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Intraspecific variation in leaf traits facilitates the occurrence of trees at the Amazonia–Cerrado transition

Igor Araújo [a,*], Beatriz S. Marimon [a,b], Marina C. Scalon [a,c], Wesley J.A. Cruz [a], Sophie Fauset [d], Tiffani C.S. Vieira [e], David R. Galbraith [f], Manuel U. Gloor [f]

[a] Programa de Pós-Graduação em Ecologia e Conservação, Universidade do Estado de Mato Grosso, Campus Nova Xavantina, Nova Xavantina, MT, Brazil
[b] Universidade do Estado de Mato Grosso, Departamento de Ciências Biológicas, Nova Xavantina, Brazil
[c] Programa de Pós-Graduação em Ecologia e Conservação, Universidade Federal do Paraná, Curitiba, PR, Brazil
[d] University of Plymouth, School of Geography, Earth and Environmental Sciences, Plymouth, Devon, United Kingdom
[e] Academic of the degree course in Biological Sciences, Universidade do Estado de Mato Grosso, Campus Nova Xavantina, Nova Xavantina, MT, Brazil
[f] School of Geography, University of Leeds, Leeds, LS2 9JT, UK

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ABSTRACT

The ability of plant species to adjust key functional traits through intraspecific variation may determine their success in persisting on our planet in the future, especially in unstable habitats, such as the Amazonia–Cerrado transition zone. We assessed intraspecific variation in 12 leaf morphological and anatomical traits for four tree species along a savanna–forest gradient, including rocky *cerrado*, typical *cerrado* and *woodland savanna*. Generally, all evaluated species showed great intraspecific variation. Our findings demonstrate that trees occurring in the *woodland savanna* are potentially more vulnerable to climate change, while in the *cerrado* the individuals presented higher tolerance to water deficit and high temperatures. Trees occurring in open-canopy habitats showed smaller stomata, higher stomata and trichome densities, compared to the same species growing in the *woodland savanna*. In contrast, the individuals in the *woodland savanna* shift leaf traits to increase resource acquisition (e.g. light), showing higher specific leaf area and larger stomata, compared to *cerrado* individuals. We have shown that vegetation-induced shifts in leaf morphological and anatomical traits are a major effect in within-species variability, with consequences for persistence and tolerance of species under future climatic conditions.

1. Introduction

Current and future climate change are among the main threats to ecosystems globally (Yu et al., 2019), and it is expected that their impacts and direct consequences will lead to global losses in biodiversity, carbon stocks and ecosystem services (Allen et al., 2010; Collins et al., 2013; Law, 2014; IPCC, 2019; Araújo et al., 2021). The forecast is for increased mortality risk of species across biomes (Van Mantgem et al., 2007; Phillips et al., 2009; Peng et al., 2011; McDowell, 2018), which are already sensitive to the current climate and may be even more vulnerable in the future (Araújo et al., 2021). Climate has changed enough to exceed the physiological limits to which some plants are adapted (Shaw and Etterson, 2012; Araújo et al., 2021) and led to negative effects that compromise survival and persistence of species over time (Becklin et al., 2016). In this context, even greater concerns arise regarding the ability of species to adapt quickly to climate change (Burrows et al., 2011; Dawson et al., 2011; Hoffmann and Sgrò, 2011). This is especially so for plants, which are sessile organisms with a long lifespan. Thus, trees will need flexibility during their lifetime to deal with rapid environmental and climatic changes, unrelated to genetic changes, and therefore intraspecific variation and phenotypic plasticity can be decisive for the persistence of trees and thus the risk of extinction due to future climate change (Nicotra et al., 2010; Benito Garzón et al., 2011). In this context, functional traits have advanced our understanding of tree physiology, community structure and ecosystem functioning (Wright et al., 2004; De la Riva et al., 2015; Volf et al., 2016).

Recent studies have integrated the variation of intraspecific characteristics in functional traits to investigate the responses of trees along environmental gradients (Laforest-Lapointe et al., 2014; Siefert et al., 2015). Intraspecific variation allows trees to establish under new

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environmental conditions (Joshi et al., 2001; Byars et al., 2007; Maracahipes et al., 2018). In particular, environmental variation induces changes in the functional traits of trees (Anderson and Gezon, 2015; Lázaro-Nogal et al., 2015; Niinemets, 2016), at morphological (Capuzzo et al., 2012), anatomical (Rossatto and Kolb, 2010, 2012; De Paula et al., 2018) and physiological levels (Rossatto et al., 2010). This scenario highlights the set of functional traits that allow plants to survive in contrasting environments has fundamental consequences for our understanding of the dynamics of vegetation, especially in highly complex and hyperdiverse areas such as savanna–forest transitions (Marimon et al., 2014; Oliveras and Yadvinder, 2016).

