legrand	1	Absent
	Eugenia umbelliflora O.Berg	2	Absent
	Gomidesia affinis (Cambess) D.Legrand	2	Absent
	Gomidesia schaueriana O.Berg	1	Present
	Marlierea eugeniopsoides (Kausel & D.Legrand) D.Legrand	1	Absent
	Marlierea reitzii D.Legrand	2	Absent
	Myrceugenia campestris (D.C.) D.Legrand & Kausel	2	Absent
	Myrceugenia reitzii D.Legrand & Kausel	2	Absent
	Myrcia acuminatissima Hieron.	2	Absent
	Myrcia fallax DC.	2	Present
	Psidium cattleyanum Sabine	3	Absent
Ochnaceae	*Ouratea parviflora* Engl.	1	Absent
Olacaceae	*Heisteria silvianii* Schwake	2	Absent
Olacaceae	*Tetrastylidium grandifolium* (Baill.) Sleumer	2	Absent
Pentaphylacaceae	*Ternstroemia brasiliensis* Cambess.	3	Absent
Phyllantaceae	*Hieronyma alchorneoides* Allem.	4	Present
Polygonaceae	*Coccoloba warmingii* Meisn.	4	Present
Primulaceae	*Conomorpha peruviana* A.DC.	1	Absent
	Rapanea ferruginea Mez	4	Absent
	Rapanea venosa Elmer	2	Absent
Rosaceae	*Prunus sellowii* Koehne	2	Absent
Rubiaceae	*Amaioua guianensis* Aubl.	2	Present
	Faramea marginata Mart.	1	Absent
	Rudgea villiflora K.Schum. ex Standl.	1	Absent
Rutacea	*Esenbeckia grandiflora* Mart.	1	Absent
Sapindaceae	*Cupania oblongifolia* Turcz.	2	Absent
	Matayba guianensis Aubl.	1	Absent
Sapotaceae	*Manilkara subsericea* (Mart.) Dubard	3	Absent
	Pouteria beaurepairei (Glaz. & Raunk) Baehni	2	Absent
	Pouteria venosa (Mart.) Baehni	2	Absent
Solanaceae	*Solanum inaequale* C. Presl	2	absent
Styracaceae	*Styrax glabratus* Warb.	3	Absent
Winteraceae	*Drimys brasiliensis* Miers.	2	Absent

Table 3. Presence and absence of bundle sheath extension (BSE) on tree species from Mixed Ombrophilous Forest, by stratum.

Family	Species	Stratum	BSE
Anacardinaceae	*Lithraea molleoides* (Vell.) Engl.	2	Absent
	Schinus terebinthifolius Raddi	2	Present
Aquifoliaceae	*Ilex paraguariensis* A.St.-Hil.	1	Absent
Araucariaceae	*Araucaria angustifolia* (Bertol.) Kuntze	4	Absent
Asteraceae	*Gochnatia polymorpha* (Less.) Cabrera	2	Present
Bignoniaceae	*Jacaranda puberula* Cham.	1	Absent
Canellaceae	*Capsicodendron dinisii* (Schwacke) Occhioni	3	Absent
Cannabaceae	*Celtis iguanaea* (Jacq.) Sarg.	1	Absent
Cardiopteridaceae	*Citronella paniculata* (Mart.) R.A.Howard	2	Absent
Celastraceae	*Maytenus ilicifolia* Mart. ex Reissek	1	Absent
Elaeocarpaceae	*Sloanea monosperma* Benth.	1	Present
Euphorbiaceae	*Sebastiania brasiliensis* Spreng.	1	Absent
	Sebastiania commersoniana (Baill.) L.B.Sm. & Downs	1	Absent
Fabaceae	*Dalbergia brasiliensis* Vogel	2	Absent
	Erythrina falcata Benth.	3	Present
	Inga marginata Willd.	2	Absent
	Lonchocarpus muehlbergianus Hassl.	3	Present
Lamiaceae	*Vitex megapotamica* (Spreng.) Moldenke	2	Present
Lauraceae	*Nectandra megapotamica* Mez	1	Present
	Ocotea porosa (Nees) Angely	3	Present

Table 3. Cont.

