Leaf Anatomical Traits of Lianas and Trees at the Canopy of Two Contrasting Lowland Tropical Forests in the Context of Leaf Economic Spectrum

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The leaf economic spectrum describes a comprehensive framework of how the surrounding environment modulates leaf functional traits (LFT) and how these are associated between them. This framework has traditionally focused on physiological, chemical, and biomass assignation traits, but rarely in leaf anatomical traits. Here we compare leaf anatomical metrics and traits of 40 liana and tree species from two lowland tropical forests in Panama with contrasting rainfall regimes: Parque Natural Metropolitano (dry-forest) and Parque Nacional San Lorenzo (wet-forest). Then we evaluate how anatomical traits are associated with well-established LFTs. Anatomical metrics were collected from leaf cross-section images estimating the area, thickness, cell count, and size of the upper and lower epidermis and palisade and spongy mesophyll. Ratios between metrics were performed as potential anatomical traits to reduce the leaf size effect between species. Our results suggest that anatomical changes between life forms are associated with increases in the palisade area and thickness of trees in comparison with lianas, while anatomical changes between forest type species are related to increases in the spongy area and thickness of wet-forest species than dry-forest. These differences could be associated with the high photosynthetic rates of trees or the need to enhance the gas exchange in humid environments. Our results also suggest that anatomical traits are related to well-established LFT; however, the degree of association between them may depend on the life forms and forest type. For example, our results suggest that reductions in the palisade and spongy cell density are associated with increases in leaf mass area and maximum photosynthetic capacity, but this association was not observed when we compared life forms or forest types. The use of leaf anatomical information may facilitate to describe the mechanism that drives the leaf economy, improving our understanding of the resource allocation strategies embedded in functional groups.

Keywords: life forms, functional traits, leaf mass per area, leaf anatomy, leaf economic spectrum

INTRODUCTION

Leaf economic spectrum (LES) provides a useful framework to describe how leaf traits are inter-correlated and how they are modulated by their surrounding environment (Wright et al., 2004). This theory attempts to transform discrete groups of plant functional types to a more continuum-oriented approach to assess plant functional diversity (Reich, 2014). Currently, the LES has been expanded towards other traits related to wood (Chave et al., 2009), organs such as roots or stems (Reich, 2014; Kong et al., 2019), plant structure (Verbeeck et al., 2019), or even to decomposition rates (Santiago, 2007). Overall, the traits founded in this spectrum are directly associated with the acquisition, processing, and conservation of resources (Reich, 2014); herein, the economic perspective. There are several traits that integrate the current economic spectrum, but those normalized by leaf area or mass have been used predominantly to group functional types based off of resource-investment strategies (Osnas et al., 2013). The most common traits used are leaf mass per area, nitrogen concentration, or maximum photosynthetic capacity that are strongly modulated by climate, latitude, or biome (Reich et al., 1997, 1999; Reich and Oleksyn, 2004; Wright et al., 2005; Reich, 2014). This modulation tends to be a consequence of internal adaptations of the leaf anatomy (upper epidermis, palisade mesophyll, spongy mesophyll, and lower epidermis) responsible for particular processes (i.e., protective cells, light capture, or conductance) (Villar et al., 2013).

The conceptual framework of the LES is rooted in the selective pressures associated with trade-off between the acquisition and conservation of carbon, water, and nutrients (Reich, 2014). Co-variances between the common functional traits, such as Leaf-Mass Area (LMA), leaf nitrogen concentration by mass (N) or photosynthetic capacity (A_{max}), have been used to explore the broader implications of natural selection at the community level (Osnas et al., 2013; Reich, 2014). For instance, the positive relationships between the mass-based traits N_{mass} and A_{max} (mass-based) are used to understand and equalize the productivity per leaf area or the unit of mass investment (Reich, 2014; John et al., 2017). This suggests there is a selective advantage of increased nitrogen leaf content to photosynthetic capacity across species and ecosystems. A deeper exploration of anatomical limitations and facilitations associated with resource acquisition to this spectrum-wide analysis could provide a structural and mechanical perspective of such generalized co-variances driven by selection.

Previous investigations have focused on specific functional traits' relationship and correlation with anatomy. For instance, Villar et al. (2013) and John et al. (2017) looked at co-variances between anatomy and LMA or Pyankov et al. (1999) compared anatomy to specific-leaf area and specific-leaf mass. There appears to be a gap in the literature that broadens the scope of multiple functional traits against multiple anatomical traits that relates to LES. Like the leaf, plant, or wood traits, leaf anatomy is likely to face physiological, structural, and defensive trade-offs (Javelle et al., 2011; Sack and Scoffoni, 2013; Somavilla et al., 2014; Onoda et al., 2017). At any given time, the variability of leaf anatomy depends on the species nature and the

surrounding pressure on their growth and development (Tichá, 1985; Oguchi et al., 2003; Xiao et al., 2016). The lack of systematic anatomical deconstruction associated with leaf traits creates an incomplete representation of the mechanics by how the LES varies between plants and environments. For example, several anatomical features appear to be important for photosynthesis, such as the palisade thickness, mesophyll cell density, or air spaces that mediate photosynthetic rates, to mention some (Kenzo et al., 2004; Lehmeier et al., 2017). Currently, studies by Liu et al. (2019) suggest that anatomical traits tend to present a weak correlation with leaf economic traits in temperate and subtropical species. However, in tropical species, there is little information regarding how anatomical traits influence leaf traits in different growth forms and how these relationships vary according to ecosystems. The latter is crucial because as a theory LES must be applied equally to different species within the same ecosystem; even those that coexist together and present distinguishing physiological, biochemical, and optical traits such as the case of lianas and trees (Sánchez-Azofeifa et al., 2009; Asner and Martin, 2012; Slot and Winter, 2017; Werden et al., 2017; Guzmán et al., 2018; Guzmán and Sanchez-Azofeifa, 2021).

Lianas –woody vines– are non-self-supporting plants that use host trees to access canopy light, probably affecting the availability of resources around the tree host (Stewart and Schnitzer, 2017). Typically during their development, lianas produce a higher proportion of their biomass in foliage above their host affecting the tree growth, survival, and reproduction (Phillips et al., 2005; Peña-Claros et al., 2008; Ingwell et al., 2010; Wright et al., 2015; Martínez-Izquierdo et al., 2016). Many investigations throughout the tropics have revealed dramatic increases in liana abundance in recent decades, particularly in seasonal ecosystems and dry climatic conditions (Schnitzer and Bongers, 2011; Schnitzer, 2015; Wright et al., 2015). Moreover, some investigations have attributed this recent proliferation to the resource investment strategies of lianas, namely, light capture and growth traits (Asner and Martin, 2012; Wyka et al., 2013). This appears to be a viable investment without the burden of costs associated with self-supporting structures provided by their host tree. Conversely, trees have been reported to invest in traits associated with defence, maintenance, and metabolism (Asner and Martin, 2012; Wyka et al., 2013; Liu et al., 2021).

