

2021-06

Intraspecific variation in leaf traits facilitates the occurrence of trees at the AmazoniaCerrado transition

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<http://hdl.handle.net/10026.1/20032>

10.1016/j.flora.2021.151829

Flora

Elsevier BV

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33 **Abstract**

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

50

51 **Keywords:** Leaf Morpho-anatomical Traits; Savanna–Forest Gradient; Microclimate;
52 Ecological Strategies; Tropics; Climate Change

53

55 **1. Introduction**

56 Current and future climate change are among the main threats to ecosystems
57 globally (Yu et al., 2019), and it is expected that their impacts and direct consequences
58 will lead to global losses in biodiversity, carbon stocks and ecosystem services (Allen et
59 al., 2010; Collins et al., 2013; Law, 2014; IPCC, 2019; Araújo et al., 2021). The
60 forecast is for increased mortality risk of species across biomes (Van Mantgem et al.,
61 2007; Phillips et al., 2009; Peng et al., 2011; McDowell, 2018), which are already
62 sensitive to the current climate and may be even more vulnerable in the future (Araújo
63 et al., 2021). Climate has changed enough to exceed the physiological limits to which
64 some plants are adapted (Shaw and Etterson, 2012; Araújo et al., 2021) and led to
65 negative effects that compromise survival and persistence of species over time (Becklin
66 et al., 2016). In this context, even greater concerns arise regarding the ability of species
67 to adapt quickly to climate change (Burrows et al., 2011; Dawson et al., 2011;
68 Hoffmann and Sgrò, 2011). This is especially so for plants, which are sessile organisms
69 with a long lifespan. Thus, trees will need flexibility during their lifetime to deal with
70 rapid environmental and climatic changes, unrelated to genetic changes, and therefore
71 intraspecific variation and phenotypic plasticity can be decisive for the persistence of
72 trees and thus the risk of extinction due to future climate change (Nicotra et al., 2010;
73 Benito Garzón et al., 2011). In this context, functional traits have advanced our
74 understanding of tree physiology, community structure and ecosystem functioning
75 (Wright et al., 2004; De la Riva et al., 2015; Volf et al., 2016).

76 Recent studies have integrated the variation of intraspecific characteristics in
77 functional traits to investigate the responses of trees along environmental gradients
78 (Laforest-Lapointe et al., 2014; Siefert et al., 2015). Intraspecific variation allows trees
79 to establish under new environmental conditions (Joshi et al., 2001; Byars et al., 2007;
80 Maracahipes et al., 2018). In particular, environmental variation induces changes in the
81 functional traits of trees (Anderson and Gezon, 2015; Lázaro-Nogal et al., 2015;
82 Niinemets, 2016), at morphological (Capuzzo et al., 2012), anatomical (Rossatto and
83 Kolb, 2010, 2012; De Paula et al., 2018) and physiological levels (Rossatto et al., 2010).
84 This scenario highlights the set of functional traits that allow plants to survive in
85 contrasting environments has fundamental consequences for our understanding of the

86 dynamics of vegetation, especially in highly complex and hyperdiverse areas such as
87 savanna–forest transitions (Marimon et al., 2014; Oliveras and Yadvinder, 2016).

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

112 Although interest in functional traits has grown more and more in plant ecology,
113 in hyperdiverse and complex tropical systems, such as the most diverse transition on the
114 planet to the Amazonia and the Cerrado, we still do not know how the variation in
115 functional traits, especially the anatomical traits (e.g., stomata density, stomata size and
116 maximum opening of the stomatal pore) differ between ecological scales, such as
117 vegetation, species and individuals (Albert et al., 2010; Messier et al., 2010; Salazar et
118 al., 2018). This fact has made it difficult to determine the scale-dependent aspects of the
119 variation in functional traits (Messier et al., 2010). At the individual level, the

120 variability in the functional traits of trees is influenced by climatic drivers (e.g.,
121 temperature and precipitation) (Fajardo and Piper, 2011; Richardson et al., 2013). In
122 addition, soil texture, successional stage and disturbance regimes can also contribute to
123 this variation (Salazar et al., 2018). In terms of vegetation, the availability of water and
124 nutrients, soil compaction, salinity and microclimate variations can induce variations in
125 traits (Messier et al., 2010). However, at the species level, the variation may result from
126 interspecific competition for resources such as water, light and nutrients (Schemske et
127 al., 2009; Albert et al., 2010; Messier et al., 2010). As far as we know, no research has
128 used a wide scan of leaf anatomical traits to measure the change in traits on a series of
129 ecological scales. Knowing which scales have the greatest variation in functional traits
130 will provide important explanations about the causes of variability in functional traits
131 and may contribute to subsidies on patterns and processes on spatial and temporal scales
132 that are ecologically more significant (McGill et al., 2006).

