

1 **Shade alters savanna grass layer structure and function along a gradient of canopy**
2 **cover**

3 **Running title: Savanna grasses turnover across the light gradient**

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30

31 **Abstract**

32

33 **Aim.** In savannas, a grass dominated ground layer is key to ecosystem function via
34 grass-fire feedbacks that maintain open ecosystems. With woody encroachment, tree
35 density increases, thereby decreasing light in the ground layer and potentially altering
36 ecosystem function. We investigated how light availability can filter individual grass
37 species distributions and whether different functional traits are associated with response
38 to a shade gradient in a landscape experiencing woody encroachment.

39 **Location.** Savanna-forest mosaic in the Cerrado domain, southeastern Brazil.

40 **Methods.** Along an encroachment gradient of increasing tree Leaf Area Index (LAI) and
41 shade, we determined how changing light availability alters grass diversity and ground
42 layer structure relative to grass cover and grass functional traits (photosynthetic
43 pathway, underground storage organs, bud protection and traits related to grass shape,
44 size and leaf dimensions).

45 **Results.** Increasing shade led to a decrease in grass cover and grass species richness,
46 and also compositional and functional changes. We found that where tree LAI reached
47 1, grass cover was reduced by 50% and species richness by 30%. While C₄ grass species
48 abundances decreased with increasing shade, the opposite pattern was true for C₃
49 grasses. There were only small differences in light preferences among C₄ subtypes, with
50 PCK species tolerating slightly more shaded conditions. Persistence of some C₄ species
51 under more shaded conditions was possible likely due to an ability to store starch
52 reserves via underground storage organs.

53 **Conclusions.** Woody encroachment changes diversity and structure of the grassy layer
54 that is critical to the functioning of savanna ecosystems, highlighting the dependence of

55 the diverse grass layer on open and sunny conditions. Our results suggest a threshold of
56 tree cover close to $LAI \approx 1$ as being critical to cerrado grassy-layer conservation.

57

58 **Key-words:** cerrado conservation, functional traits, habitat filtering, photosynthetic
59 pathway, shade tolerance, species co-occurrence, underground storage organs

60

61 **Introduction**

62

63 Woody encroachment is one of the major threats to savanna conservation, a
64 globally widespread phenomenon across the savanna biome (Stevens et al., 2017). As
65 tree density increases, forming a closed-canopy system, the species adapted to open and
66 sunny ecosystems are lost, leading to landscape homogenization and local extinction of
67 species specialized to savanna (Pinheiro and Durigan, 2009; Parr et al., 2012; Abreu et
68 al., 2017). Arguably, the most important impact of woody encroachment on savanna
69 function is the elimination of C_4 grasses, and, consequently, of fire (Hoffmann et al.,
70 2012a). Reduced burning further improves conditions for trees, leading to a cascade of
71 events promoting further tree invasion (Hoffmann et al., 2012a). Therefore, clarifying
72 how the grassy-layer responds to the increase of shade is crucial to management actions
73 aimed at reestablishing fire feedbacks in landscapes under woody encroachment (Case
74 and Staver, 2017). However, surprisingly, the relationship between tree cover and grass
75 diversity and structure is poorly defined in savanna ecosystems.

76 Savannas are complex ecosystems with a continuous ground layer dominated by
77 C_4 grasses and a discontinuous tree canopy (Lehmann et al., 2011; Ratnam et al., 2011).
78 Ground layer light availability, that is, shading by trees canopies, is an environmental
79 filter that impacts this grassy-layer structure and composition over just meters (Abdallah

80 et al., 2016). In turn, changes in the grassy layer alters ecosystem flammability, where
81 fire acts to limit tree canopy cover (Hoffmann et al., 2012a, b). Examination of these
82 feedbacks in savannas between trees and grasses, have tended to focus on grass biomass
83 or woody plant recruitment (e.g., Belsky, 1994; Higgins et al., 2012; Hoffmann et al.
84 2012a; Vadigi and Ward, 2013), rather than grassy ground layer composition or the
85 functional traits of the composing flora (Cardoso et al., 2018). However, differences in
86 the light/shade tolerance of grass species are fundamental to unpacking the resilience of
87 savanna ecosystem function to increases in tree cover.