Family	Species	Stratum	BSE
Lauraceae	*Ocotea puberula* Nees	2	Present
Lythraceae	*Lafoensia pacari* A.St.-Hil.	2	Absent
Malvaceae	*Luehea divaricate* Mart.	2	Present
Melastomataceae	*Miconia sellowiana* Naudin	1	Absent
	Tibouchina sellowiana Cogn.	1	Absent
Meliaceae	*Cedrela fissilis* Vell.	3	Present
Monimiaceae	*Mollinedia clavigera* Tul.	1	Absent
Moraceae	*Ficus luschnathiana* Miq.	2	Present
Myrtaceae	*Calyptranthes concinna* DC.	1	Absent
	Campomanesia guaviroba (DC.) Kiaersk	2	Present
	Campomanesia xanthocarpa O.Berg	2	Absent
	Eugenia pluriflora Mart.	2	Absent
	Eugenia pyriformis Cambess.	2	Present
	Eugenia uniflora O.Berg	2	Absent
	Myrceugenia miersiana (Gardner) D.Legrand & Kausel	1	Absent
	Myrcia cymoso-paniculata Kiaersk	3	Absent
	Myrcia hatschbachii D.Legrand	2	Absent
	Myrcia rostrata DC.	3	Present
	Psidium cattleyanum Sabine	1	Absent
Oleaceae	*Chionanthus filiformis* (Vell.) P.S.Green	3	Absent
Picramniaceae	*Picramnia parvifolia* Engl.	2	Absent
Piperaceae	*Piper gaudichaudianum* Kunth ex C.DC.	1	Absent
Podocarpaceae	*Podocarpus lambertii* Klotzsch ex Endl.	2	Absent
Primulaceae	*Myrsine coriacea* Nadeaud	1	Present
	Myrsine umbellate Mart.	1	Absent
Proteaceae	*Roupala Montana* Willd.	2	Absent
Rosaceae	*Prunus brasiliensis* Schott ex Spreng.	2	Absent
	Prunus sellowii Koehne	2	Absent
Salicaceae	*Casearia decandra* Jacq.	1	Absent
	Casearia sylvestris Sw.	1	Absent
Sapindaceae	*Allophylus edulis* Radlk. ex Warm.	2	Absent
	Allophylus guaraniticus Radlk.	2	Absent
	Cupania vernalis Cambess.	1	Absent
	Matayba elaeagnoides Radlk.	2	Present
Solanaceae	*Solanum pseudoquina* A.St.-Hil.	1	Absent
	Solanum sanctae-catharinae Dunal	2	Absent
	Solanum swartzianum Roem. & Schult.	2	Absent
Verbenaceae	*Duranta vestita* Cham.	3	Absent

heterobaric leaves of each forest type were compared using t-test. Both analyses were performed on PAST software (Hammer *et al*. 2001). The independent distribution analysis of heterobaric leaves among forests and strata employed the χ^2 test ($P < 0.05$). Since MOF Stratum 4 included only one species, *Araucaria angustifolia* (Bertol.) Kuntze, it was excluded from this test, which aimed to verify the distribution of heterobaric leaves among plant families. The independent distribution analysis was performed using the RCMDR package (2, 1-7) for R program (version 3.1.2, R Foundation for Statistical computing, Vienna Austria).

Results

Of the 89 LLODF species studied, 22 (25%), belonging to 10 families, had heterobaric leaves (Fig.1A-B), while 67 species (75%) from 30 families had homobaric leaves (Fig.1C-D; Tab. 4). Out of the 58 MOF species studied, 16 (28%), belonging to 11 families, had heterobaric leaves, while 42 (72%) from 26 families had homobaric leaves (Fig. 1A-B; Tab. 4). The proportion of species with each leaf type (homobaric/heterobaric) did not differ between the studied forests (χ^2 test, $P = 0.75$, GL = 1, N = 146 species). For MOF, all heterobaric leaves had sclerenchymatous BSEs (Fig. 1B), except *Ficus luschnathiana* and *Myrsine coriacea,* which had parenchymatous BSEs. In LLODF, all heterobaric leaves had sclerenchymatous BSE, and *Gomidesia schaueriana* had incomplete BSE; in this species, BSE occurred only on the adaxial side of the lamina.