133 In this study we report how the variation in leaf functional traits (e.g.,
134 morphological and anatomical) changes in three nested ecological scales (vegetations,
135 species and individuals) along a savanna–forest gradient in the Amazonia–Cerrado
136 transition, which is making it increasingly dry and hot and where some species can
137 already be affected by climate change (Tiwari et al., 2020; Araújo et al., 2021). In
138 addition, we have introduced little-explored valuable information on the variability of
139 intraspecific characteristics in leaf functional traits of co-occurring trees in contrasting
140 environments in tropical systems. We test two hypotheses: (1) among ecological scales,
141 vegetation will contribute to greater variation in leaf traits, since the distinct habitats
142 present contrasting environmental aspects, as shown in Marimon-Junior and Haridasan
143 (2005) and Figure S1; (2) species have significant intraspecific variability in leaf
144 functional traits irrespective of the habitat. We expected smaller and thicker leaves with
145 higher trichome density in habitats under higher incidence of light, high temperature,
146 and low water availability. On the other hand, larger and thinner leaves with larger
147 stomata will be found in *woodland savanna* vegetation, a habitat with lower light
148 incidence, lower temperature, and higher water availability (Rossatto et al., 2010;
149 Rossatto and Kolb, 2010, 2012; Capuzzo et al., 2012; Reich et al., 2014; Souza et al.,
150 2015).

151

152 **2. Materials and methods**

153

154 *2.1. Study area and species description*

155 We carried out the study at the Bacaba Municipal Park (BMP), a conservation
156 unit of approximately 500 ha, in the municipality of Nova Xavantina-MT, Brazil
157 (Figure 1), located in the transition region between the Amazonia and Cerrado biomes
158 (Marimon-Junior and Haridasan, 2005; Marimon et al., 2014). The climate shows
159 marked seasonality with two well-defined periods, one rainy (October to March) and the
160 other dry (April to September; Alvares et al., 2013), with average annual rainfall of
161 1.500 mm, elevation of 340 m (Marimon et al., 2010). Mean monthly temperature is 25
162 °C, ranging from 18.7 to 33.5 °C, according to data obtained from INMET
163 (Instituto Nacional de Meteorologia –<http://www.inmet.gov.br>) considering data from
164 14 years period (2004-2019).

165

>>insert Fig. 1 here

166 The typical *cerrado* occupies 153.77 ha of the BMP, is a savanna
167 phytophysiology composed of low vegetation cover, with trees spaced from each
168 other, and also presents a dense low stratum (Mews et al., 2011; Gomes et al., 2016).
169 Generally, it occurs on deep, well-drained, dystrophic, acidic Latosols and Neosols
170 (Marimon-Junior and Haridasan, 2005). In contrast, the rocky *cerrado* occupies about
171 25.44 ha of the BMP area, on shallow soils and with rocky outcrops (Litholic Neosols).
172 In this environment, trees are positioned in the gaps between the rocks, where
173 accumulation and decomposition of organic matter and sand deposition resulting from
174 weathering of the rocks occurs (Maracahipes et al., 2011; Gomes et al., 2016). The
175 rocky *cerrado* has a flora with a high degree of endemism (Alves and Kolbek, 2010)
176 and many endangered species (Mendonça and Lins, 2000). The *woodland savanna*
177 occupies about 162.02 ha of the BMP area, comprising large trees, with thick litter layer
178 (Marimon et al., 1998; Franczak et al., 2011; Reis et al., 2015) and dystrophic soil
179 (Marimon-Junior and Haridasan, 2005).