88 Grasses utilizing the C₄ photosynthetic pathway dominate over the tropics and
89 subtropics and are particularly adapted to hot, sunny and seasonally dry environments
90 (Lehmann et al., 2019). At fine scales, the limits of C₄ grasses are strongly determined
91 by light availability (Klink and Joly, 1989; Charles-Dominique et al., 2018) and it has
92 been suggested that photosynthetic subtypes of C₄ grasses (C₄ NADPme, C₄ NADme
93 and C₄ PCK) sort along small-scale gradients in light availability (Veenendaal et al.,
94 1993; Cabido et al., 2008; Charles-Dominique et al., 2018). While the abundance of C₃
95 and C₄ grasses most likely have largely non-overlapping distributions along gradients of
96 light availability, there has been limited investigation of the tree cover threshold at
97 which C₄ grasses are lost from savanna ecosystems and of the impacts of this on ground
98 layer diversity, turnover in functional traits, ecosystem function and patterns of species
99 co-occurrence.

100 In the Cerrado region (Brazilian savanna), we investigated how light availability
101 can filter individual grass species distributions and whether different functional traits
102 are associated with response to a shade gradient in a landscape experiencing woody
103 encroachment (Abreu et al., 2017). We quantified grass composition, richness and
104 ground cover related to Leaf Area Index (LAI) as a surrogate for ground layer light

105 availability. Specifically, we aimed to answer the following questions: i) How does
106 grass cover and species richness change as tree canopy cover increases?; ii) Do C₃ and
107 C₄ grass-communities sort along a gradient in tree cover?; iii) Is variability in grass
108 species distributions associated with different C₄ photosynthetic sub-types or other grass
109 functional traits?; and iv) What is the relative role of environmental filtering versus
110 biotic interactions in shaping ground layer community composition across the light
111 gradient? Because grass subfamilies have been shown to explain habitat preferences and
112 morphological traits better than photosynthetic pathways, we also tested for
113 phylogenetic signal in grass light preference (Liu et al., 2012; Ripley et al., 2015). We
114 expected to find a decrease in grass cover and compositional changes as canopy cover
115 increases and light availability decreases, with grass species more adapted to open
116 conditions being gradually replaced by shade-adapted ones. At a fine scale, we also
117 expected to detect competitive exclusion between grass species that occupy the same
118 light range, and that consequently both environmental filtering and biotic interactions
119 shape grass distribution and functional traits.

120

121 **Methods**

122

123 *Study area*

124

125 This study was conducted at Santa Barbara Ecological Station (SBES) (22°46'
126 33'' to 22°50'3'' S, 49°10'27'' to 49°15'36'' W), São Paulo State, Brazil. The SBES
127 lies near the southeastern edge of the Cerrado Domain, with dry winters and rainy
128 summers. Monthly mean temperatures range from 16° to 24°C and the mean annual
129 precipitation is about 1300 mm. The soils of the study area are deep oxisols with high

130 sand content, low nutrients and high aluminum saturation (Abreu et al., 2017). There
131 were no exotic cattle or large native grazers in the study site, which could affect plant
132 communities and species distribution. Native cervid species in the Cerrado are solitary
133 or live in small groups. The vegetation in the SBES ranges from open cerrado
134 grasslands to closed canopy forest (Abreu et al., 2017). Along this gradient, the open
135 ecosystem, composed of a continuous grass layer with scattered savanna trees, is
136 replaced by closed-canopy forests with the ground covered by a litter layer (Appendix
137 S1). This vegetation gradient is a result of at least 30 years of woody encroachment due
138 to fire suppression. Importantly, all the closed-canopy vegetation studied here was a
139 savanna or grassland three decades ago (see Abreu et al., 2017 for details).

140

141 *Experimental design and data collection*

142

143 Three savanna-forest transitions were selected at distances of two to five km
144 from each other (Abreu et al., 2017). The vegetation types in these areas were classified
145 as recently formed forest (cerradão) due to encroachment, tree-dominated savanna
146 (cerrado *sensu stricto*) and grass-dominated savanna (campo cerrado) (Abreu et al.,
147 2017). A total of 30 plots were distributed across the three savanna-forest transitions, 12
148 in the recently formed forest, 12 in tree-dominated savanna and 6 in the grass-
149 dominated savanna. Each plot was 20 m × 50 m (0.1 ha). To sample the grass
150 community, we used 40 1 m × 1 m subplots distributed in a grid within each 0.1-ha plot.
151 In each 1m x 1m subplot, all grass individuals were counted and identified to species
152 level (see Appendix S2 for subplots distribution across the light gradient).

153 Cerrado grasses are essentially caespitose (Sarmiento, 1972), making it easy to
154 distinguish individual tussocks above the soil surface. We considered individuals as

155 plants clearly separated from each other at soil surface. We acknowledge that in some
156 cases these may not represent genetically or physiologically distinct individuals. Percent
157 of ground cover by grasses was estimated visually (Wikum and Shanholtzer, 1978),
158 with only living plant material included in cover estimation. Vegetation sampling took
159 place in April 2015, at the end of the rainy season and peak greenness, before grasses
160 cured.