The aim of this study is to determine whether associations between anatomical and well-established leaf functional traits provide a mechanistic point of view to the trade-off theory of the LES. The associations between different anatomical traits and functional traits are the result of evolutionary and ecological pressures driving patterns along the spectrum as a continuous entity. Here we consider leaf functional traits as those morpho-physiological and structural traits that modulate the fitness of the individual (e.g., growth, reproduction, and survival) (Violle et al., 2007). The leaf anatomical traits studied here, which could also be functional, are focused on fractions of each tissue type and their density (i.e., epidermis, palisade, and spongy mesophyll) based on the cellular organization (i.e., number of cells, cell size, air spaces, and tissue cross area and thickness) in order to normalize the potential differences in leaf size. We attempt to address this by examining the LES using two perspectives: the

spectrum as a single axis (i.e., investment patterns regardless of relatedness or climate) and a comparison of potential end members along the spectrum. Overall, the liana–tree co-existence and their contrasting traits make for a convenient comparison as contrasting endmembers of the spectrum. This is also explored at two different forest types (tropical wet forest and tropical dry forest) as a potential descriptor of environmental drivers that modulate the variation in leaf traits. Specifically, here we address two hypotheses: (i) liana and tree species present contrasting leaf anatomical traits exhibiting of resource acquisitive-conservative (fast-slow) strategies according to the forest type, and (ii) the trade-off investment strategies of the leaf anatomical traits are associated with those described in well-established leaf traits. For the first hypothesis, we expect that liana investment in leaf anatomical traits resemble those related to resource acquisitive strategies (fast), while trees to resource conservative (slow) (List of species **Table 1**). For the second hypothesis, we predict that the variability in leaf anatomical traits have a meaningful correlation, positive or negative, with the variability of well-establish traits, in accordance with investment payoff of a particular trait (**Table 2**). For example, as palisade cell density increases, A_{max} and R_d

should also increase; this is because palisade anatomical traits which have light capture and growth strategies should exhibit positive relationships with A_{max} and R_d because they also exhibit light capture and growth functional strategies; or as spongy mesophyll density decreases nitrogen leaf content should increase because there two sets of traits have opposing strategic investments, maintenance and metabolism vs. light capture and growth, respectively (**Table 2**); The negative association is because there is an inherent energy requirement to maintain the leaf at the cost of resource acquisition ability. The anatomical traits studied here may represent the mechanism by which LES strategies are achieved, improving our understanding of the resource allocation hypothesis embedded in the LES framework.

MATERIALS AND METHODS

Study Area

This research took place at two canopy crane systems in Panama during the rainy season, August 2004: (i) Parque Natural Metropolitano (PNM, 8° 59′ 39.95″ N, 79° 32′ 34.68″ W, 150 m

TABLE 1 | List of species of lianas and trees collected at the Parque Natural Metropolitano and Parque Nacional San Lorenzo, Panama.

Parque Natural Metropolitano		Parque Nacional San Lorenzo	
Family	**Species**	**Family**	**Species**
Lianas			
Aristolochiaceae	*Aristolochia maxima* Jacq.	Apocynaceae	*Forsteronia myriantha* Donn. Sm.
Asteraceae	*Mikania leiostachya* Benth.		*Odontadenia puncticulosa* (Rich.) Pulle.
Bignoniaceae	*Arrabidaea candidans* DC.	Bignoniaceae	*Arrabidaea verrucose* Kranzl.
	Stizophyllum riparium (Kunth) Sandwith.		*Phryganocydia corymbosa* (Vent.) L.G. Lohmann
Convolvulaceae	*Bonamia maripoide* Hallier f.		*Pleonotoma variabilis* (Jacq.) Miers.
	Jacquemontia perryana Robertson.	Celastraceae	*Tontelea ovalifolia* (Miers.) A.C.
Papilionoideae	*Machaerium riparium* Brandegee.	Convolvulaceae	*Maripa panamensis* Hemsl.
Malpighiaceae	*Stigmaphyllon hypargyreum* Triana and Planch.	Dilleniaceae	*Doliocarpus multiflorus* Standl.
Passifloraceae	*Passiflora vitifolia* Triana and Planch.	Papilionoideae	*Dioclea wilsonii* Standl.
Rhamnaceae	*Gouania lupuloides* (L) Urb.		
Sapindaceae	*Serjania mexicana* (L) Willd.		
Vitaceae	*Vitis tiliifolia* Humb. and Bonpl.		
Trees			
Anacardiaceae	*Astronium graveolens* Jacq.	Apocynaceae	*Aspidosperma cruenta* Woodson
Annonaceae	*Annona spraguei* Saff.	Boraginaceae	*Cordia bicolor* A. DC.
Boraginaceae	*Cordia alliodora* (Ruiz and Pav.) Oken.	Clusiaceae	*Marila laxiflora* Rusby.
Tiliaceae	*Luehea seemannii* Triana and Planch.	Caesalpinioideae	*Tachigali versicolor* Standl. and L.O. Williams.
		Papilionoideae	*Lonchocarpus longifolium* Pitter.
		Meliaceae	*Carapa guianensis* Aubl.
		Moraceae	*Brosimum utile* (Kunth) Oken.
			Ficus insipida Willd.
			Ficus nymphaeifolia Mill.
		Myristicaceae	*Virola surinamensis* (Rol. ex Rottb.) Warb.
		Rubiaceae	*Tocoyena pittieri* Standl.
		Sapindaceae	*Matayba apetala* Radlk.
		Sapotaceae	*Manilkara bidentata* (A. DC.) A. Chev.
		Simaroubaceae	*Simarouba amara* Aubl.
		Urticaceae	*Porouma bicolor* Mart. (Standl.)

TABLE 2 | Functional strategy and primary resource invested of each anatomical tissue layer and LTF.