180

181 For our study we selected four co-occurring species (*Hymenaea stigonocarpa*
182 Mart. ex Hayne – Fabaceae, *Qualea parviflora* Mart. – Vochysiaceae, *Vatairea*
183 *macrocarpa* (Benth.) Ducke – Fabaceae and *Pseudobombax longiflorum* (Mart.)
184 A.Robyns – Malvaceae) in *cerrado* vegetation (rocky *cerrado* and typical *cerrado*) and
185 a *woodland savanna* in BMP. These species have extensive geographic distributions and
186 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,

187 frequency and relative dominance (Mews et al., 2011; Reis et al., 2015; Gomes et al.,
188 2016). For each species, we haphazardly selected five individuals per vegetation type,
189 totaling 15 individuals per species.

190

191 *2.2. Leaf morphological and anatomical parameters*

192 For each individual of each species, we selected eight leaves, five for
193 morphological characterization and three leaves for anatomical determinations (Table
194 1). As a standardization criterion, we collected fully expanded leaves, exposed to full
195 sunlight, and free of pathogens (i.e. leaf standardization protocol). We measured leaf
196 thickness, specific leaf area (ratio between leaf area and leaf dry mass) and leaf water
197 mass content for each species. We determined the thickness of the fresh leaves with an
198 electronic digital micrometer and digitized them with a scanner to determine leaf area
199 and calculate the specific leaf area (Abràmoff et al., 2004). We estimated the wet weight
200 of the leaves with a precision balance (± 0.001 g), then placed them in paper bags in an
201 oven at 60 °C, and after 72 h determined the dry weight. The leaf water content was
202 calculated as the ratio between wet and dry weights (Pérez-Harguindeguy et al., 2013).

203 For anatomical characterization, we took imprints of the leaf surface with high-
204 tech molding silicon (Speedex), as proposed by Weyers and Johansen (1985).
205 Subsequently, we used colorless enamel to make the impression of the mold on slides,
206 which was visualized with an optical microscope, with an attached camera, to view
207 stomata and trichomes. We took stomatal size and stomatal density measurements under
208 10 \times microscopic amplification selecting haphazardly 10 fields per leaf. Microscope
209 images were processed with the ImageJ software (Abràmoff et al., 2004).

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

220 For each of the three leaves described above, we took 2 cm × 2 cm sample from
221 the median portion of the leaf blade and used a freehand cross-section. We fixed the leaf
222 fragment on a half-open petiole (*Cecropia* sp.) and cut it freehand with the help of a
223 razor blade. Afterwards, we stored the samples in Petri dishes and with the aid of a
224 Pasteur pipette we added 3 ml of sodium hypochlorite, and we waited for 5 to 10 min
225 until the samples became translucent. Then, we rinsed three times with distilled water
226 and stained the samples with a solution of 50% methylene blue and 50% safranin. We
227 photographed the slides with a camera attached to an optical microscope, haphazardly
228 selecting 10 fields from each leaf with a 10× magnification objective to assess the
229 thickness of the adaxial cuticle, adaxial epidermis, and palisade and spongy parenchyma
230 (Roeser 1962).

231

232 2.3. Data analysis

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

248 To understand controls on trait variability, we used different groupings of mixed
249 linear models, adjusting the separate models for each trait (Rosas et al., 2019).
250 Vegetation types, species, and individuals were introduced as nested random factors to
251 assess how the variability of functional traits was distributed among these different
252 levels of organization. All variables were checked for normality and log₁₀-transformed

253 whenever necessary to ensure normality. All data analyses were performed using the R
254 program, version 3.6.1 (R Core Team, 2019) with a 5% significance level.

255

256

257 **3. Results**

258

259 *3.1. Distribution of leaf morphological and anatomical traits*

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

268

>>insert Fig. 2 here

269

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

275

>>insert Fig. 3 here

276

277

278 *3.2. Variability of leaf morphological and anatomical traits at different organizational* 279 *levels*

280

For most of the traits evaluated, vegetation type and species together explained
281 most of the variance (Figure 4). The vegetation type explained between 42 and 85% of
282 the variation in adaxial epidermis thickness, adaxial cuticle thickness, and specific leaf
283 area. Species explained between 68 to 91% of the variation in stomatal size, stomatal
284 density, leaf water mass content, and maximum opening of the stomatal pore. On the
285 other hand, some traits showed low variation at the individual level, which varied from
286 4 to 11% of the variation in adaxial cuticle thickness, leaf water mass content and

287 adaxial epidermis thickness (Figure 4). To access the decomposition of variance for all
288 organizational levels, see Table S2.