161 To quantify the gradient of light availability in the ground layer, Leaf Area
162 Index (LAI) of the overstory was measured using a hemispherical photograph of the
163 canopy at each 1 m × 1 m subplot. Photos were taken before sunrise, after sunset, or
164 under homogeneous sky to ensure conditions of diffuse light, with a tripod to position
165 the camera (Canon EF 8-15mm fisheye lens) at a height of 1 m. Photos were taken with
166 an underexposure of one f-stop, the colour images were converted to black and white
167 and using maximum blue contrast (Nobis and Hunziker, 2005; Thimonier et al., 2010;
168 Macfarlane et al., 2014). Images were analyzed with Hemisfer 2.12 using an automatic
169 threshold for closed-canopy vegetation and with a supervised manual threshold under
170 open canopies (Abreu et al., 2017).

171

172 *Data analyses*

173

174 The photosynthetic pathway of each species was classified based on the
175 literature (Davidse, 1978; Sage and Monson, 1999; Giussani et al., 2001; Alvarez et al.,
176 2005; Zuloaga et al., 2018) (Appendix S3). Further, species functional traits known to
177 impact flammability, resilience to fire or to otherwise potentially play a role in
178 distribution along a light gradient were compiled (Table 1) (Charles-Dominique et al.,
179 2018; Archibald et al., 2019). We examined grass functional traits including: the

180 presence of underground storage organs with capacity to store nutrients and
181 carbohydrates (rhizome, *sensu* Pausas et al., 2018); grass architecture (cespitose:
182 growing in dense tufts versus mat-forming: horizontal and highly spread growth due to
183 long rhizomes); leaf sheath persistence for protection of meristematic tissue (tunic
184 grasses, *sensu* Rachid-Edwards, 1956); maximum height; maximum culm height;
185 maximum leaf width and length. Data on these seven traits were compiled from the
186 literature (Wanderley et al., 2001), herbarium specimens and field observations (Table
187 1, Appendix S3). Traits were classified as response traits and effect traits following
188 Violle et al. (2007).

189

190 *Structural changes and species distribution through the light gradient*

191 To characterize the general patterns in grass community changes along the
192 woody encroachment gradient, total grass cover and grass species richness per subplot
193 along the light gradient were modeled using generalized additive models (GAM). The
194 models were fitted using the ‘*mgvc*’ package (Wood, 2017). For both response variables
195 (cover and richness) we modeled the average values sampled at each LAI value
196 registered (predictor variable). For example, for all subplots with overstory LAI values
197 equal to zero, we have taken the average richness and grass cover. This method reduces
198 the extremely high variances inherent to the numerous small subplots (1m²) and is a
199 common procedure in nested designs (Quinn and Keough, 2002). It is important to
200 clarify that we did not group subplots within a plot (20 m x 50 m plot) due to the large
201 variation in canopy cover among subplots. Within a plot, it was possible to find subplots
202 (1 m²) with very low overstory LAI and also high LAI values (mainly due to the
203 nucleation process that is particular to cerrado landscapes in woody encroachment). To
204 analyze average subplot-values from a big plot would obscure the ecological patterns.

205 In addition, in these models we deleted subplots with grass richness and cover equal to
206 zero due to the monodominance of shrubs (e.g., *Campomanesia adamantium* or *Psidium*
207 *australe*), which eliminated the noise related to open plots (low overstory LAI) without
208 grass species or cover.

209 To test for association between species frequency per subplot and overstory LAI
210 along the gradient, we fitted generalized linear mixed models (GLMM), with a binomial
211 distribution and a logit link function, for species with more than 10 individuals sampled,
212 site (20 m x 50 m plot) being considered as the random effect. The models were fitted
213 using the ‘*mgvc*’ package (Bates et al., 2015).

214 Permutational analysis of variance (PERMANOVA) was conducted to test for
215 differences in the light distribution among photosynthetic pathways, with overstory LAI
216 as the dependent variable, and photosynthetic pathway as the factor (C₄ NADPme, C₄
217 NADme, C₄ PCK and C₃) (Legendre and Legendre, 1998).