Tissue	Resource (Carbon, nutrients, and water)	Selective mechanism/functional strategy
Epidermis	Water	Structural and defensive
Palisade	Carbon	Photosynthetic capacity; light capture, and growth
Spongy	Water and gas exchange	Maintenance and metabolism
Air space	Water and gas exchange	Maintenance and metabolism

Trait	Resource (Carbon, nutrients, and water)	Selective mechanism/functional strategy
A_{max}	Carbon	Photosynthetic capacity; light capture and growth
R_{dark}	Carbon	Light capture and growth
N	Nutrients	Light capture and growth
LMA	Carbon	Maintenance and metabolism; light capture and growth; Maintenance and metabolism
LT	Carbon	Structural and defensive; resource capture
LD	Carbon and water	Structural and defensive; resource capture
LL	Carbon and water and nutrients	Structural and defensive; Maintenance and metabolism

Acronyms represent the maximum photosynthetic capacity (A_{max}), dark respiration (R_{dark}), leaf mass area (LMA), leaf thickness (LT), leaf density (LD), and leaf lifespan (LL).

a.s.l.), and (ii) Parque Nacional San Lorenzo (SL, 9° 16′ 51.71″ N, 79° 58′ 28.27″ W, 130 m a.s.l.). The PNM is located in Panama City on the Pacific coast of the Republic of Panama. This site presents a seasonally dry tropical forest with a mean annual temperature of 26.5°C and an annual rainfall of 1740 mm. The PNM contains a 265 ha of a natural reserve with an old secondary forest of 80–150 years with tree heights up to 40 m (Wright et al., 2003). The PNM site has a 42 m standing crane with a 51 m long jib with a suspended cage that can be used to access the top of the canopy. The crane covers approximately 0.85 ha of forest in which more than 60 species of trees and lianas can be reached (Wright et al., 2003). On the other hand, the SL is a wet evergreen forest located on the Caribbean coast of Panama with a mean annual temperature of 25.3°C and an annual rainfall of 3000 mm. This site presents a weak dry season from January to March with rainfalls below 45 mm per month. The SL contains a 12,000 ha of old forest (∼300 years) that is part of the Mesoamerican Biological Corridor (Wright et al., 2003). This site also presents a standing crane of 52 m tall with a 54 m jib that can be used to access the top of the canopy. This crane provides access to 0.92 ha of the forest where more than 240 species of trees and lianas can be found (Wright et al., 2003).

Sample Collection and Leaf Histology

Three individuals (i.e., trees or lianas) were selected to collect three sun leaves from the top of the canopy using both cranes in the wet season of 2004; therefore, nine leaves per species were sampled. Leaves were collected from 40 species in total (**Table 1**). Twelve liana species and four tree species were collected from the

PNM crane, while nine liana species and fifteen tree species were collected from the SL crane. Each leaf sample immediately upon clipping (early morning) was placed in a plastic bag with a moist paper towel and then transferred within 1-h to a dry ice cooler for transportation to a laboratory. Three small sections (5 mm in width and 20 mm in length) were cut from each leaf while avoiding the presence of prominent vascular areas. The cut-out sections were treated for 2 weeks with formalin aceto-alcohol in a vacuum oven. After the 2 weeks, the samples were run through an ethanol processing centre and transferred to a hot paraffin bath for embedding into paraffin blocks. A detailed description of the process can be found in Sánchez-Azofeifa et al. (2009).

Slides were prepared following Bancroft and Cook (1984) histological preparation protocols. On these samples, seven cross-sections were prepared with a Leica microtome from a paraffin block and mounted onto microscope slides. Each cross-section has a thickness of 7 µm and a width and height of 22 by 22 mm. The slides were placed in an oven at 37°C overnight for roughly 10–12 h. The following morning, slides were de-waxed with a series of diluted ethanol concentrations and then soaked in toluene for 2 min. The slides were then submerged in a toluene blue stain for 22 s. Coverslips were placed over stained samples using a DPX mounting medium. Slides were again placed in 37°C oven for 10–12 h to dry and set the DPX. Finally, cross-sectional photos were taken using a Zeiss M1 Axio Imager microscope at 400 times magnification. All samples had a field of view of 450 µm by 450 µm. For each species, nine leaves were mounted, stained, and photographed.

Estimation of Anatomical Metrics and Traits

The cross-section photographs previously obtained were used to derive metrics in order to quantify the anatomy of leaf tissues. Specifically, the used metrics are focused on describing the area, thickness, number of cells, and cell size of four-leaf sections: (i) upper epidermis (EU), (ii) lower epidermis (EL), (iii) palisade mesophyll (MP), and (iv) spongy mesophyll (MS). These anatomical metrics derived from photos that were previously enhanced by colour and contrast and transformed to black and white using ImageJ 1.46 (Schneider et al., 2012). The area and percentage of the blackened and non-blackened region were used to estimate the cell-occupied space and leaf airspace using the "analysed particles" tools. Likewise, the area and thickness of each leaf section were estimated, making a manual selection of each blackened section using the "region of interest" tool. The area and thickness of each section were calculated by taking the average of 5 measurements per sample. On the other hand, the number of cells was estimated using the edge effect rules with a counting frame (Mbf Bioscience, 2015). For this, a counting frame of 3,000 µm² central area was established from the centre of the photograph with a 1,500 µm² buffer zone surrounding the perimeter of the cross-section. Next, each cell at each layer was counted in the selected area. For cells to be included, they needed to be entirely encompassed in the central area and the buffer zone. Once the cell count was established, the number of cells was estimated per mesophyll layer (MP and MS). The

estimated cell count was accomplished by multiplying the total area of the mesophyll layers by the estimates cell counts. Likewise, the individual cell size was then estimated with the cell count results. This was accomplished by dividing the cell counts by the area of the mesophyll layers. To eliminate pseudo-replication bias, the sample mean for the individual (three individuals per species) was used as a descriptor of the anatomical metrics.

Once the anatomical metrics were done, we calculated anatomical relationships between metrics to reduce the collinearity among them for future analysis. In general, these relationships are a group of ratios or fractions that can be used to normalize the variation of anatomical metrics, which could be affected by the leaf size. These anatomical relationships are called hereinafter as leaf anatomical traits. We calculated eight anatomical traits based on their potential significance to describe physiological, structural, and defensive trade-offs. Specifically, we calculated (**Table 3**): (i) palisade area fraction ($f\text{MP}_{area}$), (ii) spongy area fraction ($f\text{MS}_{area}$), (iii) airspace area fraction ($f\text{AIR}_{area}$), (iv) palisade density (ρMP_{cell}; number of cells per μm^2), (v) spongy density (ρMS_{cell}; number of cells per μm^2), (vi) epidermis thickness fraction ($f\text{E}_{thickness}$), (vii) palisade thickness fraction ($f\text{MP}_{thickness}$), and (viii) palisade/spongy cell count ratio ($\text{MP}_{count}/\text{MS}_{count}$). For the following traits, $f\text{MP}_{area}$, $f\text{MS}_{area}$, $f\text{AIR}_{area}$, $f\text{E}_{thickness}$, $f\text{MP}_{thickness}$, and $\text{MP}_{count}/\text{MS}_{count}$ they do not have units because they are fractions. However, area and thickness are measured in μm and cell counts are the number of cells in a given area. Descriptions on how to derive these traits and their potential relevance are described in **Table 3**.