The Amazonia–Cerrado transition is a natural laboratory in the open, as it presents a marked gradient (savanna–forest) with high diversity of species and contrasting environmental aspects (Marimon-Junior and Haridasan, 2005; Marimon et al., 2014; Araújo et al., 2021), that allow investigating the relationships between functional traits and the environment, providing a step forward to better understand and predict ecological patterns in a changing environment (Hoffmann et al., 2005; Maracahipes et al., 2018). Savannas have low soil fertility, lower air humidity, high temperatures and light intensity, seasonal droughts and frequent fires (Marimon-Junior and Haridasan, 2005; Reis et al., 2015) and can favour trees with conservative resource use strategies (Pausas et al., 2017; Maracahipes et al., 2018) that tolerate such environmental conditions, reflected in leaf traits such as the high leaf water content to avoid desiccation (Bündchen et al., 2015), long petioles to reduce leaf surface temperature preventing thermal leaf damage (Yates et al., 2010), thick leaves to reduce leaf damage (Dahlgren et al., 2006; Rossatto et al., 2010). In addition, savanna-adapted species can display a set of anatomical traits to prevent excessive water loss such as the high density of trichomes and stomata (Gianoli and Gonzalez-Teuber, 2005; Galmés et al., 2007) and also smaller stomata sizes and maximum opening of the stomata pore that benefits more efficient control in the use of water (Franco, 2002; Rossatto et al., 2009). On the other hand, trees in forests tend to present resource acquisition strategies (Hoffmann et al., 2012), with a combination of functional traits linked to greater uptake of light, water and nutrients. For example, it is expected to find species with high specific leaf area, thin leaves, larger stomata sizes and maximum opening of the stomatal pore (Grime, 1979; Westoby, 1998; Ogburn and Edwards, 2010) that promote higher growth rates.

Although interest in functional traits has grown more and more in plant ecology, in hyperdiverse and complex tropical systems, such as the most diverse transition on the planet between the Amazonia and the Cerrado, we still do not know how the variation in functional traits, especially the anatomical traits (e.g., stomata density, stomata size and maximum opening of the stomatal pore) differ between ecological scales, such as vegetation, species and individuals (Albert et al., 2010; Messier et al., 2010; Salazar et al., 2018). This fact has made it difficult to determine the scale-dependent aspects of the variation in functional traits (Messier et al., 2010). At the individual level, the variability in the functional traits of trees is influenced by climatic drivers (e.g., temperature and precipitation) (Fajardo and Piper, 2011; Richardson et al., 2013). In addition, soil texture, successional stage and disturbance regimes can also contribute to this variation (Salazar et al., 2018). In terms of vegetation, the availability of water and nutrients, soil compaction, salinity and microclimate variations can induce variations in traits (Messier et al., 2010). However, at the species level, the variation may result from interspecific competition for resources such as water, light and nutrients (Schemske et al., 2009; Albert et al., 2010; Messier et al., 2010). As far as we know, no research has used a wide scan of leaf anatomical traits to measure the change in traits on a series of ecological scales. Knowing which scales have the greatest variation in functional traits will provide important explanations about the causes of variability in functional traits and may contribute to subsidies on patterns and processes on spatial and temporal scales that are ecologically more significant (McGill et al., 2006).

In this study we report how the variation in leaf functional traits (e.

g., morphological and anatomical) changes in three nested ecological scales (vegetations, species and individuals) along a savanna–forest gradient in the Amazonia–Cerrado transition, which is making it increasingly dry and hot and where some species can already be affected by climate change (Tiwari et al., 2020; Araújo et al., 2021). In addition, we have introduced little-explored valuable information on the variability of intraspecific characteristics in leaf functional traits of co-occurring trees in contrasting environments in tropical systems. We test two hypotheses: (1) among ecological scales, vegetation will contribute to greater variation in leaf traits, since the distinct habitats present contrasting environmental aspects, as shown in Marimon-Junior and Haridasan (2005) and Figure S1; (2) species have significant intraspecific variability in leaf functional traits irrespective of the habitat. We expected smaller and thicker leaves with higher trichome density in habitats under higher incidence of light, high temperature, and low water availability. On the other hand, larger and thinner leaves with larger stomata will be found in *woodland savanna* vegetation, a habitat with lower light incidence, lower temperature, and higher water availability (Rossatto et al., 2010; Rossatto and Kolb, 2010, 2012; Capuzzo et al., 2012; Reich et al., 2014; Souza et al., 2015).

2. Materials and methods

2.1. Study area and species description

We carried out the study at the Bacaba Municipal Park (BMP), a conservation unit of approximately 500 ha, in the municipality of Nova Xavantina-MT, Brazil (Fig. 1), located in the transition region between the Amazonia and Cerrado biomes (Marimon-Junior and Haridasan, 2005; Marimon et al., 2014). The climate shows marked seasonality with two well-defined periods, one rainy (October to March) and the other dry (April to September; Alvares et al., 2013), with average annual rainfall of 1.500 mm, elevation of 340 m (Marimon et al., 2010). Mean monthly temperature is 25 °C, ranging from 18.7 to 33.5 °C, according to data obtained from INMET (Instituto Nacional de Meteorololologia –http://www.inmet.gov.br) considering data from 14 years period (2004–2019).

The typical *cerrado* occupies 153.77 ha of the BMP, is a savanna phytophysiognomy composed of low vegetation cover, with trees spaced from each other, and also presents a dense low stratum (Mews et al., 2011; Gomes et al., 2016). Generally, it occurs on deep, well-drained, dystrophic, acidic Latosols and Neosols (Marimon-Junior and Haridasan, 2005). In contrast, the rocky *cerrado* occupies about 25.44 ha of the BMP area, on shallow soils and with rocky outcrops (Litholic Neosols). In this environment, trees are positioned in the gaps between the rocks, where accumulation and decomposition of organic matter and sand deposition resulting from weathering of the rocks occurs (Maracahipes et al., 2011; Gomes et al., 2016). The rocky *cerrado* has a flora with a high degree of endemism (Alves and Kolbek, 2010) and many endangered species (Mendonça and Lins, 2000). The *woodland savanna* occupies about 162.02 ha of the BMP area, comprising large trees, with thick litter layer (Marimon et al., 1998; Franczak et al., 2011; Reis et al., 2015) and dystrophic soil (Marimon-Junior and Haridasan, 2005).