218

219 *Evolutionary patterns in light regime preference*

220 To investigate the possibility of niche conservatism in light regime preference
221 we calculated phylogenetic signal using Pagel’s λ to estimate the degree that LAI
222 maximum, median and minimum values (for each species occurrence) show “niche
223 similarity” depending on phylogeny, following the prediction of a simple Brownian
224 model of trait evolution (Pagel, 1999; Münkemüller et al., 2012). In the estimation of
225 Pagel’s λ , a value of $\lambda=0$ means that there is no phylogenetic dependence in the trait
226 evaluated, while $\lambda=1$ indicate a pattern of phylogenetic dependence expected under
227 Brownian motion evolution of the trait. The phylogenetic tree for the grass species
228 studied was generated using the Angiosperm megaphylogeny of Zanne et al. (2014)
229 updated and corrected by Quian and Jin (2016). We used the “*SPhyloMaker*” function to

230 prune our species list from the megatree, which includes the updated version of the
231 Zanne’s tree (Quian and Jin, 2016) and *phytools* package (Revell, 2012). For the species
232 not present in the original megatree, their phylogenetic relationships were resolved
233 using taxon-specific molecular phylogenies (Soreng, 2015; Saarela et al., 2018). This
234 approach reduces the bias in tree building and gives a more precise calculation of
235 phylogenetic indices (Vega-Álvarez et al., 2019). As the tree was constructed based on
236 the “Scenario 2” of Quian and Jin (2016) (*i.e.* polytomies are randomly resolved inside
237 the specific genera), 100 phylogenetic trees were generated and the Pagel’s λ was
238 estimated for each tree, to account for phylogenetic uncertainty in the analysis. We
239 report here the average λ values.

240 In addition, we investigated if the presence of C₄ grass species in shadier
241 conditions is related with functional traits other than photosynthetic pathway (traits
242 described in table 1), while accounting for phylogenetic relatedness in the analysis. In
243 this analysis, we did not separate C₄ subtypes. We performed a phylogenetic logistic
244 regression for the traits: presence of underground storage organs and bud protection. As
245 all C₄ grasses sampled have caespitose architecture (Appendix S3), we did not perform
246 analysis using this trait. For the continuous traits (maximum height, maximum culm
247 height, maximum leaf width and length) we fitted a linear phylogenetic regression. The
248 phylogenetic regression models were fitted using the “*phylolm*” package (Ho & Ane,
249 2014). As the maximum values of overstory LAI for each species can be interpreted as a
250 measure of shade tolerance, the maximum LAI values for species distribution were
251 considered the independent variable in the phylogenetic regression models.

252

253 *Environmental filtering and species interaction*

254 To determine the relative role of environmental filtering and biotic interactions
255 (positive or negative association) in determining community composition, we fitted
256 multi-variate abundance models that combined generalized linear models for each
257 species, using LAI as a covariate and site as a random factor (Hui, 2018). Models were
258 fitted with a negative binomial distribution. Site, which corresponded to a 20 m x 50 m
259 plot, was included to account for plot-level differences in the residual analyses.
260 Correlations between species were then able to be distinguished as due to environmental
261 responses or species interactions, where the residual correlation matrix is used to
262 approximate species interactions (Hui, 2016). For these analyses, the “*boral*” package
263 was used (Hui, 2016; Hui, 2018), while species correlations were plotted using the
264 “*corrplot*” package (Wei and Simko, 2017). All analyses were carried out using the R
265 version 3.5.1 environment (R Development Core Team, 2018).

266

267 **Results**

268

269 *Structural changes and species distribution through the light gradient*

270

271 Across all plots, decreasing light availability corresponded to an overall decrease
272 in both grass cover ($F= 54.62$, $P<0.001$, $R^2_{(adj)}= 0.50$, Deviance explained= 50.6%) and
273 grass species richness ($F= 62.73$, $P<0.001$, $R^2_{(adj)}= 0.57$, Deviance explained= 57.5%)
274 and these relationships are non-linear (Figure 1). Grass cover was reduced by 48% as
275 tree LAI reached 1, and by 70% at LAI = 2. Grass-species richness reduced by 30% at
276 LAI =1 and 45% as LAI reaches 2. At overstory LAI = 0, the most frequent grass cover
277 is 70 to 90% and 4 to 6 species/m².

278 A total of 8110 individual plants from 35 grass species were sampled across the
279 1200 1 m² plots (nested within the 30 larger 0.1 ha plots). These 35 species were from
280 the subfamilies Aristidoideae (9%), Chloridoideae (14%) and Panicoideae (77%), from
281 the PACMAD clade. Considering the number of individuals sampled across the entire
282 study, 95% belong to Panicoideae, with 40% from tribe Paniceae, 33% Paspaleae and
283 19% Andropogoneae. Considering all species, 91% were C₄, of which 75% were
284 classified as C₄ NADPme, 19% NADme and 6% PCK (Appendix S3).