The distribution of species with heterobaric leaves among forest strata differed significantly in LLODF ($\chi2$ test, P=0.013, DF=3, N=89 species). The highest proportion of

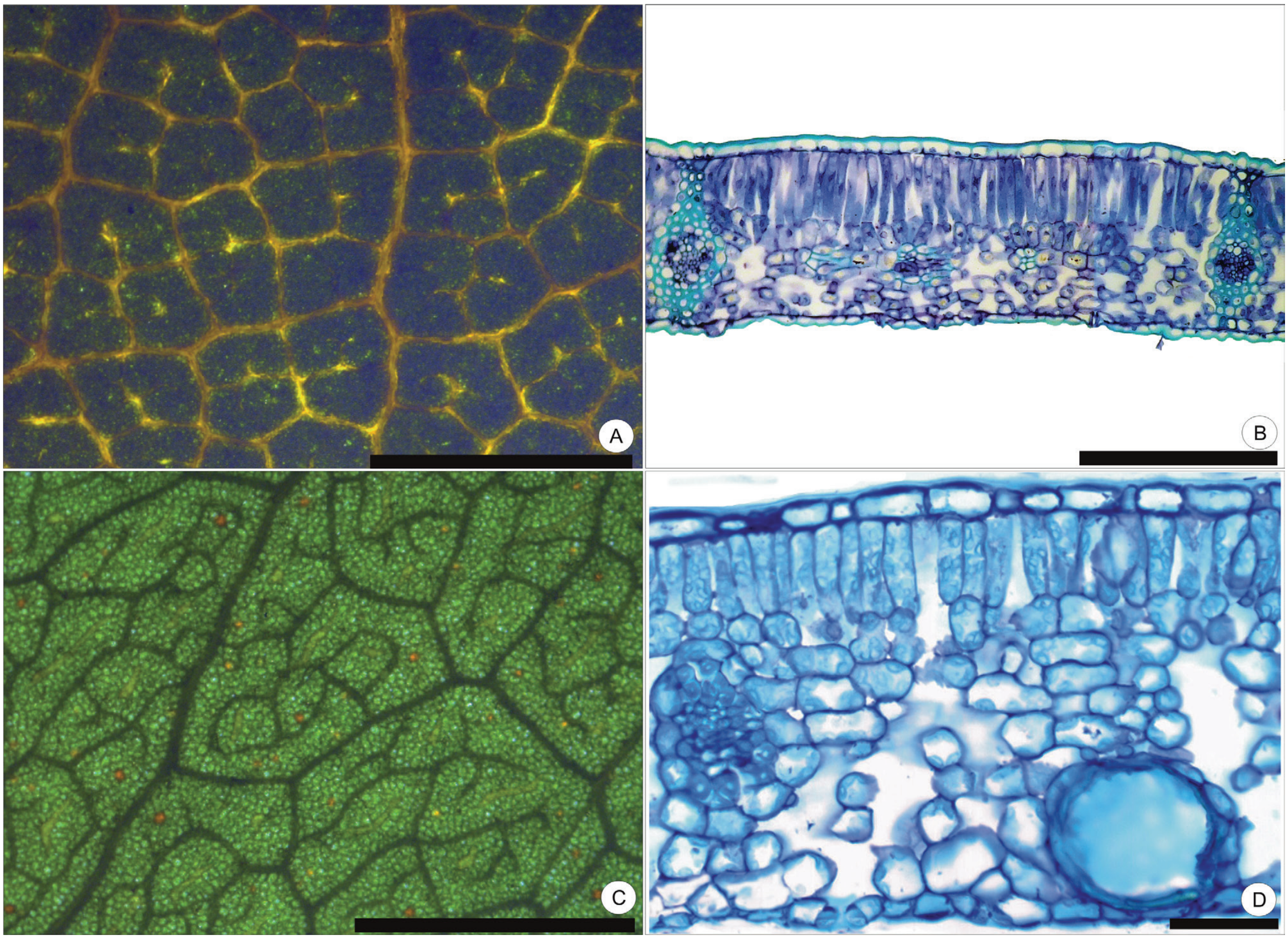

Figure 1. Surface and cross sections of homobric and heterobaric leaves. A. Surface of heterobaric leaf of *Ocotea porosa*, showing the division of the lamina in small areas by bundle sheath extension (BSE). B. Cross section of heterobaric leaf of *Ocotea porosa*, showing BSE. C. Surface of homobaric leaf of *Lonchocarphus muehlbergianus*, showing a homogeneous lamina. D. Cross section of homobaric leaf of *Tibouchina sellowiana*. Bars: A and C = 2 mm; B = 30 µm; D = 50 µm

heterobaric leaves was found in Stratum 4 (Tab. 4), and the number of heterobaric leaves was directly proportional to light stratification. In MOF, despite the fact that heterobaric leaves were not significantly distributed among strata ($\chi 2$ test, P=0.0662, DF=3, N=57 species), an increase in heterobaric leaves was directly related to light intensity, with the highest proportion of heterobaric of leaves being in Stratum 3, since the frequency of heterobaric leaves was 0% in Stratum 4, which was composed of a single species (*A. angustifolia*) with only homobaric leaves. The lower strata had higher proportions of homobaric leaves in both forests (Tab. 4).