Well-Established Leaf Functional Traits

We used eight well-established LFTs for each species and forest type obtained from published records from Osnas et al. (2018). Specifically, we used: maximum net photosynthetic rate (A_{max}, μmol CO_2 m^{-2} s^{-1}), dark respiration rate (R_{dark}, μmol CO_2 m^{-2} s^{-1}), nitrogen concentration (N, g m^{-2}), and phosphorus concentration (P, g m^{-2}), leaf mass per area (LMA,

g m^{-2}), leaf thickness (LT, mm), leaf density (LD, g cm^{-3}) and leaf lifespan (LL, days). In general, these traits were collected at the same sites under two canopy positions (canopy and understory) from different species, including trees, treelets, lianas, vines, epiphytes, and hemi-epiphytes. For this research, only sun leaves were used to compare against leaf anatomical traits. A detailed description of the data collection and trait calculations can be found at Osnas et al. (2018).

Data Analysis

We first compared the effect of life forms and forest type using MANOVAs at three levels of evaluation: (i) anatomical metrics, (ii) leaf anatomical traits, and (iii) well-established LFTs. For each MANOVA, we extracted the univariate analysis of variance (ANOVA) to describe the effects on each trait. For these analyses, we considered each species as the sample unit using the average of leaves per species, and thus meet the assumption of independent observations. In addition, for these analysis leaf traits variables were initially transformed using Box-Cox or log transformation in order to reach the univariate normality and homogeneity of variance assumptions. The application of the Mahalanobis distance tests did not reveal multivariate outliers at the three levels of evaluation. However, the multivariate normality assumption was not meet in all the cases; probably to the sample size. Therefore, Pillai trace was used as measure of variance given that it is relative robust to multivariate normality deviations, assumptions of homogeneity of the variance–covariance matrices across groups, and unbalanced datasets (Johnson and Field, 1993; Quinn and Keough, 2002). These analyses were performed following the recommendations of Quinn and Keough (2002). On the other hand, we performed bootstrapped pairwise Pearson's correlation analysis between the leaf anatomical traits and the well-established LFTs to see their association. These correlations were first performed using all the species, and then on separate by life forms and forest type. The previous analyses were conducted in R software version 3.6.1 (R Core Team, 2020) using the "manova" and "aov" functions to compute the MANOVAs and ANOVAs, respectively, the "corr.test" function of the *psych* package (Revelle, 2017) to estimate the Pearson correlations and their significance, and the *boot* package for bootstrapping (Canty and Ripley, 2019). Each trait was previously transformed using the logarithmic transformation when the normality of the data was not reached.

RESULTS

Anatomical Metrics

According to the MANOVA, the comparisons of the anatomical metrics between life forms, forest type, and their interactions suggested that the multivariate effect do not contribute to the differences between life forms (Pillai trace$_{(15,21)}$ = 0.55; $p = 0.12$), forest types (Pillai trace$_{(15,21)}$ = 0.56; $p = 0.11$), or the life form $\times$ forest type interaction (Pillai trace$_{(15,21)}$ = 0.41; $p = 0.53$). Despite this, the univariate analyses of these comparisons suggested the several anatomical metrics tend to differ between

TABLE 3 | Description of the leaf anatomical traits.

Leaf anatomical trait	Acronyms	Estimation
Palisade area fraction	$f\text{MP}_{area}$	Palisade cross-section area/total cross-section area
Spongy area fraction	$f\text{MS}_{area}$	Spongy cross-section area/total cross-section area
Air space area fraction	$f\text{AIR}_{area}$	Air space cross-section area/total cross-section area
Palisade cell density	ρMP_{cell}	Palisade cell count/total cross-section area
Spongy cell density	ρMS_{cell}	Spongy cell count/total cross-section area
Epidermis thickness fraction	$f\text{E}_{thickness}$	Epidermis thickness/total cross-section thickness
Palisade thickness fraction	$f\text{MP}_{thickness}$	Palisade thickness/total cross-section thickness
Palisade/Spongy cell count ratio	$\text{MP}_{count}/\text{MS}_{count}$	Palisade cell count/Spongy cell count

life forms as well as forest types (**Table 4**). Between life forms specially, the results revealed that liana leaves tend to have less cross-sectional area and are thinner than trees. Likewise, lianas presented lower palisade cross-section area and thickness than trees. However, at the cellular level, lianas seemed to have larger cells and fewer cells in the palisade and spongy leaf tissues. Between forest types on the other hand, species at PNM showed leaves with the less cross-sectional area and thinner that species at SL. Species at SL seemed to have higher spongy cross-section area and thickness than species at PNM. Likewise, the cross-section area of airspaces and the cell count of the spongy leaf tissue seemed to be higher in species at SL than PNM. The univariate comparisons did not reveal an effect of the interaction of life form and forest type in any anatomical metric.

Leaf Anatomical Traits

The comparisons on the anatomical traits between life forms, forest type, and their interactions using a MANOVA revealed that these traits tend to be affected by life form (Pillai trace$_{(8,28)}$ = 0.46; p = 0.01), but not by the forest type (Pillai trace$_{(8,28)}$ = 0.26; p = 0.31) or interaction of life form and forest type (Pillai trace$_{(8,28)}$ = 0.22; p = 0.45). The univariate comparisons showed that only ρMP_{cell} and ρMS_{cell} were affected by the life forms (**Table 5**), where lianas tended to present higher values than trees (**Figures 1D,E**). Likewise, the ρMS_{cell} is the only trait that seemed to be affected by forest type, where species at PNM presented higher values than species at SL (**Figure 1E**). Overall, lianas seemed to show higher values of fMS_{area}, $fAIR_{area}$, and $fE_{thickness}$ and lower values of fMP_{area}, $fMP_{thickness}$, and MP_{count}/MS_{count} than trees (**Figures 1A–C,F–H**); however, these differences did not appear to be statistically supported (**Table 5**).

TABLE 5 | Results of the ANOVA comparison of the effect of life forms, forest type, and their interaction on the derived leaf anatomical traits.