For our study we selected four co-occurring species (*Hymenaea stigonocarpa* Mart. ex Hayne – Fabaceae, *Qualea parviflora* Mart. – Vochysiaceae, *Vatairea macrocarpa* (Benth.) Ducke – Fabaceae and *Pseudobombax longiflorum* (Mart.) A.Robyns – Malvaceae) in *cerrado* vegetation (rocky *cerrado* and typical *cerrado*) and a *woodland savanna* in BMP. These species have extensive geographic distributions and belong to three important botanical families of the *Cerrado* biome (Ratter et al., 2006), in addition to having high importance value indices locally, in terms of density, frequency and relative dominance (Mews et al., 2011; Reis et al., 2015; Gomes et al., 2016). For each species, we haphazardly selected five individuals per vegetation type, totalling 15 individuals per species.
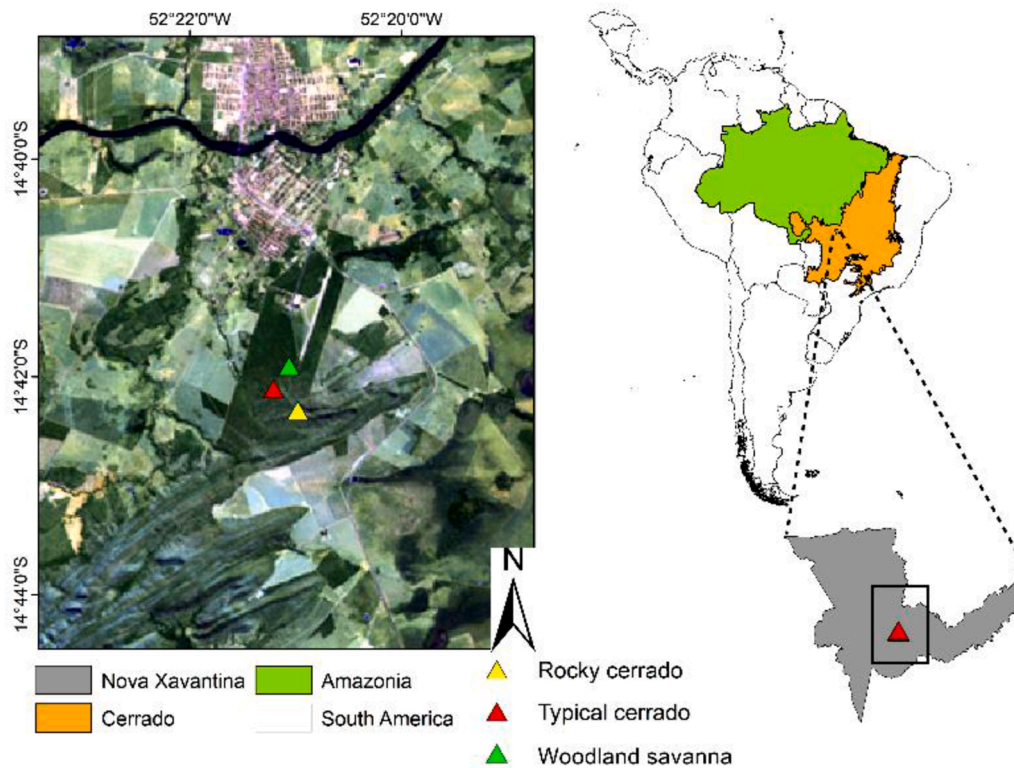
Fig. 1. Neotropical savannas and *woodland savanna* in the Amazonia–Cerrado transition, Brazil, South America.

2.2. Leaf morphological and anatomical parameters

For each individual of each species, we selected eight leaves, five for morphological characterization and three leaves for anatomical

Table 1
Leaf morphological and anatomical traits evaluated for tropical trees in the Amazonia–Cerrado transition. [1] Grime, 1979; [2] Bündchen et al., 2015; [3] Poorter and Bongers, 2006; [4] Turner, 1994; [5] Pallardy, 1981; [6] Gratani et al., 2006; [7] Westoby, 1998; [8] Pearce et al., 2006; [9] Weyers and Meidner, 1990; [10] Ehleringer and Björkman, 1978; [11] Abrams et al., 1994; [12] Rossatto et al., 2009.

Traits	Acronym	Unit	Functional significance
Specific leaf area	SLA	$cm^2 g^{-1}$	Resource uptake, resource use efficiency, structural defense, and growth strategies[1];
Leaf water mass content	LWMC	$mg\ g^{-1}$	Leaf temperature reduction and protection against desiccation[2];
Petiole length	PEL	mm	Light capture and heat dissipation[3];
Leaf thickness	LT	mm	Resource acquisition and use, and resistance to physical damage[4];
Adaxial cuticle thickness	ACT	μm	Water conservation[5];
Adaxial epidermis thickness	AET	μm	Light and heat dissipation[6];
Palisade parenchyma thickness	PPT	μm	Photosynthetic rates[7];
Spongy parenchyma thickness	SPT	μm	Efficient gas exchange[8];
Stomatal density	STO	mm^{-2}	Carbon assimilation and water use efficiency[9];
Trichome density	TRD	mm^{-2}	Preventing the increase in leaf temperature[10];
Stomatal size	STS	μm	Water use efficiency[11];
Maximum opening of the stomatal pore	AMAX	$μm^2$	Prevent water loss[12];

determinations (Table 1). As a standardization criterion, we collected fully expanded leaves, exposed to full sunlight, and free of pathogens (i. e. leaf standardization protocol). We measured leaf thickness, specific leaf area (ratio between leaf area and leaf dry mass) and leaf water mass content for each species. We determined the thickness of the fresh leaves with an electronic digital micrometer and digitized them with a scanner to determine leaf area and calculated the specific leaf area (Abràmoff et al., 2004). We estimated the wet weight of the leaves with a precision balance ($\pm$ 0.001 g), then placed them in paper bags in an oven at 60 °C, and after 72 h determined the dry weight. The leaf water content was calculated as the ratio between wet and dry weights (Pérez-Harguindeguy et al., 2013).