289 >>insert Fig. 4 here

290

291 3.3. Intraspecific variability of leaf morphological and anatomical traits

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

297 Patterns within species across vegetation types showed that individuals
298 occurring on *cerrado* formations generally have more conservative strategies than those
299 in *woodland savanna* formations. For example, specific leaf area, a trait associated with
300 resource acquisition, was higher for individuals in the *woodland savanna* ($P < 0.001$;
301 Figure 2) while leaf water mass content, associated with resistance to desiccation, was
302 higher for individuals of the typical *cerrado* and rocky *cerrado* ($P = 0.010$; Figure 2).
303 Similarly, individuals occurring on *cerrado* formations, especially those on the rocky
304 *cerrado*, generally had higher spongy parenchyma, compared to individuals of the same
305 species in the *woodland savanna* ($P < 0.001$; Figure 2). On the other hand, the
306 trichomes density and the thickness of the cuticle and adaxial epidermis and the palisade
307 parenchyma showed differences with the species and vegetation interaction. ($P < 0.001$;
308 Figure 2).

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

319 In general, for all traits, the coefficient of variation was variable at the species
320 level (Figure S2): higher values for *P. longiflorum* in the rocky *cerrado*, for *Q.*

321 *parviflora* in the typical *cerrado* and for *V. macrocarpa* in the *woodland savanna*
322 (Figure S2). *Hymenaea stigonocarpa* generally showed the lowest variability in terms
323 of species and vegetation (Figure S2). The coefficient of variation was always higher for
324 species in the *woodland savanna* and decreased with the canopy opening (Figure S2).
325 For all species, the most variable trait in the rocky *cerrado* was the spongy parenchyma
326 thickness while palisade parenchyma thickness was the most variable in the typical
327 *cerrado* and leaf thickness was most variable in the *woodland savanna* (Figure S2).
328

329 **3. Discussion**

330 The species here evaluated in the largest and most diverse transition between
331 biomes on the planet, the Amazonia–Cerrado, showed variability in their leaf
332 morphological and anatomical traits. Spongy parenchyma thickness, palisade
333 parenchyma thickness and leaf thickness were the most variable traits at the
334 intraspecific level. On the other hand, petiole length and adaxial epidermis thickness are
335 the least variable. These variations between the leaf morphological and anatomical traits
336 are driven by different factors such as species, environmental and genetic modification
337 (Garnier et al., 2001; Cássia-Silva et al., 2017; Smedt et al., 2018). Variations in leaf
338 traits diverged widely between organizational levels. The variance explained by
339 vegetation, species, and individuals allowed us to understand separate contribution to
340 leaf traits' variability. In this study, intraspecific variation of the thickness of the cuticle
341 and the adaxial epidermis were mostly explained by vegetation, which may be driven by
342 water availability, nutrients, and microclimate variations (Marimon-Junior and
343 Haridasan, 2005). Surprisingly, vegetation did not influence leaf water content, which
344 >90% of its variability was explained by species, which may be related to interspecific
345 differences in ecological strategies to use and compete for resources (Albert et al.,
346 2010; Messier et al., 2010). The stomatal dimensions (i.e., the maximum opening of the
347 stomatal pore, stomata size and stomata density) exhibited greater variation also
348 explained at the species level, which may be more related to water and nutrient
349 limitations (Marimon-Junior and Haridasan, 2005; Schemske et al., 2009; Albert et al.,
350 2010). On the other hand, a relatively small percentage of variation (4–35%) was
351 explained by the individual level for all leaf traits, which may be due to the marked
352 climate seasonality and edaphic contrasts in this region (Marimon et al., 2014).