Traits	ANOVA		
	Life form	Forest type	Life form × Forest type
fMP_{area}	0.19	1.51	1.38
fMS_{area}	0.04	3.28	0.82
$fAIR_{area}$	0.18	3.38	2.37
ρMP_{cell}	11.59**	1.25	1.19
ρMS_{cell}	23.87***	6.10*	0.11
$fE_{thickness}$	2.56	2.26	0.56
$fMP_{thickness}$	0.67	3.84	0.25
MP_{count}/MS_{count}	0.02	1.37	0.06

The ANOVA results represented by the F-ratios and the asterisks represent their significance: * p < 0.05; ** p < 0.01; *** p < 0.001.

Well-Established Leaf Functional Traits

On the other hand, the comparisons of LFT between life forms, forest type, and their interactions revealed that the LFT were affected by the life forms (Pillai trace$_{(8,21)}$ = 0.66; p < 0.01), forest type (Pillai trace$_{(8,21)}$ = 0.69; p < 0.001), and their interaction (Pillai trace$_{(8,21)}$ = 0.58; p < 0.05). The univariate effects of this analysis suggest that only A_{max}, N, P, LMA, LD, and LL were affected by life forms, where lianas showed lower values than trees (**Table 6**). Likewise, the univariate effect of this analysis suggested that only P, LMA, and LT were affected by the forest type, where species at PNM showed higher values of P and lower values of LMA and LT than species at SL (**Table 6**). Despite the presence of multivariate interaction, the univariate analysis did not reveal statistically significant interaction within LFTs.

TABLE 4 | Comparison of anatomical metrics between lianas and trees summarized by forest type: seasonally dry forest (Parque Natural Metropolitano) and wet forest (Parque Nacional San Lorenzo).

Metrics	Region or tissue	Parque Natural Metropolitano		Parque Nacional San Lorenzo		ANOVA		
		Lianas	Trees	Lianas	Trees	Life form	Site	Interaction
Area (μm²)	Leaf	51945.51 ± 9073.22	52971.91 ± 12256.89	69092.59 ± 15151.32	78999.87 ± 24544.74	5.74*	13.88***	0.32
	EU	6970.79 ± 2345.18	8518.13 ± 3355.51	8807.57 ± 3892.54	9988.36 ± 4022.03	3.42	2.29	0.06
	MP	19262.97 ± 4516.09	23051.87 ± 5863.77	24719.91 ± 11031.43	28319.36 ± 9080.87	5.93*	3.57	0.01
	MS	17481.74 ± 4691.01	16304.92 ± 6353.04	25404.92 ± 11373.29	29189.57 ± 10778.74	3.33	11.56**	0.93
	EL	5205.21 ± 1263.88	4079.67 ± 871.66	5414.98 ± 1197.84	5478.14 ± 1444.78	0.09	2.14	1.64
	Airspace	3024.80 ± 2624.25	1017.32 ± 1133.68	4745.21 ± 4117.84	6024.45 ± 6443.05	0.04	5.67*	2.21
Thickness (μm)	Leaf	299.93 ± 54.73	306.23 ± 67.97	402.91 ± 77.45	454.37 ± 138.04	5.82*	15.78***	0.22
	EU	19.95 ± 6.75	22.54 ± 9.14	25.32 ± 11.24	23.28 ± 7.90	0.20	1.64	0.50
	MP	55.94 ± 13.09	65.25 ± 7.87	66.58 ± 28.22	81.30 ± 22.71	8.94**	2.65	0.08
	MS	58.65 ± 20.92	53.05 ± 27.65	93.58 ± 37.85	101.73 ± 47.94	1.70	14.08***	0.52
	EL	13.88 ± 3.90	12.00 ± 2.58	15.99 ± 4.50	14.77 ± 4.47	0.22	2.63	0.05
Cell size (μm²)	MP	261.75 ± 58.25	228.40 ± 99.92	353.89 ± 161.02	214.46 ± 81.50	7.40*	0.95	0.98
	MS	202.70 ± 41.91	135.09 ± 29.23	207.67 ± 60.27	164.57 ± 37.92	10.68**	0.84	0.58
Cell count	MP	80.57 ± 29.48	113.17 ± 37.46	98.79 ± 85.34	150.46 ± 67.17	10.28**	0.12	0.46
	MS	91.28 ± 27.25	130.14 ± 55.90	130.22 ± 53.43	179.90 ± 50.64	17.05***	7.25*	0.11