For anatomical characterization, we took imprints of the leaf surface with high-tech molding silicon (Speedex), as proposed by Weyers and Johansen (1985). Subsequently, we used colorless enamel to make the impression of the mold on slides, which was visualized with an optical microscope, with an attached camera, to view stomata and trichomes. We took stomatal size and stomatal density measurements under 10 × microscopic amplification selecting haphazardly 10 fields per leaf. Microscope images were processed with the ImageJ software (Abràmoff et al., 2004).

We calculated stomatal density, for each individual, as the average number of stomata counted in the same fields of view recorded previously, and then we estimated the average stomatal densities, lengths, and widths per species, measuring 25 stomatal complexes from 3 leaves per individual. We measured the length of the guard cell ("L" in μm), the width of the pair of guard cells ("W", μm), the size of the stomata ("S", estimated as S = L * W, according to Franks et al., 2009, 2012) and the maximum area of the stomatal pore ("a_{max}", $μm^2$). We calculated the maximum area of the stomatal pore as $a_{max} = \alpha * S$, $\alpha = 0.12$ (Franks and Beerling, 2009). We determined the trichome density (when present) as the average number of trichomes counted in the same fields of view recorded previously and estimated the densities.

For each of the three leaves described above, we took 2 cm × 2 cm sample from the median portion of the leaf blade and used a freehand

cross-section. We fixed the leaf fragment on a half-open petiole (*Cecropia* sp.) and cut it freehand with the help of a razor blade. Afterwards, we stored the samples in Petri dishes and with the aid of a Pasteur pipette we added 3 ml of sodium hypochlorite, and we waited for 5 to 10 min until the samples became translucent. Then, we rinsed three times with distilled water and stained the samples with a solution of 50% methylene blue and 50% safranin. We photographed the slides with a camera attached to an optical microscope, haphazardly selecting 10 fields from each leaf with a 10 × magnification objective to assess the thickness of the adaxial cuticle, adaxial epidermis, and palisade and spongy parenchyma (Roeser, 1962).

2.3. Data analysis

Before the analysis, we tested for normality and homoscedasticity of the data using the Shapiro-Wilk and Levene tests (Levene, 1961; Shapiro-Wilk, 1965). To compare leaf traits between species and vegetation we used split-plot ANOVAs, where species and the interaction between species and vegetation were nested within vegetation. We used the lmer function from nlme package (Pinheiro et al., 2017) and lsmeans (Lenth and Lenth, 2018) and multcomp (Hothorn et al., 2008) packages for the post-hoc analyses. To test whether humidity and air temperature and the photosynthetic photon flux density (PPFD) were different between vegetation types, we performed univariate analysis of variance (ANOVA) individually for each variable. Subsequently, we used Tukey post-hoc tests to identify significant differences between specific vegetation types. We also calculated the coefficient of variation for the functional traits to observe which traits are more variable and how they varied in terms of species and vegetation types (Garnier et al., 2001). We further performed a principal component analysis (PCA) to verify which functional traits were associated with vegetation types and species.