353 Therefore, our findings show that plants co-occurring in open and closed-canopy
354 environments adopt different ecological strategies to establish in vegetation with
355 contrasting environmental properties (Hoffmann and Franco, 2003; Laureto and
356 Cianciaruso, 2015; Araújo et al., 2021). Our results are corroborated by another large-
357 scale study conducted by Maracahipes et al. (2018), who found intraspecific differences
358 in five traits across generalist species occurring in savanna and forest formations in the
359 Cerrado Biome. Here, we provide a new set of little-explored leaf characteristics,
360 including anatomical traits, which can be integrated into the group of key characteristics
361 that describe the ecological strategies of plant species, not only in transition areas but to
362 understand the responses of vegetation facing climate change worldwide (Gillison,
363 2019). In addition, we show that, in savanna-forest systems that occur side by side,
364 regardless of the size of the scale, environmental differences drive changes in functional
365 diversity of vegetation.

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

378 There was a clear distinction between *woodland savanna* and other vegetation
379 types, mainly driven by differences in leaf morphology and anatomy, reflected in the
380 principal component analysis (Figure 3). While *woodland savanna* individuals showed
381 higher specific leaf area compared to individuals of the same species occurring in the
382 other *cerrado* formations, rocky *cerrado* individuals showed higher thicker cuticles,
383 epidermis, palisade and spongy parenchyma reflecting in higher leaf thickness. High
384 specific leaf area is associated with lower investment in the biosynthesis of tissues that
385 provide support and protection (Grime, 1979; Franco et al., 2005). On the other hand,
386 low specific leaf area for individuals growing in *cerrado* is essential to reduce leaf

387 surface area and prevent water loss through transpiration (Poorter et al., 2009). The
388 lower investment in specific leaf area may be a strategy to increase resource retention
389 and allocation to other organs of the plant (Reich et al., 1992; Westoby, 1998; Pérez-
390 Harguindeguy et al., 2013). In addition, the greater investment for the formation of
391 protective tissues and structures, such as the cuticle and epidermis, with thicker cell
392 walls (Fahn and Cutler, 1992), help to reduce the harmful effects of ultraviolet radiation
393 and to prevent leaf damage caused by herbivores and high temperatures (Turner, 1994;
394 Rozendaal et al., 2006). Under high temperature, vapor pressure deficit, and light leaf
395 mesophyll tissues can be affected (Grime, 1979; Reich et al., 1999; Dahlgren et al.,
396 2006; Gratani et al., 2006; Rossatto and Kolb, 2010), resulting in leaf area and tissue
397 thickness changes (Goulet and Bellefleur, 1986) as observed for the evaluated species
398 (Figure S3).

399 In the *cerrado* formations, individuals also showed higher trichome density and
400 higher stomata density for *Q. parviflora* and *V. macrocarpa*. These traits represent an
401 adaptive strategy to prevent or control the increase in leaf temperature (Ehleringer and
402 Björkman, 1978), as well as reducing water loss (Gianoli and Gonzalez-Teuber, 2005).
403 On the other hand, in *P. longiflorum* stomata density was higher for individuals in the
404 *woodland savanna*, which represents greater CO₂ assimilation and, consequently, higher
405 evapotranspiration rates that promote greater growth of the species (Ogburn and
406 Edwards, 2010). Stomata size and petiole length differences varied depending on the
407 species considered. *V. macrocarpa* and *P. longiflorum*, showed smaller stomata in
408 individuals from *cerrado* formations. Smaller stomata and stomatal openings allow
409 more efficient control over water use (Abrams et al., 1994; Franco, 2002; Goldstein et
410 al., 2008), especially in areas under high temperatures, intense light and low humidity
411 (Shields, 1950; Ackerly, 2004; Poorter et al., 2009), as the studied *cerrados*. *H.*
412 *stigonocarpa* and *P. longiflorum* showed longer petioles in individuals located in the
413 *woodland savanna* and shorter petioles for those from *cerrado* formations. In general,
414 petioles with greater length allow greater light uptake (Poorter and Bongers, 2006),
415 which is an advantageous trait in *woodland savanna* environments, where species are
416 subject to greater competition for light (Carswell et al., 2000). Moreover, longer petioles
417 also contribute to reducing leaf clumping and overlapping around the stem (Takenaka,
418 1994; Poorter, 2009), which can be a key feature in *woodland savanna*. On the other
419 hand, *Q. parviflora* showed longer petioles in individuals that grow in the rocky
420 *cerrado*, which could probably contribute to reduce the leaf surface temperature,

421 dissipating heat and preventing thermal leaf damage (Yates et al., 2010; Ye et al., 2011;
422 Leigh et al., 2012).