The regions or tissues refers to the upper epidermis (EU), lower epidermis (EL), palisade mesophyll (MP), and spongy mesophyll (MS). Leaf and airspaces refer to regions occupied or not occupied by cells in the leaves.
The ANOVA values are represented by the F-ratios and the asterisks represent their significance: * p < 0.05; ** p < 0.01; *** p < 0.001.
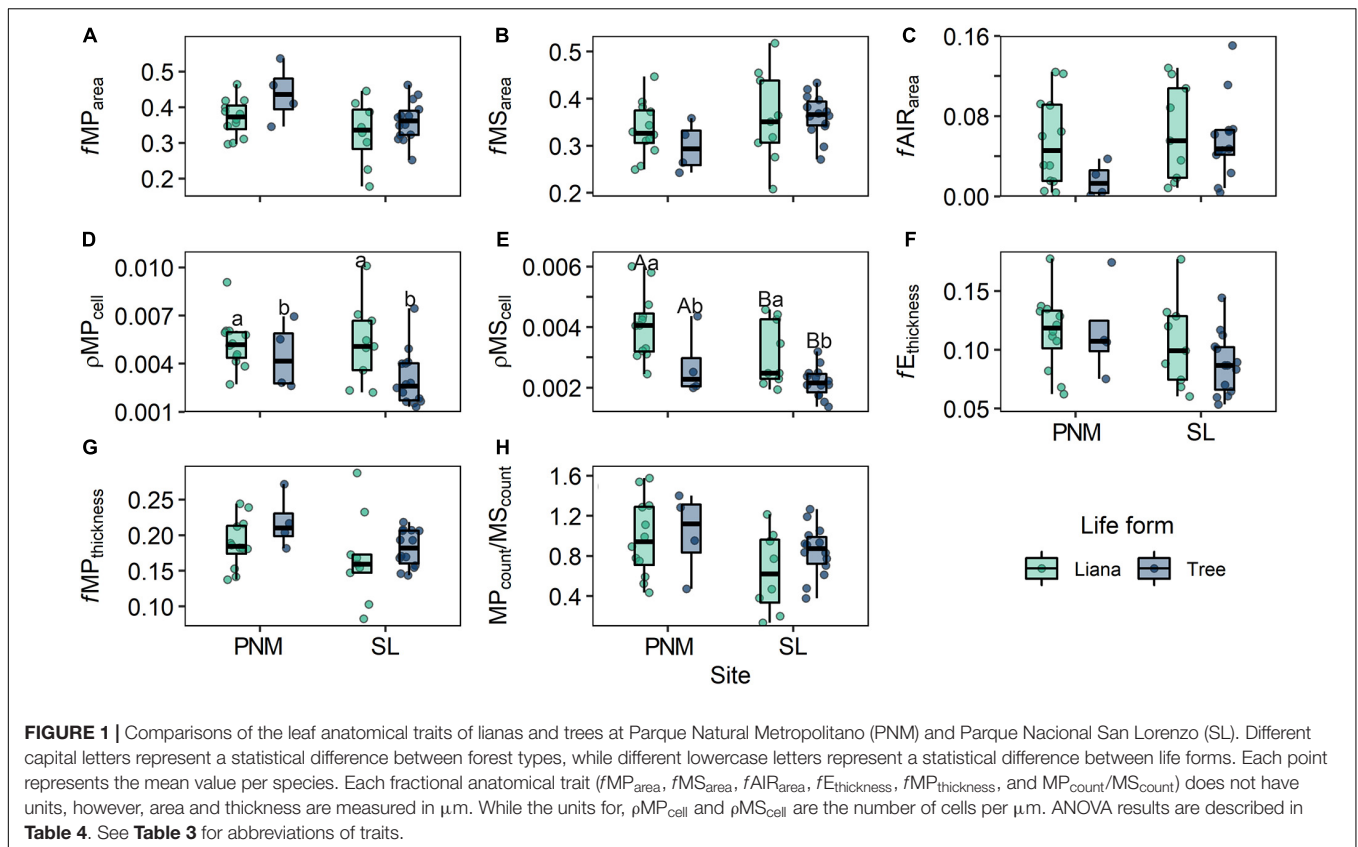
FIGURE 1 | Comparisons of the leaf anatomical traits of lianas and trees at Parque Natural Metropolitano (PNM) and Parque Nacional San Lorenzo (SL). Different capital letters represent a statistical difference between forest types, while different lowercase letters represent a statistical difference between life forms. Each point represents the mean value per species. Each fractional anatomical trait (fMP$_{area}$, fMS$_{area}$, fAIR$_{area}$, fE$_{thickness}$, fMP$_{thickness}$, and MP$_{count}$/MS$_{count}$) does not have units, however, area and thickness are measured in μm. While the units for, ρMP$_{cell}$ and ρMS$_{cell}$ are the number of cells per μm. ANOVA results are described in **Table 4**. See **Table 3** for abbreviations of traits.

TABLE 6 | Comparison of the well-established leaf traits between lianas and trees summarized by forest type: seasonally dry forest, Parque Natural Metropolitano, and wet forest, Parque Nacional San Lorenzo.

Traits	Parque Natural Metropolitano		Parque Nacional San Lorenzo		ANOVA		
	Lianas	Trees	Lianas	Trees	Life form	Site	Interaction
A_{max}	9.84 ± 3.25	16.43 ± 3.13	13.92 ± 2.26	14.95 ± 6.38	5.17*	0.65	2.54
R_{dark} (10^{-2})	1.02 ± 0.45	1.35 ± 1.50	1.22 ± 0.49	0.89 ± 0.57	0.23	0.36	1.60
N (10^{-2})	1.47 ± 0.38	1.99 ± 0.57	1.51 ± 0.23	2.02 ± 0.48	16.58***	0.18	0.31
P (10^{-2})	0.10 ± 0.02	0.14 ± 0.03	0.08 ± 0.02	0.11 ± 0.04	16.17***	8.04**	0.31
LMA	58.02 ± 17.45	82.26 ± 16.42	85.16 ± 13.78	120.15 ± 29.66	28.84***	8.64**	0.06
LT	0.23 ± 0.07	0.20 ± 0.06	0.28 ± 0.07	0.31 ± 0.08	2.22	5.63*	0.72
LD	0.27 ± 0.05	0.48 ± 0.16	0.32 ± 0.09	0.40 ± 0.08	16.44***	0.01	3.74
LL	146.09 ± 53.41	212.00 ± 42.98	286.00 ± 115.98	318.75 ± 136.90	6.18*	10.54**	0.76

*The ANOVA values are represented by the F-ratios and the asterisks represent their significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.*

Association Between Anatomical Traits and Well-Established Leaf Functional Traits

The correlations revealed that some leaf anatomical traits were associated with well-established traits (**Figure 2A**); however, the presence or degree of correlations seemed to be dependent on the life forms and forest type (**Figures 2B,C**). For instance, reductions in ρMP$_{cell}$, ρMS$_{cell}$, and fE$_{thickness}$ seemed to be associated with increases in LMA regardless of the life form or forest type (**Figure 2A**). Likewise, increases in fE$_{thickness}$ tended to be correlated with reductions in LD and LL. Increases in