To understand controls on trait variability, we used different groupings of mixed linear models, adjusting the separate models for each trait (Rosas et al., 2019). Vegetation types, species, and individuals were introduced as nested random factors to assess how the variability of functional traits was distributed among these different levels of
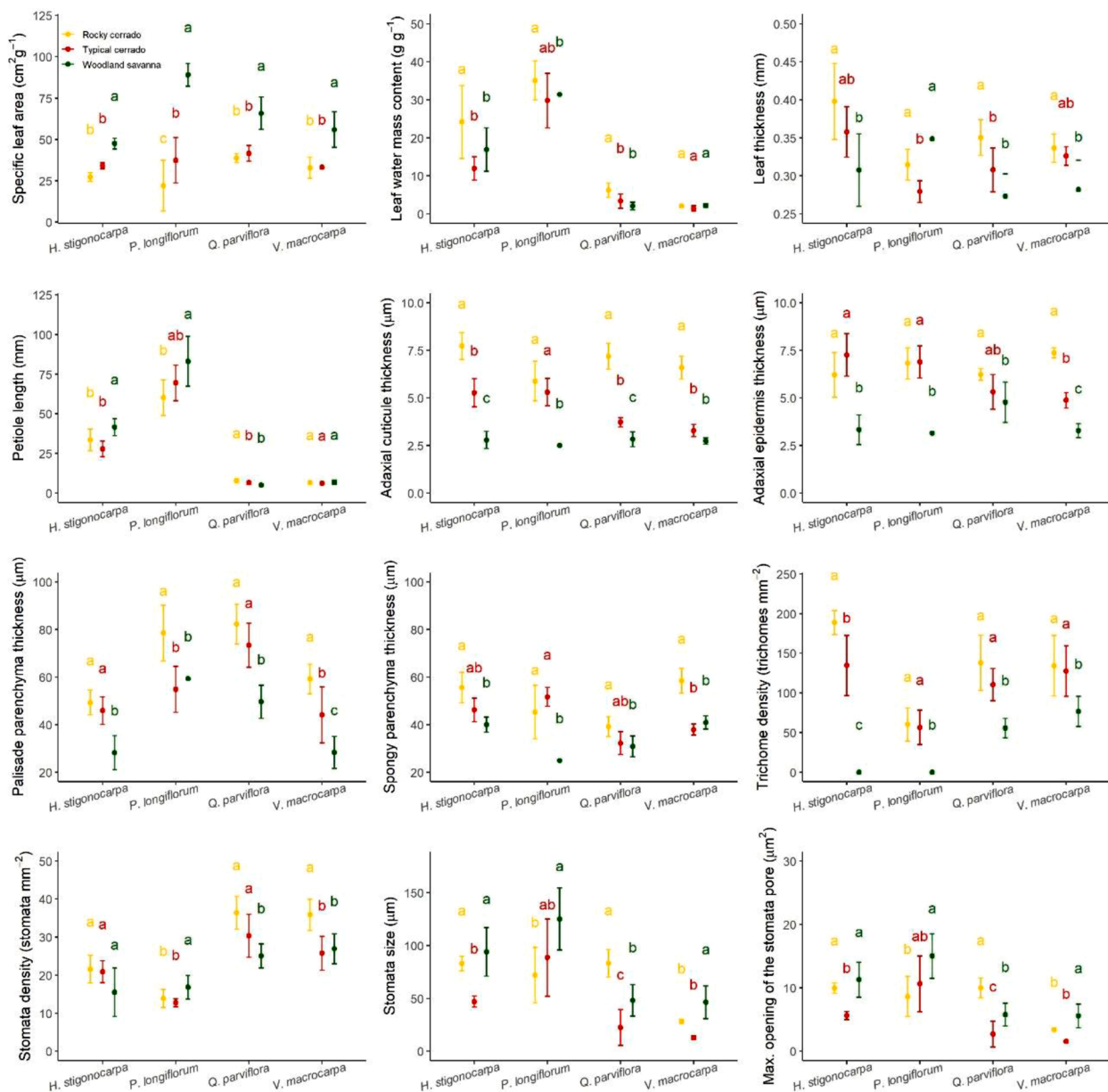

Fig. 2. Trait differences of four tree species from the Amazonia–Cerrado transition that co-occur in three contrasting vegetation types. Different lowercase letters denote significant differences (Split-plot ANOVA, P < 0.05).

organization. All variables were checked for normality and log$_{10}$-transformed whenever necessary to ensure normality. All data analyses were performed using the R program, version 3.6.1 (R Core Team, 2019) with a 5% significance level.

3. Results

3.1. Distribution of leaf morphological and anatomical traits

Qualea parviflora and *V. macrocarpa* showed higher stomatal density and trichome density, regardless of the vegetation type they occurred, while *H. stigonocarpa* and *P. longiflorum* were generally characterized by higher petiole length and leaf water mass content (Fig. 2). The first two axes of the PCA explained 65.1% of the variation and presented a very evident difference between species, which allowed identification of different groups (Fig. 3). PC1 explained 37.4% of the variation and was related to differences between species, while PC2 explained 27.7% of the variation, and separated the vegetation types.

We verified the separation of the three vegetation types (PC2), with a clear distinction between those with extreme environmental conditions such as the rocky *cerrado* (shallow and rocky soil, high temperature and high light intensity) and the *woodland savanna* (deep soil, low light intensity and high humidity), while the typical *cerrado* occupied an intermediate position (Fig. 3). Adaxial cuticle thickness presented the highest correlation with PC2 (Table S1).

3.2. Variability of leaf morphological and anatomical traits at different organizational levels

For most of the traits evaluated, vegetation type and species together explained most of the variance (Fig. 4). The vegetation type explained between 42 and 85% of the variation in adaxial epidermis thickness, adaxial cuticle thickness, and specific leaf area. Species explained between 68 to 91% of the variation in stomatal size, stomatal density, leaf water mass content, and maximum opening of the stomatal pore. On the other hand, some traits showed low variation at the individual level, which varied from 4 to 11% of the variation in adaxial cuticle thickness, leaf water mass content and adaxial epidermis thickness (Fig. 4). To access the decomposition of variance for all organizational levels, see Table S2.
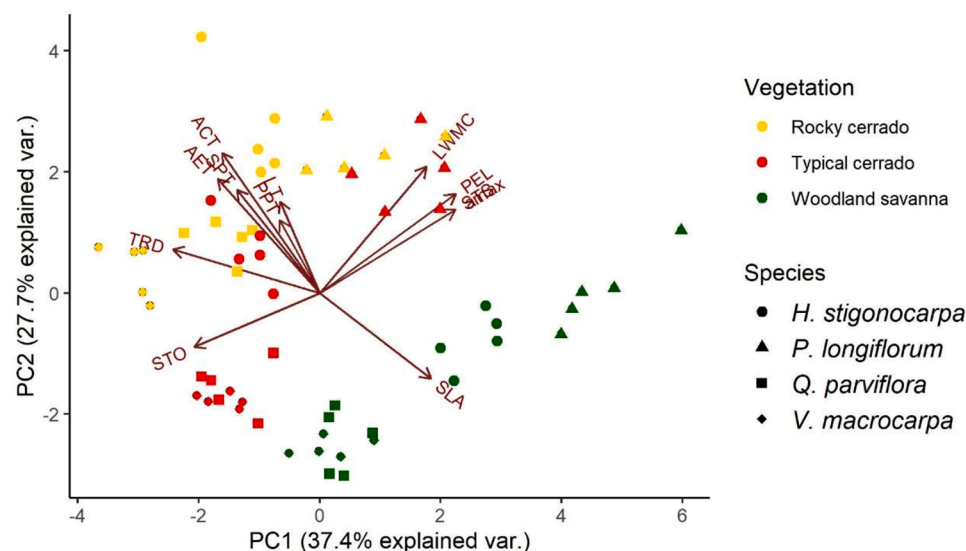
3.3. Intraspecific variability of leaf morphological and anatomical traits