285 Among the 28 species that had distributions analyzed by generalized mixed
286 models, 71% (20 species) were significantly negatively related to increasing overstory
287 LAI. All C₄ species were negatively affected by shading (see negative β_1 estimates for
288 models in Appendix S3). In contrast, the frequency of the three C₃ species (*Lasiacis*
289 *ligulata*, *Panicum millegrana* and *Panicum sellowii*) was positively related to shading
290 ($\beta_1=2.5$, $Z=4.13$, $P<0.001$; $\beta_1=1.89$, $Z= 2.48$, $P<0.001$, $\beta_1=2.32$, $Z= 7.07$, $P<0.001$,
291 respectively). The frequency occurrences of only five C₄ species were unrelated to LAI
292 (Appendix S3). Parameters from all logistic regressions are reported in Appendix S3.

293 In general, a strong dichotomous pattern was found with C₄ species decreasing,
294 regardless of the photosynthetic subtype, and C₃ species increasing in frequency with an
295 increase in shade (Figure 2). All C₄ subtypes exhibited a preference for greater light
296 availability, with median LAI values of 0.45 (C₄-NADme), 0.64 (C₄-NADPme) and
297 0.78 (C₄-PCK), compared to C₃ species in which median LAI was 2.88 ($F= 12160$,
298 $P<0.001$, $df= 3$, Figure 3). Among C₄ subtypes, C₄-NADme were restricted to sites with
299 lower tree cover than C₄-PCK and C₄-NADPme (Figure 3).

300

301 *Phylogenetic background in light preferences*

302

303 Grass species exhibited a strong phylogenetic signal for median overstory LAI
304 ($\lambda=0.83$, $P<0.01$) as closely related species tend to have the same photosynthetic
305 pathway (Figure 4), and effects of phylogenetic structure and photosynthetic pathways
306 in the light preferences can be difficult to disentangle. However, *Panicum olyroides*, a
307 C_4 species typical of open cerrado vegetation, has distribution mainly in $LAI < 1$ that
308 differs from other *Panicum* C_3 species studied (Figure 4, Appendix S3). There was no
309 significant phylogenetic signal for the minimum and maximum LAI ($\lambda<0.001$, $P=1$).

310 Along the light gradient, the distribution of some C_4 species extended into closed
311 canopy conditions ($LAI > 1$), where light at the ground level becomes scarce (Figure 4).
312 Exploring the functional traits that could explain these distributions by phylogenetic
313 regression, the presence of underground storage organs was positively correlated with
314 the maximum LAI values, with a strong phylogenetic effect in these correlations (Table
315 2). Contrary to our expectations, we did not find taller C_4 grasses, or those with larger
316 leaves to be better able to tolerate shaded conditions.

317

318 *Environmental filtering and species interaction*

319

320 The correlation between species due to plot and LAI showed a clear habitat
321 separation for C_3 and C_4 species (Figure 5a). With few exceptions, all C_4 species were
322 positively associated, therefore occurring in similar environments. The same pattern was
323 found for C_3 species (Figure 5a). The negative correlations in Figure 5a occur only in
324 comparisons between C_3 and C_4 species. These results highlight the effect of the
325 environmental filtering in the grass-distribution through the gradient studied. There was
326 some evidence for competitive exclusion among grass species in some cases, based on
327 patterns of co-occurrence (Figure 5b). The big-tussock species *Elionurus muticus*,

328 *Axonopus pressus*, *Loudetiopsis chrysothrix* and *Paspalum lachneum* were negatively
329 correlated with some short species (see species functional traits in Appendix S3). We do
330 not find a pattern in the grass traits that is correlated with the positive associations in
331 Figure 5b.

332

333 **Discussion**

334

335 With woody encroachment, shading by trees increases, and grass cover and
336 species richness decrease in this Brazilian savanna alongside a turnover in the functional
337 traits of the composing flora. There is a dichotomy in the patterns of C₄ versus C₃
338 species diversity as ground layer light availability becomes limiting. The lack of
339 phylogenetic signal for shade tolerance (as inferred from the maximum canopy LAI
340 under which the species occurred) showed that other proximate causes likely explain C₄
341 grass persistence in more shaded conditions. For example, grass species with
342 underground storage structures were positively correlated with the maximum LAI of
343 species distributions. Our results show a strong effect of environmental filtering shaping
344 grass species distributions at the landscape scale (across savanna-forest transitions), and
345 at the local scale (community level), where there is evidence for competitive exclusion
346 of some small and delicate grasses by species that form large tussocks.