For all species considered in LLODF, leaf area did not differ among Strata 1, 2, and 3, but was higher in Stratum 4 (Tab. 5). When we excluded species with leaf area > 100 cm^2, [*Ormosia arborea* in Stratum 1; *Aparisthmium cordatum* and *Cupania oblongifolia* in Stratum 2; *Miconia cabucu* in Stratum 3 and *Coccoloba warmingii* in Stratum 4], the average leaf area of Stratum 4 differed from Strata 2 and 3. Although these species occur in small numbers in each stratum, they significantly affected average leaf area, as shown by the standard deviations (Tab. 5). In MOF, there was no variation in leaf area among lower strata; only Stratum 4 differed due to the reduced leaf area of *A. angustifolia* leaves (Tab. 5).

In LLODF, leaf thickness did not exhibit the same pattern of variation among strata as leaf area. Only Stratum 3 differed by having thinner leaves than the other strata. When we excluded the species with leaf area > 100 cm^2, mean leaf thickness varied as follows: Stratum 1 = Stratum 2 > Stratum 3 > Stratum 4. In MOF, Stratum 4 had thicker leaves than the other strata (Tab. 5).

The comparison of homobaric and heterobaric leaves, independently of strata, indicates that homobaric leaves were thicker than heterobaric leaves in both forests types (LLODF - t test: t = 19.65, $P < 0.001$; MOF – t test t = 18.79, $P < 0.001$), when leaves > 100 cm^2 are excluded (Tab. 5).

The distribution of heterobaric leaves among some plant families was also evaluated (χ^2 test, $P < 0.0001$, GL = 7, N = 81 species from eight families with more than five species, Tab. S01 in supplementary material). In this study, all species of Lauraceae had heterobaric leaves, independently of strata (Fig. 2), except *Endlicheria paniculata*, which had heterobaric

Table 4. Number and percentage of species with homobaric and heterobaric leaves by forest type and stratum. Legend: LLODF – Lowland Ombrophilous Dense Forest; MOF – Mixed Ombrophilous Forest.

Forest type	Stratum	N° of species with heterobaric leaves	N° of species with homobaric leaves	Total	Species with heterobaric leaves (%)
LLODF	1 (< 5m)	0	21	21	0
	2 (5.0-9.9m)	3	35	38	7.9
	3 (10-14.9m)	5	9	14	35.7
	4 (> 15m)	14	2	16	87.5
Total		**22**	**67**	**89**	**25.0**
MOF	1 (< 7m)	3	18	21	14.3
	2 (7 – 14.9m)	4	19	23	17.4
	3 (15 – 26m)	9	4	13	69.2
	4 (> 26m)	0	1	1	0
Total		**16**	**42**	**58**	**28**

Table 5. Average height, average values and respective standard deviations of leaf area and leaf thickness by stratum, leaf types and forest type. Legend: LLODF – Lowland Ombrophilous Dense Forest; MOF – Mixed Ombrophilous Forest. (*) Leaf area and leaf thickness averages with the exclusion of leaves > 100 cm2, only in LLODF. Different letters in the same column, within the forest type, represent statistical difference, Tukey test ($p<0.05$).

Forest type	Stratum	Height (m)	Leaf area (cm²)	Leaf area *(cm²)	Leaf thickness (mm)	Leaf thickness (mm)*
LLODF	1 (<5m)	3.4 (1.4)	29.3 (25.1)b	23.1 (11.0)ab	0.31 (0.1)a	0.30 (0.1)a
	2 (5-9.9m)	7.5 (1.5)	30.5 (25.4)b	27.5 (19.6)a	0.32 (0.2)a	0.32 (0.2)a
	3 (10-14.9m)	11.7 (1.1)	35.3 (56.0)b	19.1 (11.2)b	0.23 (0.1)b	0.21 (0.1)b
	4 (> 15m)	16.3 (1.6)	81.5 (81.3)a	25.4 (30.1)a	0.31 (0.1)a	0.16 (0.1)c
Homobaric leaves	all	—	32.5 (35.6)a	15.0 (11.9)b	0.30 (0.15)a	0.31 (0.16)a
Heterobaric leaves	all	—	39.6 (46.3)a	19.6 (14.0)a	0.30 (0.12)a	0.29 (0.10)b
MOF	1 (<7 m)	5.2 (1.8)	14.7 (12.3)a		0.18 (0.1)b	
	2 (7-14.9 m)	9.2 (3.3)	18.3 (13.4)a		0.19 (0.1)b	
	3 (15-26 m)	12.7 (9.2)	16.2 (11.1)a		0.18 (0.0)b	
	4 (>26 m)	30.1(12.0)	1.04(0.2)b		0.36 (0.07)a	
Homobaric leaves	all	—	15.0 (11.9)a		0.20 (0.08)a	
Heterobaric leaves	all	—	19.6 (14.1)a		0.15 (0.04)b	