The effect of species was significant for most traits, except for adaxial epidermis thickness (Table S3). The interactions between the two effects were significant, except for leaf water mass content (Table S3). These effects (i.e. species and vegetation types) will be examined successively, combining the results shown in Table S3 with the data obtained in all individual species (Fig. 2).

Patterns within species across vegetation types showed that individuals occurring on *cerrado* formations generally have more conservative strategies than those in *woodland savanna* formations. For example, specific leaf area, a trait associated with resource acquisition, was higher for individuals in the *woodland savanna* (P < 0.001; Fig. 2) while leaf water mass content, associated with resistance to desiccation, was higher for individuals of the typical *cerrado* and rocky *cerrado* (P = 0.010; Fig. 2).

Similarly, individuals occurring on *cerrado* formations, especially those on the rocky *cerrado*, generally had higher spongy parenchyma thickness, compared to individuals of the same species in the *woodland savanna* (P < 0.001; Fig. 2). On the other hand, the trichomes density and the thickness of the cuticle and adaxial epidermis and the palisade parenchyma showed differences with the species and vegetation interaction (P < 0.001; Fig. 2).

For other traits, differences across vegetation types were less consistent across species. For example, petiole length widely varied among individuals, being highest for *Q. parviflora* individuals in the rocky *cerrado*, whereas for *H. stigonocarpa* and *P. longiflorum*, they were higher in the *woodland savanna* (P = 0.003; Fig. 2). Similarly, stomatal traits did not exhibit consistent variation across vegetation types. For *Q. parviflora* and *V. macrocarpa*, stomatal density was higher for individuals in the *cerrados*, whereas for *P. longiflorum* the stomata density values were higher in the *woodland savanna* (P = 0.002; Fig. 2). *Qualea parviflora* recorded the highest stomatal size values in the rocky *cerrado*, whereas *V. macrocarpa* and *P. longiflorum* recorded the highest values in the *woodland savanna* (P < 0.001; Fig. 2).

In general, for all traits, the coefficient of variation was variable at the species level (Figure S2): higher values for *P. longiflorum* in the rocky *cerrado*, for *Q. parviflora* in the typical *cerrado* and for *V. macrocarpa* in the *woodland savanna* (Figure S2). *Hymenaea stigonocarpa* generally showed the lowest variability in terms of species and vegetation (Figure S2). The coefficient of variation was always higher for species in the *woodland savanna* and decreased with the canopy opening (Figure S2). For all species, the most variable trait in the rocky *cerrado*

Fig. 3. Principal component analysis of the morphological and anatomical traits of four tree species from the Amazonia–Cerrado transition, co-occurring in rocky *cerrado*, typical *cerrado* and *woodland savanna*. Maximum opening of the stomatal pore (AMAX), stomata size (STS), stomata density (STO), trichome density (TRD), spongy parenchyma thickness (SPT), palisade parenchyma thickness (PPT), adaxial epidermis thickness (AET), adaxial cuticle thickness (ACT), petiole length (PEL), leaf thickness (LT), leaf water mass content (LWMC) and specific leaf area (SLA).
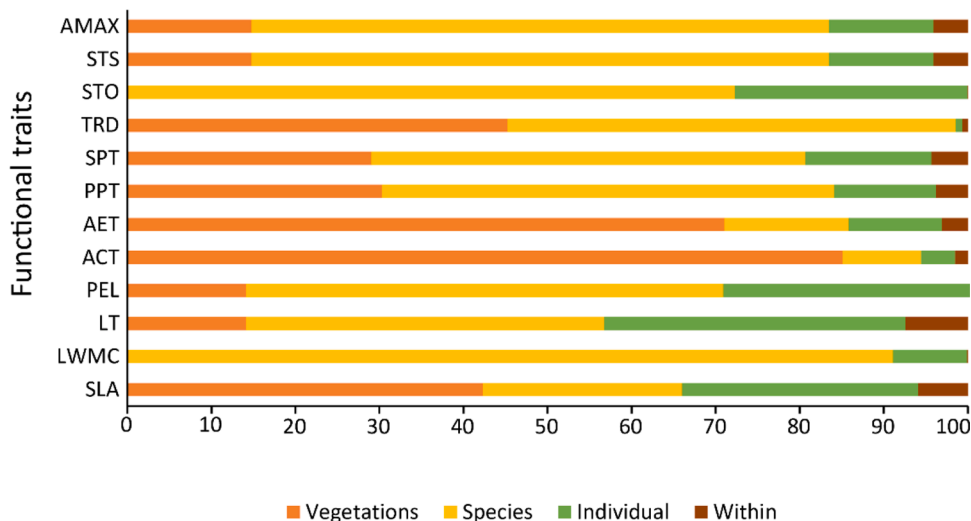
Fig. 4. Partitioning of the variance of the nested linear models of the morphological and anatomical traits of four species in the Amazonia–Cerrado transition. Maximum opening of the stomatal pore (AMAX), stomata size (STS), stomata density (STO), trichome density (TRD), spongy parenchyma thickness (SPT), palisade parenchyma thickness (PPT), adaxial epidermis thickness (AET), adaxial cuticle thickness (ACT), petiole length (PEL), leaf thickness (LT), leaf water mass content (LWMC) and specific leaf area (SLA). Within means the residual error, all data were transformed (log-10) before analysis.

was the spongy parenchyma thickness while palisade parenchyma thickness was the most variable in the typical *cerrado* and leaf thickness was most variable in the *woodland savanna* (Figure S2).