ρMP$_{cell}$ also tended to be associated with reductions in N, while increases in ρMS$_{cell}$ tended to be correlated with reductions of A_{max} and LT. Between life forms (**Figure 2B**), fMP$_{area}$ and fMP$_{thickness}$ were positively correlated with N, P, and LD in trees, but not in lianas. On the other hand, in liana species increases in ρMP$_{cell}$, ρMS$_{cell}$, and fE$_{thickness}$ seemed to be correlated with reductions in N, LT, and LD, respectively, but not in trees species. When comparing forest types, the correlations revealed that reductions in ρMP$_{cell}$, ρMS$_{cell}$ of PNM species seemed to be associated with increases in LT, but not for SL species. Likewise, increases of fMP$_{area}$ of PNM species tended to be correlated with increases in LT. In SL species, reductions in fE$_{thickness}$ seemed
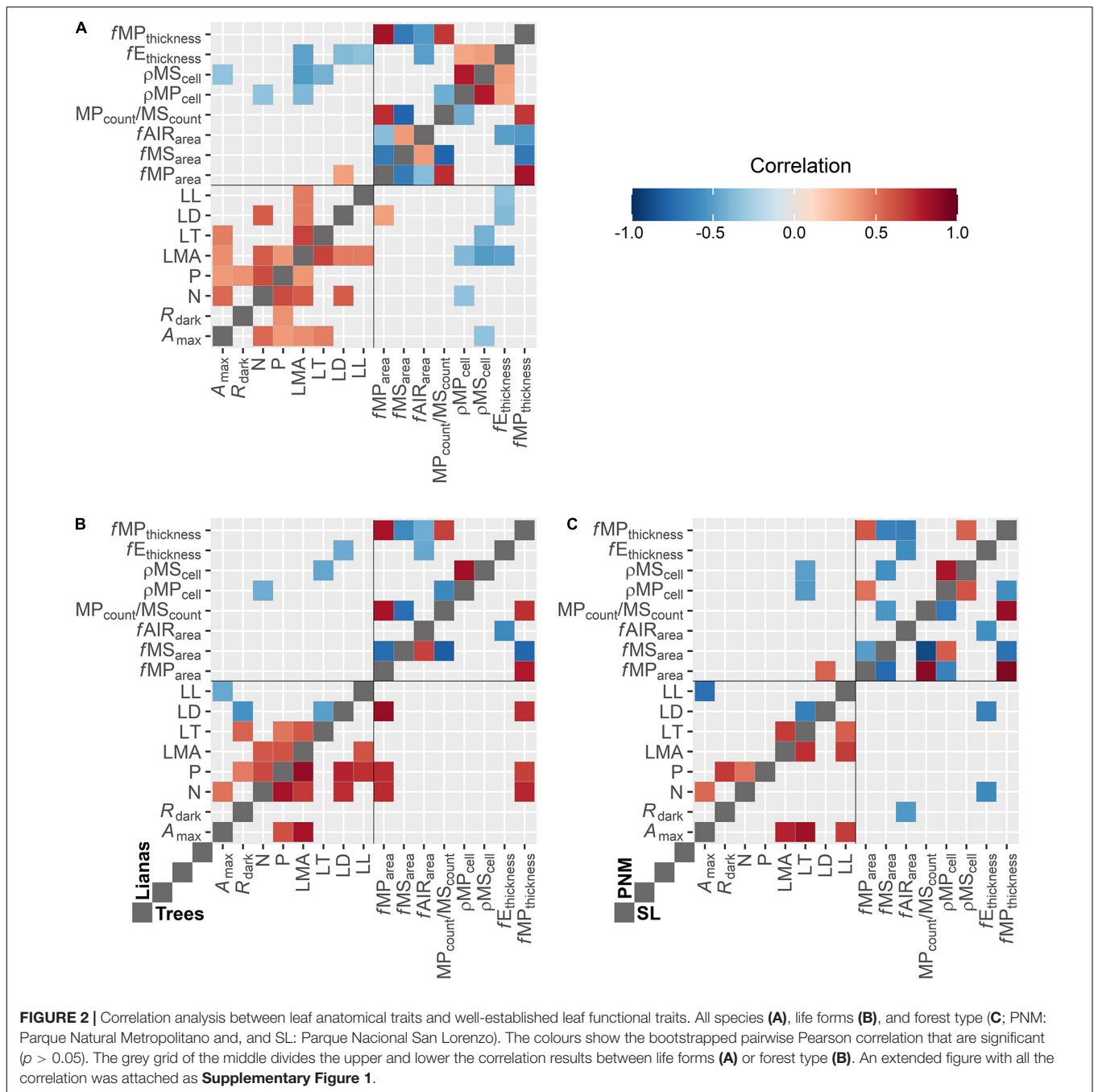
FIGURE 2 | Correlation analysis between leaf anatomical traits and well-established leaf functional traits. All species **(A)**, life forms **(B)**, and forest type **(C**; PNM: Parque Natural Metropolitano and, and SL: Parque Nacional San Lorenzo). The colours show the bootstrapped pairwise Pearson correlation that are significant (*p* > 0.05). The grey grid of the middle divides the upper and lower the correlation results between life forms **(A)** or forest type **(B)**. An extended figure with all the correlation was attached as **Supplementary Figure 1**.

to be associated with increases in N and LD, while reductions in fAIR$_{area}$ seemed to be associated with increases in R_{dark}. A complete figure with all the correlations (i.e., significant or not) was added in **Supplementary Figure 1**.

DISCUSSION

This research reveals several anatomical traits that tend to be contrasting between life forms and forest types as well as the mechanics that drive some of the well-established LFTs. Here,

we focus on highlighting the interactions between anatomical traits and LFT, and how these differences reveal potential trade-off strategies between species and environments. Albeit the discussion regarding anatomy and LES has been had before, our finding advances this argument in that are cell and tissue mechanisms associated with morpho-physiological trait investment strategies. Moreover, our results also have merit to the idea that leaf anatomical traits, as an additional scientific exploration tool, can help us better understand the functional ecology of species. From these findings, we argue that anatomical traits could complement the framework of the LES.

End of Spectrum Case Study Comparison- Growth Form and Site

The objective of the endmember perspective of this investigation was to compare groups of species that exhibit fast vs. slow functional strategies. Therefore, our first hypothesis was supported by the association between anatomical traits and LFT may depend on the life forms and forest type. For example, species with higher LMA seem to be associated with reductions in ρMP_{cell}, ρMS_{cell}, and $fMP_{thickness}$, but this association is not observed when comparing life forms or forest types. Despite this, on some occasions, ρMP_{cell} and ρMS_{cell} and appear to have a negative association with the variation of LD and LT in lianas, and both forest types. These correlations interestingly mirror the breakdown of LMA as the product of LD and LT (LMA = Leaf volume area × Leaf density) (Poorter et al., 2009).

Trade-offs in investment strategies such as the conservative-acquisitive trade-off hypothesis between lianas and trees (Asner and Martin, 2012; Wyka et al., 2013) could be dissected with the correlative exploration between leaf anatomical traits and the well-established LFT. For example, trees tend to have more in N and P per unit leaf area and higher LD as a conservative strategy. These LFTs mentioned above seem to be positively correlated with fMP_{area} and $fMP_{thickness}$; probably to boost carbon assimilation for woody structures. On the other hand, with lower metabolic demand (compared to trees) of support structures, lianas traits are generally related to acquisitive strategies with low investment in nutrients and structures for fast growth and high light capture. This strategy appears to be reflected in the increasing cellular infrastructure (ρMP_{cell}, ρMS_{cell}, and $fE_{thickness}$) with high turnover of nutrients as investment and leaf volume demands (LT and LD). In addition, trade-offs in investment strategies between forest types may also reveal how species modify their leaf anatomy to deal with contrasting rainfall regimes. For example, it appears that species at the PNM tend to increase the density of palisade and spongy cells with lower LT which would suggest a loss of airspace. This strategy of dry-forest species may prevent to break the water tension between cells avoiding the leaf hydraulic failure. A negative pressure is formed between mesophyll cells when small menisci of water are formed through adhesion to the outside of the spongy mesophyll cells creating tension and suction as water is lost through the stomata. Conversely, species at the wet-forest seem to increase $fAIR_{area}$; to be more metabolically efficient to reduce of R_{dark}.