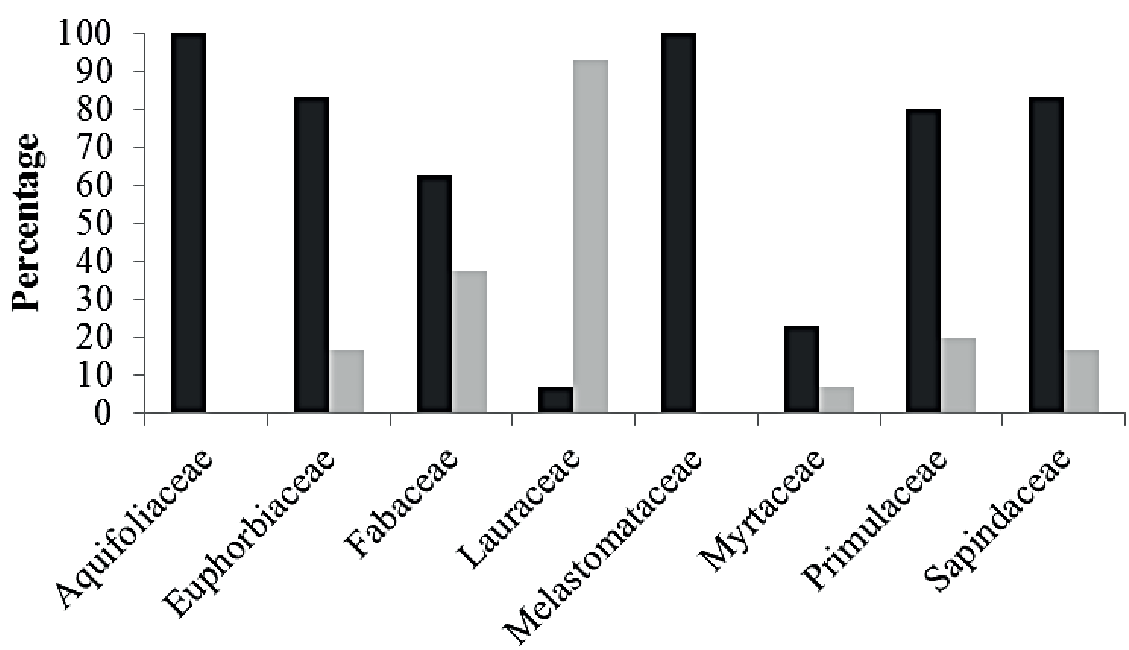

Figure 2. Percentage of homobaric and heterobaric leaves among plant families with more than five species. Black bars represent homobaric leaves and grey bars represent heterobaric leaves.

leaves in Stratum 1 at LLODF.

Besides Lauraceae, Euphorbiaceae, Fabaceae, Myrtaceae, Primulaceae, and Sapotaceae also had species with both leaf types, with heterobaric leaves being mainly distributed in Strata 2 and 3. No species of Aquifoliaceae and Melastomataceae, which occurred mainly in Strata 1 and 2, had heterobaric leaves.

Discussion

Both forest types exhibited similar proportions of species with homobaric and heterobaric leaves, with a greater occurrence of the former. Heterobaric leaves are generally associated with deciduous forests with cold or dry, well-defined seasons (Terashima 1992). On the other hand, homobaric leaves occur in evergreen forests, generally found in humid and hot regions (Kenzo ***et al***. 2007). Thus, our data corroborate a previous study that found a higher proportion of homobaric leaves in humid forests with high precipitation throughout the year (Kenzo ***et al***. 2007), such as our study sites. Although heterobaric leaves are associated with drier, deciduous forests, they are also present in humid forests, such as was found in our study sites. The distribution of these two types of leaves among strata in the present study was similar to that observed by Kenzo ***et al***. (2007) in a rain forest at Sarawak, Malasia.