347 The results indicate that an increase in tree canopy cover over open cerrado
348 vegetation will systematically change the grass community structure and composition.
349 The total grass cover decreases sharply to less than ~50% when LAI surpasses 1.
350 Meanwhile, it is still possible to find grass diversity in the understory, with more than
351 half the species remaining when LAI reaches 2, which corresponds to an almost closed-
352 canopy vegetation. In terms of function, changes in grass cover may be paramount when

353 considering the maintenance of the whole savanna system. Frequent, cool ground fire
354 conserves savannas as species-rich systems (Durigan and Ratter, 2016; Abreu et al.,
355 2017). For operational fire feedbacks, a contiguous grass-layer is necessary (De Castro
356 and Kauffman, 1998). Consequently, despite C₄ grass species being able to maintain
357 small populations in shaded conditions, the reduction of grass cover when LAI > 1 will
358 likely affect fire feedbacks (Hoffmann et al., 2012b).

359 Changes in structure of the grass layer were accompanied by changes in floristic
360 and functional composition. While the majority of C₄ grasses decreased in frequency as
361 shade increased, some species were able to maintain a reduced population at
362 intermediate light levels, with LAI between 1 and 2 (e.g. *Tristachya leiostachya*,
363 *Paspalum pectinatum*, *Paspalum carinatum*, *Elionurus muticus*) (Fig. 4). These species
364 extend their distributions into shadier conditions, but are not truly adapted to shade
365 (Rossato et al., 2018). Our results showed that underground storage organs were
366 associated with shade tolerance (Table 2) and it may be that these species are relicts
367 from the open savanna existing 30 years before sampling (Abreu et al., 2017). Storing
368 resources can represent an adaptation to fire, by facilitating rapid regrowth, and which
369 can also help species to survive for a limited time period in shaded understories (Dong
370 and Kroon, 1994; Cruz, 1997; De Moraes et al., 2016; Pinheiro et al., 2016). Savanna
371 grasses under dense canopy, however, are usually not able to reproduce, germinate or
372 propagate (Gardener et al., 2001; Kolb et al., 2016). Consequently, populations of these
373 species are likely to contract over time (Cruz, 1997). Essentially, these shaded grasses
374 are likely ‘zombies’, still living but likely dependent on stored reserves. These results
375 raise potential questions for future research, such as: for how long can C₄ grass species
376 survive under shaded conditions using their reserves? If fire returns to the system, will
377 these shaded species be able to resprout or produce seeds? Fire could also increase

378 mortality, since C₄ grasses under shaded conditions cannot replenish the stored reserves
379 (Zimmermann et al., 2010).

380 Among the C₄ subtypes, we found a narrow gradient of light preferences where
381 NADme presents a median distribution in more open conditions, followed by NADPme,
382 and PCK in shadier but still open conditions (LAI<1). This partially corroborates the
383 pattern observed in a savanna-forest mosaic in South Africa (Charles-Dominique et al.,
384 2018). Contrasting with results from Charles-Dominique et al. (2018), NADme species
385 presented less tolerance to shade, being more frequent in open and sunny patches of the
386 mosaic studied. This difference could be a product of the distinct data collection
387 procedures, since we sampled the entire grass community and not only the most
388 abundant species. However, even if we had considered only the most abundant species
389 in the subplots the results still would be different as only NADPme and PCK species
390 would be registered in this hypothetical scenario. The NADPme subtype was the most
391 abundant in terms of number of species and individuals in the study site, compared with
392 the other C₄ subtypes. Regarding the PCK subtype, one species - *Loudetiopsis*
393 *chrysothrix*, can be also quite abundant in some plots. In addition, the NADPme subtype
394 is generally associated with wetter environments (Veenendaal et al., 1993; Cabido et al.,
395 2008), while the NADme subtype increases under drier conditions (Cabido et al., 2008).
396 Thus, the remarkable differences in edaphoclimatic conditions between the two
397 savannas (southeastern Cerrado and South Africa savanna) could better explain the
398 differences than the data collection procedures. Our samples were taken in an area with
399 lower fertility soils and higher annual precipitation than the area studied by Charles-
400 Dominique et al. (2018). Further investigation is necessary to elucidate whether the
401 patterns for the photosynthetic pathways found here differ from drier savannas of the
402 Neotropics.