The LES as a Continuous Single Axis- an Anatomical Perspective

There is convincing evidence for leaf investment strategies along the LES to exhibit the same patterning of trait correlation across taxonomy and climatic gradients (Wright et al., 2004). The LES maps these universal patterns in which traits that are not energetically unfavourable are lost and thus allows for the LES to be placed along single axis. Our second perspective to the LES takes a closer look at these patterns and dissects the compositional origin. Broadly speaking, we find cellular mechanisms and organization associated with the function of a particular tissue. The anatomical traits that seem to have the most casual impacts are mesophyll densities and epidermal thickness (ρMP_{cell}, ρMS_{cell} and $fE_{thickness}$). For example, decreases in ρMP_{cell}, ρMS_{cell}, and $fE_{thickness}$ were found to be associated with an increase in LMA (**Figure 2**). This cellular structure demonstrates the trade off between metabolic function and the structural integrity of the leaf. Although LMA is thought to be a black box in the literature, John et al. (2017) complementarily found that an increase in cell size (thus decrease in density) drives greater LMA. Moreover, other compositional studies have found a breakdown of LMA by anatomy to be affected equally by protoplast and cell wall components (metabolism and structure, respectively; Shipley et al., 2006; John et al., 2017). A more specific example can be seen in the negative correlation between ρMP_{cell} and N. Given that nitrogen is an important component of chloroplast, more dense palisade results in lower N and thus there is a restriction on photosynthetic capacity. This is supported by Pyankov et al. (1999), who found that mesophyll size and numbers were negatively correlated and that leaves with large cells have more chloroplasts per cell.

When comparing investment strategies types, we found support for our second hypothesis. We found higher epidermal thickness with lower LD and LL. These correlations reflect the carbon allocation investment costs of structural defence. The cuticle and epidermis are considered the first line of defence in plants, this trade off can be seen regardless of phylogenetic and environmental factors. Simply put, this pattern arose from blanketed selective pressures on plants as stationary organisms and the of cost of protection. Moreover, Flexas et al. (2013) have found that across diverse range of species mesophyll conductance is strongly linked to gas exchange traits, stomatal conductance and leaf vein density. Supporting the idea that at the foliar traits can be linked to tissue-level dissection of these traits and there is evolution consistency in these trends.

Moreover, within the spongy mesophyll we found negative relationships between ρMS_{cell} and A_{max} which supports the trade-off hypothesis of different functional strategies (maintenance and metabolism against light capture and growth traits) (**Table 2**). Increasing spongy mesophyll density with lower A_{max} is indicative of increased gas diffusion resistance. Less intracellular space reduces the diffusion capacity of the spongy mesophyll and this shows photosynthesis capacity's limitation associated with the rate of gas diffusion. Liu et al. (2019) found high dry matter content with low SLA suggesting an increase diffusion resistance. Our findings take this one step further and show the allocation of spongy mesophyll tissue to come at a cost of decreased transpiration and photosynthetic capacity despite the wide variability around life form and site type. The application to the LES spectrum is that photosynthetic capacity is limited by its reactants' and products' the distance travelled and rate of travel across the spongy anatomy, quite literally, it is subjected to fast-slow continuum.

Future Perspectives of Leaf Anatomical Traits

Currently, there are a few studies that address the role of leaf anatomical traits on the functional ecology of the species

or communities, however, they seem to focus on only snap-shot perspectives of the LES; for instance solely discussing the usefulness of LMA (Pyankov et al., 1999; John et al., 2017), or finding a weak correlation between anatomical traits and economic and hydraulic traits (Liu et al., 2019).

The LES was a paradigm shift in plant ecology. This study provides further justification to add anatomical traits to the LES, however, we only focused on leaf traits. The future of this research should focus on anatomical deconstruction at all levels to match the grandiose nature of the LES: cellular, tissue, organ, and systems as well as root, stem, and leaf. Moreover, it seems clear that the degree of correlation of the anatomical traits depends on the different functional groups and environments. Therefore, the differentiation of the co-variation of anatomical traits between species, functional groups, or communities could reflect the different trade-off strategies of species in face to a given environment. Based on this, the use of anatomical decomposition of leaves may provide a deeper understanding of the leaf economy variation along ecological evolutionary and climatic gradients.

CONCLUSION

The discussion of traits as an explanation to the trade-off between growth and survival is long and continuous. Moreover, the discussion of including anatomy has been recited before, we have pushed this discussion in a direction that shows that leaf anatomy plays specific roles between growth and survival. The novel approach in this investigation was focusing on the LES as a spectrum, in a continuous vs. endmember dissection of the covariances of LFT and anatomy. These covariances are a result of ecological and evolutionary selective pressures. We found mesophyll density and epidermal thickness (ρMP_{cell}, ρMS_{cell}, and $fE_{thickness}$) have a more casual impact when summarizing the LES along singular axis. Mesophyll density is the proverbial stock to the currency of photosynthetic capacity traits as the currencies and epidermal thickness come at a cost of longevity and density. Such trade offs reflect unifying selective pressures along the LES: i-maintenance and metabolism for light capture and growth and ii- structure and defence for maintenance and metabolism, respectively. In contrast, our endmember case study found a group of traits selected for a specific life history. For example, lianas, resource acquisitive or at the "fast" end of the spectrum show positive covariance strategies both favourable to growth such as increase nutrients in their palisade mesophyll. The results where water was the comparative metric demonstrated that when resource was limited (water;

PMN) the anatomy reflected this source of selective pressure by reducing thickness and ultimately airspaces. Future studies should investigate deeper in the harmonization of anatomical traits to better explain the leaf anatomy and current LFTs. Likewise, future studies should address the leaf anatomical trait co-variation of different taxonomic groups and how these are adapted to different environments.

DATA AVAILABILITY STATEMENT

Data collected by the authors of leaf anatomical metrics and traits per species and forest types are available at the Tropi-Dry dataverse through https://doi.org/10.7910/DVN/ESLKNT.

AUTHOR CONTRIBUTIONS

GS-A performed the leaf collection, helped to the interpretation of the results, editing of the manuscript, and got the funding. DH carried out the leaf histology and the measurements of anatomical metrics. JG analysed the data and performed the Figures. DH and JG drafted the manuscript. All the authors agreed with the final version of the manuscript.

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