The distribution analysis indicated that heterobaric leaves are more common in Strata 3 and 4 in MOF and LLODF, respectively, while homobaric leaves were more common in Strata 1 and 2. This distribution of homobaric and heterobaric leaves in different strata appears to be due to micro-environmental gradients associated with the various forest strata (Kenzo ***et al***. 2007). Such gradients include light availability, temperature, vapor pressure deficit, and wind (Théry 2001; Kitajima & Poorter 2010; Bennett ***et al***. 2015; Inoue ***et al***. 2015). Of these, light availability is particularly important because it can influence the growth, survival, and subsequent reproduction of young individuals (Chazdon ***et al***. 1996; Valladares & Niinemets 2008).

In the canopy, for example, plants are subjected to intense light and heat, which can be stressful during some periods of the day and/or year (Théry 2001; Valladares & Niinemets 2008). The presence of heterobaric leaves in the higher strata of a forest can be advantageous because sclerenchymatous BSEs can give additional mechanical support, due to the strength given by the sclerenchyma cells (Dickson 2000; Cutler ***et al***. 2008), and help to maintain leaf shape and volume (Roth 1984), as well as protect against herbivores (Sack & Scoffoni 2013). Secondarily, BSEs can perform optic functions such as facilitating the dispersion of light within the compartments of the leaf (Karabourniotis ***et al***. 2000; Nikolopoulos ***et al***. 2002), thereby enhancing photosynthetic rate (Nikolopoulos ***et al***. 2002; Liakoura ***et al***. 2009; Buckley ***et al***. 2011).

Plants restricted to lower strata, on the other hand, are subjected to low levels of heterogeneous light (Théry 2001; Kenzo ***et al***. 2007; Valladares & Niinemets 2008). These conditions are beneficial to homobaric leaves with their well-developed spongy parenchyma (Fig. 1D), as observed in the studied species [spongy:palisade parenchyma ratio for MOF homobaric leaves (2.1 ± 0.9); for MOF heterobaric leaves (1.5 ± 0.6); for LLODF homobaric leaves (5.3 ± 3.2) and for LLODF heterobaric leaves (3.9 ± 1.7)]. A thicker spongy parenchyma is advantageous for capturing diffused light because the irregular-shaped cells reflect light rays within the mesophyll, thereby facilitating more efficient absorption (Vogelmann ***et al***. 1996). Homobaric leaves also increase the proportion of photosynthetic areas in the mesophyll (Terashima 1992), which may contribute to more efficient photosynthesis and water use (Pieruschka ***et al***. 2006; Pieruschka ***et al***. 2010; Lynch ***et al***. 2012) Thus, under limited light conditions, species with homobaric leaves perform better than those with heterobaric leaves (Kenzo ***et al***. 2007).

The distribution of species with homobaric and heterobaric leaves was weakly correlated with taxonomic group. Although Lauraceae is present in all strata of LLODF and in the first three strata of MOF, it is the only family that is represented by a larger number of heterobaric leaf species. All the species of the families Aquifoliaceae and Melastomataceae, which are commonly found in under-canopy strata, had homobaric leaves. Even though they comprise species with both leaf types, the families Euphorbiaceae, Myrtaceae, Primulaceae, and Sapindaceae did not show a distributional pattern related to strata. The one exception was Fabaceae, whose species with heterobaric leaves were present in Stratum 3 in MOF. These results indicate that the leaf types of each species are more dependent on habitat and/or life form type than phylogenetic relationships. Environmental filters have convergent effects and seem to favor functional diversity due the habitat heterogeneity, especially in tropical forest communities (Manel ***et al***. 2014).

In conclusion, the occurrence of homobaric and heterobaric leaves seems to be related to light stratification. The distribution of homobaric and heterobaric leaves in the different forest strata shows that light stratification acts as an ecological filter on the composition of the vegetation. Heterobaric leaves tend to occur in hotter strata that are more exposed to light, while homobaric leaves are more frequent in the under-canopy and more humid strata. This difference indicates that both leaf types occupy different positions on the "leaf economic spectrum", based on the balance between the cost of investment in structural tissues and the investment in photosynthetic tissues for carbon fixation via photosynthesis (sense Wright ***et al***. 2004).

Besides environmental influences, the occurrence of leaf types is weakly related to taxonomic group. Only Lauraceae included a large number of heterobaric species. These results show that these two leaf types (homobaric/heterobaric) are more dependent on habitat and/or life form than phylogenetic relationships. Environmental filters seem to shape functional diversity due to habitat heterogeneity, especially in tropical forest communities.

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