403 Within savanna ecology, investigation of plant competition has focused on
404 grasses versus trees to explain coexistence (Scholes and Archer, 1997). Much research
405 has examined the feedbacks between functional traits and disturbance to describe how
406 grasses “win” the competition via flammability promoting fire that negatively affects
407 the growth of sapling trees in the “fire-trap” (Hoffman et al., 2012a). But, is there
408 competition among grass species? In humid and productive savanna ecosystems, where
409 resources are abundant (e.g. water), species are prone to compete (Liu et al., 2012).
410 Most species documented here are caespitose, some of which do not seem to invest in
411 large root biomass (Sarmiento, 1992; Taylor et al., 2010-for Paniceae; Aires et al.,
412 2014) and rapidly senesce aboveground biomass in the dry season (Sarmiento, 1992).
413 Consequently, these grass species are unlikely to compete for water later into the dry
414 season. Rather, competition is likely to be for space and light through times of high-
415 water availability, and both tall and large grasses with dense canopies and high annual
416 production of biomass (such as *Paspalum lachneum*) can exclude smaller and more
417 slender grasses (such as *Agenium leptocladum* or *Eragrostis leucosticta*) in the absence
418 of fire (Figure 5b). The higher diversity observed in high light conditions may well be
419 mediated by fire, which reduces the overall grass biomass creating space and conditions
420 for the small grasses to propagate (Coutinho, 1990, Fidelis et al., 2012; Pilon et al.,
421 2019). We do not interpret the positive associations between species (Figure 5b) as a
422 trend towards facilitation processes. In our interpretation, the positive association is a
423 strong signal for the same habitat preferences, and absence of competitive exclusion, in
424 most cases.

425 There is a turnover of grass species at the landscape and community level in a
426 savanna-forest mosaic in the Neotropics, driven by light availability. As the ecology and
427 plant community dynamics of the savanna ground-layer are a “black-box” in the

428 knowledge of savanna ecosystem processes, our results highlight the importance of
429 habitat filtering as a major force shaping the assemblage of grasses in savanna-forest
430 mosaics. In addition, considering the increase in canopy cover and landscape
431 homogenization due to the woody encroachment process, the reduction in grass cover as
432 tree LAI surpasses a value of 1 has strong implications for fire management and
433 savanna conservation, an important threshold with regards to grass cover and diversity.

434

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439

440 **Authors' Contributions**

441 CL, GD, WAH, NALP conceived and designed the research, NALP, GD, RCRA
442 collected the data. NALP analyzed the data, and NALP, CL, KD, JR, RTP, GD, WAH
443 interpreted the results. NALP, CL, JR wrote the paper. All authors revised the paper and
444 gave final approval for publication.

445

446 **Data Availability statement**

447 Data used for this study are available as supporting information and additional data are
448 available through contacting the primary author in the form of Excel spreadsheets.

449

450 **References**

451

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673

674 **Supporting information**

675 **Appendix S1.** Mosaic of vegetation types studied in São Paulo Cerrado area, Brazil.

676 **Appendix S2.** Subplot distribution along the light gradient in the experimental areas.

677

678 **Appendix S3.** Grass species and functional traits sampled in Brazilian savanna.

679

680 **Table 1:** Description and predicted environmental role of traits associated to fire and
 681 shade tolerance for savanna grass species.

Trait	Attribute	Description and ecological meaning	References
Photosynthetic pathway	C ₃ , C ₄ NADme, C ₄ NADPme and C ₄ PCK	Effect trait: The flammability associated to the high C:N ratio in C ₄ species that can act in the fire feedbacks	Archibald et al. 2019
		Response trait: Shade and drought tolerance may be associated to different photosynthetic pathways	Charles-Dominique et al. 2018, Veenendaal et al. 1993, Cabido et al. 2008
Underground storage organs	Rhizomes (presence vs. absence)	Response trait: Allows for quick resprouting after elimination of aboveground biomass by disturbance	Sarmiento 1992, de Moraes et al. 2016, Pausas et al. 2018, Archibald et al. 2019
Bud protection	Presence vs. absence	Response trait: Persistent old leaf-sheath at the plant base, protecting the meristematic tissue during fire passage	Rachid-Edwards 1956, Warming (1892)
Maximum height	Continuous (cm)	Response trait: Light capture efficiency	Lebrija-Trejos et al. 2010
Maximum culm height	Continuous (cm)	Response trait: Higher culms may represent advantages in seed dispersal in post-fire environment	Thomson et al. 2011, Geissler et al. 2019
Maximum leaf width	Continuous (cm)	Response trait: When light is a scarce resource, <i>e.g.</i> under an overstory, grass species with wide leaves are predicted to be favored. In an opposite way, in open vegetation types, leaves are predicted to be narrow	Lebrija-Trejos et al. 2010, Solofondranohatra et al. 2018
Maximum leaf length	Continuous (cm)	Effect trait: Long leaves in fire prone grassland can propagate the fire far away from the meristematic tissue during fire passage, and produces a less compact fuel enhancing fire spread	Hoffmann et al. 2012b, Solofondranohatra et al. 2018, Archibald et al. 2019
		Response trait: It is expected to find longer leaves in grass species in open vegetation types than in under close canopy	
Architecture	Caespitose or mat forming	Response trait: Vertical growth (caespitose) increases height gain, light capture and moves flammable material away from the buds. Horizontal growth (mat forming) allows lateral spread and quick ground cover, however bud is less protected from fire	Solofondranohatra et al. 2018, Archibald et al. 2019