4. Discussion

The species here evaluated in the largest and most diverse transition between biomes on the planet, the Amazonia–Cerrado, showed variability in their leaf morphological and anatomical traits. Spongy parenchyma thickness, palisade parenchyma thickness and leaf thickness were the most variable traits at the intraspecific level. On the other hand, petiole length and adaxial epidermis thickness are the least variable. These variations between the leaf morphological and anatomical traits are driven by different factors such as species, environmental and genetic modification (Garnier et al., 2001; Cássia-Silva et al., 2017; Smedt et al., 2018). Variations in leaf traits diverged widely between organizational levels. The variance explained by vegetation, species, and individuals allowed us to understand separate contribution to leaf traits' variability. In this study, intraspecific variation of the thickness of the cuticle and the adaxial epidermis were mostly explained by vegetation, which may be driven by water availability, nutrients, and microclimate variations (Marimon-Junior and Haridasan, 2005). Surprisingly, vegetation did not influence leaf water content, which >90% of its variability was explained by species, which may be related to interspecific differences in ecological strategies to use and compete for resources (Albert et al., 2010; Messier et al., 2010). The stomatal dimensions (i.e., the maximum opening of the stomatal pore, stomata size and stomata density) exhibited greater variation also explained at the species level, which may be more related to water and nutrient limitations (Marimon-Junior and Haridasan, 2005; Schemske et al., 2009; Albert et al., 2010). On the other hand, a relatively small percentage of variation (4–35%) was explained by the individual level for all leaf traits, which may be due to the marked climate seasonality and edaphic contrasts in this region (Marimon et al., 2014).

Therefore, our findings show that plants co-occurring in open and closed-canopy environments adopt different ecological strategies to establish in vegetation with contrasting environmental properties (Hoffmann and Franco, 2003; Laureto and Cianciaruso, 2015; Araújo et al., 2021). Our results are corroborated by another large-scale study conducted by Maracahipes et al. (2018), who found intraspecific differences in five traits across generalist species occurring in savanna and forest formations in the Cerrado Biome. Here, we provide a new set of little-explored leaf characteristics, including anatomical traits, which can be integrated into the group of key characteristics that describe the ecological strategies of plant species, not only in transition areas but to

understand the responses of vegetation facing climate change worldwide (Gillison, 2019). In addition, we show that, in savanna-forest systems that occur side by side, regardless of the size of the scale, environmental differences drive changes in functional diversity of vegetation.

Variations in the traits of trees that occur in vegetation with contrasting abiotic stresses can be interpreted as a result of the pressure of disruptive selection (Lemos-Filho et al., 2008) which reflects the functional divergence between individuals of the same species that are adapted to different conditions (Hoffmann and Franco, 2008). Therefore, intraspecific variation found for the species evaluated here can provide a role in adapting to environmental and climatic changes. It also contributes to expanding the ecological and geographical distribution of the species (Bedetti et al., 2011). We showed that, within species, traits associated with drought tolerance (such as cuticle thickness and trichome density) showed higher values in habitats with greater canopy opening, while traits related to productivity (e.g. specific leaf area) were higher in closed-canopy habitats. In addition, species with high variation in traits usually occupy broader ecological niche and can occur in different habitats (Jung et al., 2010).

There was a clear distinction between *woodland savanna* and other vegetation types, mainly driven by differences in leaf morphology and anatomy, reflected in the principal component analysis (Fig. 3). While *woodland savanna* individuals showed higher specific leaf area compared to individuals of the same species occurring in the other *cerrado* formations, rocky *cerrado* individuals showed thicker cuticles, epidermis, palisade and spongy parenchyma reflecting in higher leaf thickness. High specific leaf area is associated with lower investment in the biosynthesis of tissues that provide support and protection (Grime, 1979; Franco et al., 2005). On the other hand, low specific leaf area for individuals growing in *cerrado* is essential to reduce leaf surface area and prevent water loss through transpiration (Poorter et al., 2009). The lower investment in specific leaf area may be a strategy to increase resource retention and allocation to other organs of the plant (Reich et al., 1992; Westoby, 1998; Pérez-Harguindeguy et al., 2013). In addition, the greater investment for the formation of protective tissues and structures, such as the cuticle and epidermis, with thicker cell walls (Fahn and Cutler, 1992), helps to reduce the harmful effects of ultraviolet radiation and to prevent leaf damage caused by herbivores and high temperatures (Turner, 1994; Rozendaal et al., 2006). Under high temperature, vapor pressure deficit and light leaf mesophyll tissues can be affected (Grime, 1979; Reich et al., 1999; Dahlgren et al., 2006; Gratani et al., 2006; Rossatto and Kolb, 2010), resulting in leaf area and tissue thickness changes (Goulet and Bellefleur, 1986) as observed for the evaluated species (Figure S3).