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

431 Future climate changes are predicted to increase extreme weather events, such as
432 droughts and heatwaves (Meehl and Tebaldi, 2004; Collins et al., 2013). These changes
433 may negatively affect the ecophysiological fitness of the trees and, consequently,
434 increase the risk of mortality, especially for species in the Amazonia–Cerrado transition
435 that already experience critical levels of temperature increase (Araújo et al., 2021),
436 about 2-fold greater than the average heating recorded for the planet (Coe et al., 2016;
437 Hoegh-Guldberg et al., 2018). Therefore, intraspecific variation may represent a key
438 mechanism for species survival in future climates. In this case, functional traits that
439 allow greater water storage and water use efficiency, and assist in the dissipation of heat
440 may be more advantageous for trees subject to extreme weather events (Nicotra et al.,
441 2010; Benito Garzón et al., 2011).

442 The high intraspecific variation observed for the species in this study is
443 important for persistence over time, especially in this region, where climate change is
444 already affecting trees (Tiwari et al., 2020; Araújo et al., 2021). Therefore, we suggest
445 that these species are good candidates for the recovery of degraded areas in the
446 Amazonia–Cerrado transition. This aspect is of ultimate importance, since this region is
447 known as the “Arc of deforestation” due to seasonal droughts, fires and uncontrolled
448 deforestation occurring concurrently (Schmidt and Eloy, 2020; Brando et al.,
449 2020). Moreover, the Amazonia–Cerrado transition, which suffers more deforestation
450 than forests and savannas in each individual biome, is close to collapse due to the
451 intense use of land for crops and pastures (Marques et al., 2020). This scenario is
452 particularly concerning because this transitional region comprises greater local
453 biodiversity than each biome separately (Marimon et al., 2006; Mews et al., 2012;
454 Marimon et al., 2014).

455 Adding up to previous studies across distinct environmental gradients (Scalon et
456 al. 2017; Silva et al. 2019; Xu et al., 2020), we showed that intraspecific variability in
457 leaf traits is substantial and should not be ignored in trait-based studies, especially in
458 heterogeneous environment, such as transitional zones (Albert et al. 2010; Jung et al.
459 2010). Our findings showed that widespread species in the Amazonia–Cerrado
460 transition have different functional strategies to establish and maintain populations in
461 contrasting environments through their high potential for intraspecific variation in their
462 functional traits. Whereas trees in the *cerrado* have leaf morphological and anatomical
463 traits linked to competition for water and conservation of resources, trees of the same
464 species growing in *woodland savanna*, have traits that increase efficiency in the
465 competition for light and generate higher productivity. Our results highlight the
466 importance of the interaction between the environment and the leaf morphological and
467 anatomical traits for understanding how selective pressures affect the functional
468 diversity of species in tropical ecosystems.

469

470 **Acknowledgements**

471 We are thank CAPES (Coordination for the Improvement of Higher Education
472 Personnel) - Finance Code 001 for granting the author's scholarship.

473

474 **4. References**

475

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858

859 **Figure Legends**

860

861 **Figure 1.** Neotropical savannas and *woodland savanna* in the Amazonia–Cerrado
862 transition, Brazil, South America.