In the *cerrado* formations, individuals also showed higher trichome density and higher stomata density for *Q. parviflora* and *V. macrocarpa.* These traits represent an adaptive strategy to prevent or control the increase in leaf temperature (Ehleringer and Björkman, 1978), as well as reducing water loss (Gianoli and Gonzalez-Teuber, 2005). On the other hand, in *P. longiflorum* stomata density was higher for individuals in the *woodland savanna*, which represents greater CO_2 assimilation and, consequently, higher evapotranspiration rates that promote greater growth of the species (Ogburn and Edwards, 2010). Stomata size and petiole length differences varied depending on the species considered. *Vatairea macrocarpa* and *P. longiflorum* showed smaller stomata in individuals from *cerrado* formations. Smaller stomata and stomatal openings allow more efficient control over water loss (Abrams et al., 1994; Franco, 2002; Goldstein et al., 2008), especially in areas under high temperatures, intense light and low humidity (Shields, 1950; Ackerly, 2004; Poorter et al., 2009), as the studied *cerrados*. *Hymenaea stigonocarpa* and *P. longiflorum* showed longer petioles in individuals located in the *woodland savanna* and shorter petioles for those from *cerrado* formations. In general, petioles with greater length allow greater light uptake (Poorter and Bongers, 2006), which is an advantageous trait in *woodland savanna* environments, where species are subject to greater competition for light (Carswell et al., 2000). Moreover, longer petioles also contribute to reducing leaf clumping and overlapping around the stem (Takenaka, 1994; Poorter, 2009), which can be a key feature in *woodland savanna*. On the other hand, *Q. parviflora* showed longer petioles in individuals that grow in the rocky *cerrado*, which could probably contribute to reduce leaf surface temperature, dissipating heat and preventing thermal leaf damage (Yates et al., 2010; Ye et al., 2011; Leigh et al., 2012).

Together, these characteristics are important to increase the efficiency of plants in the conservation and use of water (Pallardy, 1981; Rossatto and Kolb, 2010), dissipate excessive light and heat (Feller, 1996; Gratani et al., 2006) and promote an increase in photosynthetic rates (Pearce et al., 2006). In fact, the greater water availability in the *woodland savanna* soil (Marimon-Junior and Haridasan, 2005) may allow individuals to invest in traits not only related to water storage and drought tolerance (Larcher, 2003; Monteiro et al., 2016; Lin et al., 2017), while in *cerrado* formations these strategies could be crucial for the individual survival.

Future climate changes are predicted to increase extreme weather events, such as droughts and heatwaves (Meehl and Tebaldi, 2004; Collins et al., 2013). These changes may negatively affect the ecophysiological fitness of the trees and, consequently, increase the risk of mortality, especially for species in the Amazonia–Cerrado transition that already experience critical levels of temperature increase (Araújo et al., 2021), about 2-fold greater than the average heating recorded for the planet (Coe et al., 2016; Hoegh-Guldberg et al., 2018). Therefore, intraspecific variation may represent a key mechanism for species survival in future climates. In this case, functional traits that allow greater water storage and water use efficiency, and assist in the dissipation of heat may be more advantageous for trees subject to extreme weather events (Nicotra et al., 2010; Benito Garzón et al., 2011).

The high intraspecific variation observed for the species in this study is important for persistence over time, especially in this region, where climate change is already affecting trees (Tiwari et al., 2020; Araújo et al., 2021). Therefore, we suggest that these species are good candidates for the recovery of degraded areas in the Amazonia–Cerrado transition. This aspect is of ultimate importance, since this region is known as the "Arc of deforestation" due to seasonal droughts, fires and uncontrolled deforestation occurring concurrently (Schmidt and Eloy, 2020; Brando et al., 2020). Moreover, the Amazonia–Cerrado transition, which suffers more deforestation than forests and savannas in each individual biome, is close to collapse due to the intense use of land for crops and pastures (Marques et al., 2020). This scenario is particularly concerning because this transitional region comprises greater local biodiversity than each biome separately (Marimon et al., 2006; Mews et al., 2012; Marimon et al., 2014).

Adding up to previous studies across distinct environmental gradients (Scalon et al., 2017; Silva et al., 2019; Xu et al., 2020), we showed that intraspecific variability in leaf traits is substantial and should not be ignored in trait-based studies, especially in heterogeneous environments, such as transitional zones (Albert et al., 2010; Jung et al., 2010). Our findings showed that widespread species in the Amazonia–Cerrado transition have different functional strategies to establish and maintain populations in contrasting environments through their high potential for intraspecific variation in their functional traits. Whereas trees in the *cerrado* have leaf morphological and anatomical traits linked to competition for water and conservation of resources, trees of the same species growing in *woodland savanna* have traits that increase efficiency in the competition for light and generate higher productivity. Our results highlight the importance of the interaction between the environment and the leaf morphological and anatomical traits for understanding how selective pressures affect the functional diversity of species in tropical ecosystems.

CRediT authorship contribution statement

Igor Araújo: Conceptualization, Formal analysis, Investigation, Methodology, Writing - original draft. **Beatriz S. Marimon:** Conceptualization, Methodology, Writing - review & editing. **Marina C. Scalon:** Conceptualization, Methodology, Writing - review & editing. **Wesley J. A. Cruz:** Formal analysis. **Sophie Fauset:** Writing - review & editing. **Tiffani C.S. Vieira:** Methodology, Investigation. **David R. Galbraith:** Formal analysis, Writing - review & editing. **Manuel U. Gloor:** Formal analysis, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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