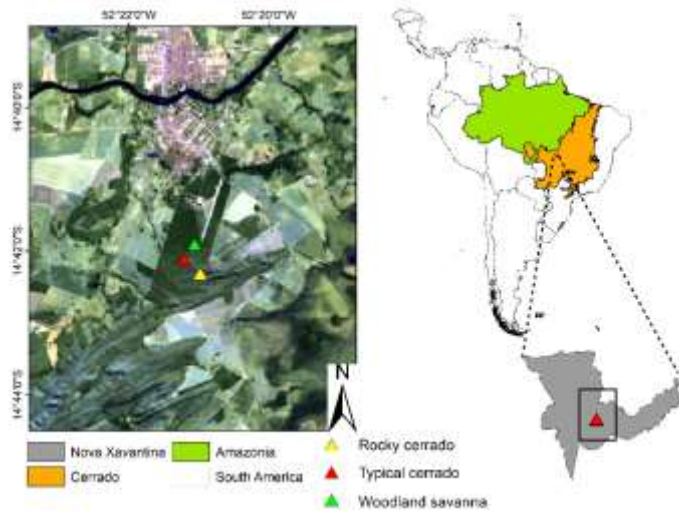
863 **Figure 2.** Trait differences of four tree species from the Amazonia–Cerrado transition
864 that co-occur in three contrasting vegetation types. Different lowercase letters denote
865 significant differences (Split-plot ANOVA, $P < 0.05$).

866 **Figure 3.** Principal component analysis of the morphological and anatomical traits of
867 four tree species from the Amazonia–Cerrado transition, co-occurring in rocky *cerrado*,
868 typical *cerrado* and *woodland savanna*. Maximum opening of the stomatal pore (a_{max}),
869 stomata size (STS), stomata density (STO), trichome density (TRD), spongy
870 parenchyma thickness (SPT), palisade parenchyma thickness (PPT), adaxial epidermis
871 thickness (AET), adaxial cuticle thickness (ACT), petiole length (PEL), leaf thickness
872 (LT), leaf water mass content (LWMC) and specific leaf area (SLA).

873 **Figure 4.** Partitioning of the variance of the nested linear models of the morphological
874 and anatomical traits of four species in the Amazonia–Cerrado transition. Maximum
875 opening of the stomatal pore (a_{max}), stomata size (STS), stomata density (STO),
876 trichome density (TRD), spongy parenchyma thickness (SPT), palisade parenchyma
877 thickness (PPT), adaxial epidermis thickness (AET), adaxial cuticle thickness (ACT),
878 petiole length (PEL), leaf thickness (LT), leaf water mass content (LWMC) and specific
879 leaf area (SLA). Within means the residual error, all data were transformed (log-10)
880 before analysis.

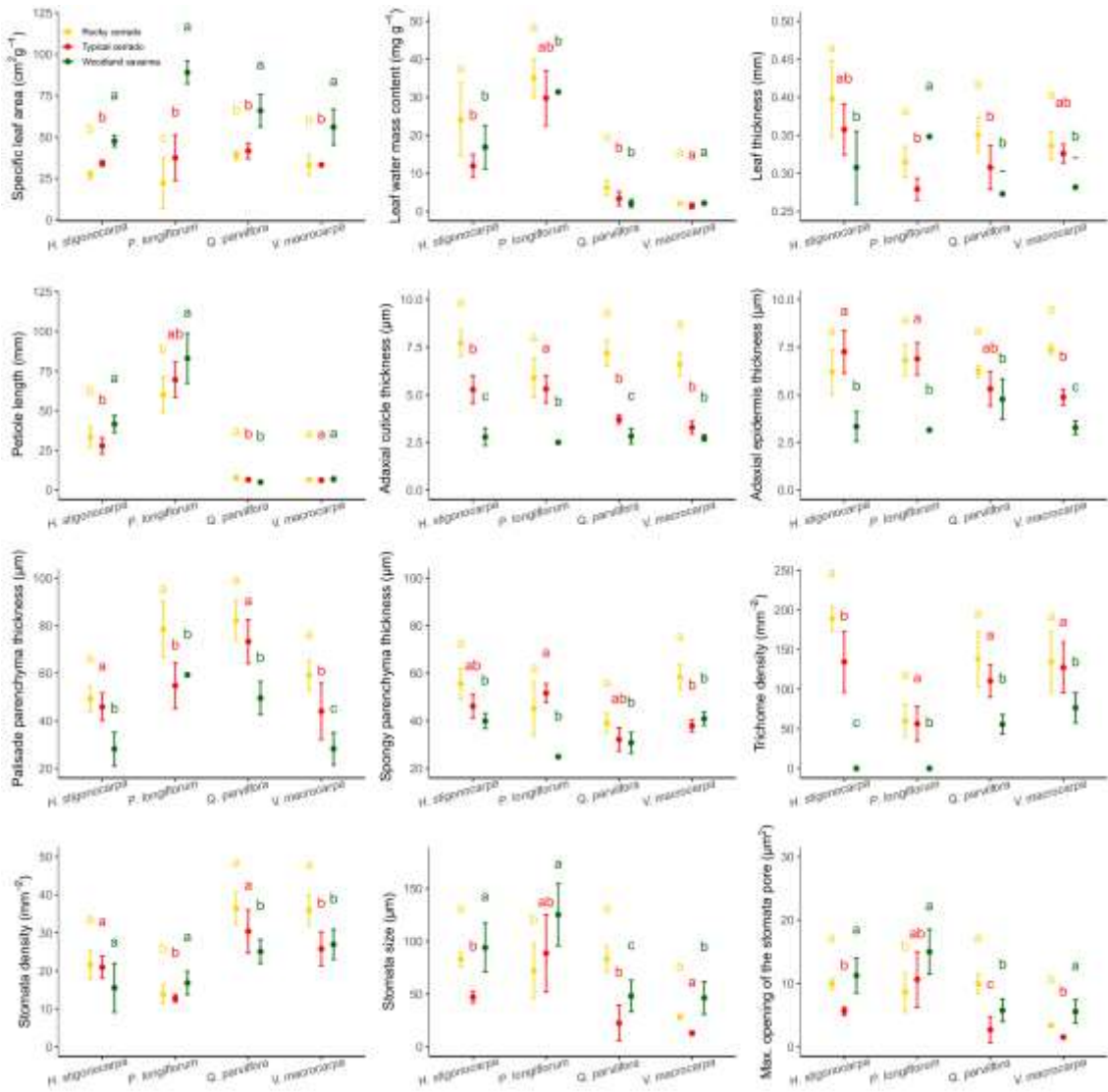
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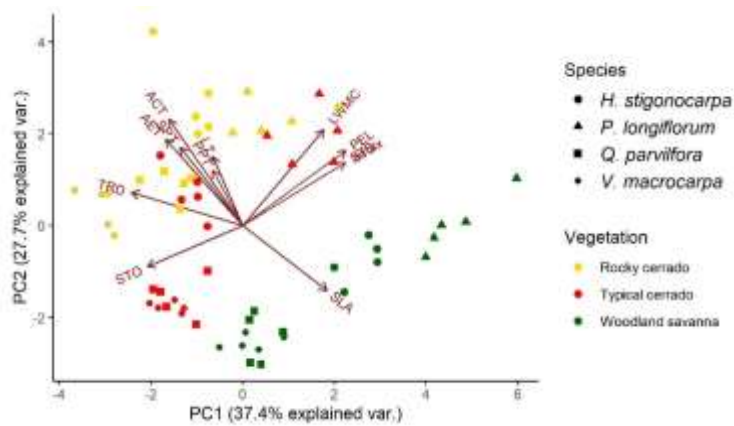
884 **Figure 1.**



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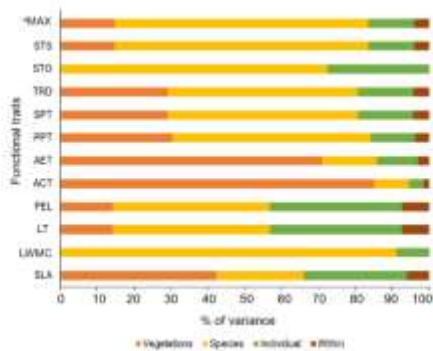
886 **Figure 2.**

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889 **Figure 3.**



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891 **Figure 4.**

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893 **Table 1.** Leaf morphological and anatomical traits evaluated for tropical trees in the
 894 Amazonia–Cerrado transition. ¹ Grime, 1979; ² Bündchen et al., 2015; ³ Poorter and
 895 Bongers, 2006; ⁴ Turner, 1994; ⁵ Pallardy, 1981; ⁶ Gratani et al., 2006; ⁷ Westoby, 1998;
 896 ⁸ Pearce et al., 2006; ⁹ Weyers and Meidner, 1990; ¹⁰ Ehleringer and Björkman, 1978; ¹¹
 897 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 ;

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