682

683

684 **Table 2:** Phylogenetic regression parameters for correlations of species' traits with
 685 shade tolerance (maximum Leaf Area Index values). (*Z-value for logistic regression, t-
 686 value for linear regression)

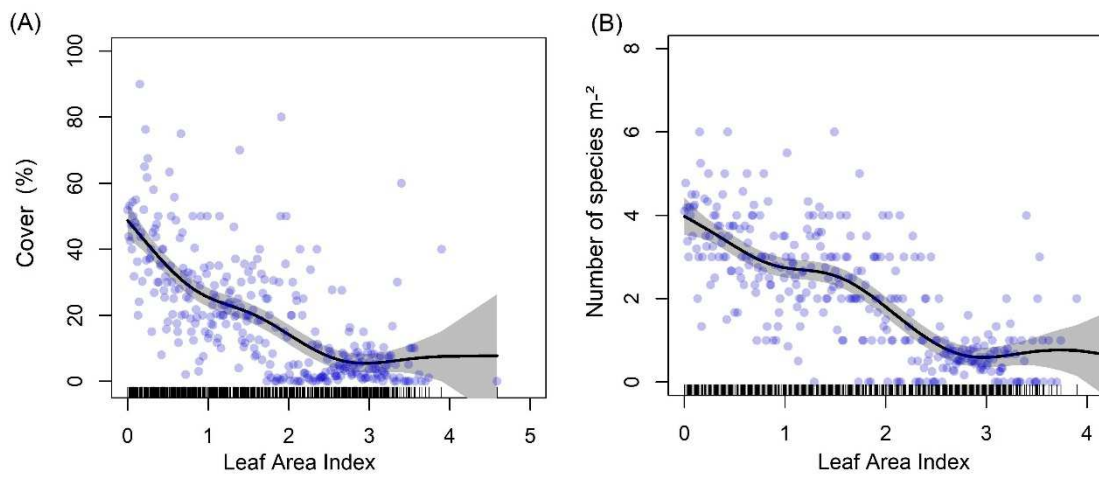
687

Trait	β_1	Phylogenetic correlation parameter	Statistic*	P-value
Underground storage organ	1.10	0.4	2.15	0.03
Tunic Grass	0.97	5.6	0.56	ns
Maximum height	1.76	< 0.000	0.48	ns
Maximum culm height	-9.46	< 0.001	-0.89	ns
Maximum leaf width	-0.16	< 0.001	-1.95	ns
Maximum leaf length	-0.14	0.1	-0.03	ns

688

689

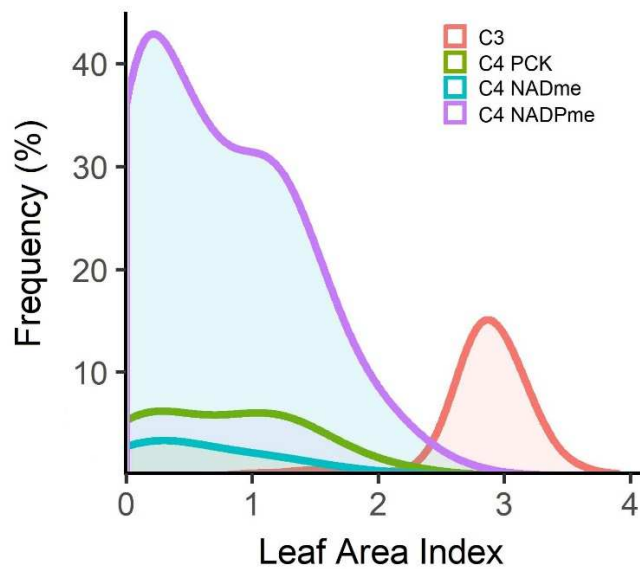
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691

692 Figure 1. Changes in grass cover (a) and grass species richness (b) in the light gradient
693 in a savanna-forest mosaic. Both C₃ and C₄ species are included. Solid lines represent
694 the predicted distribution, with the grey shaded area giving the 95% confidence interval.

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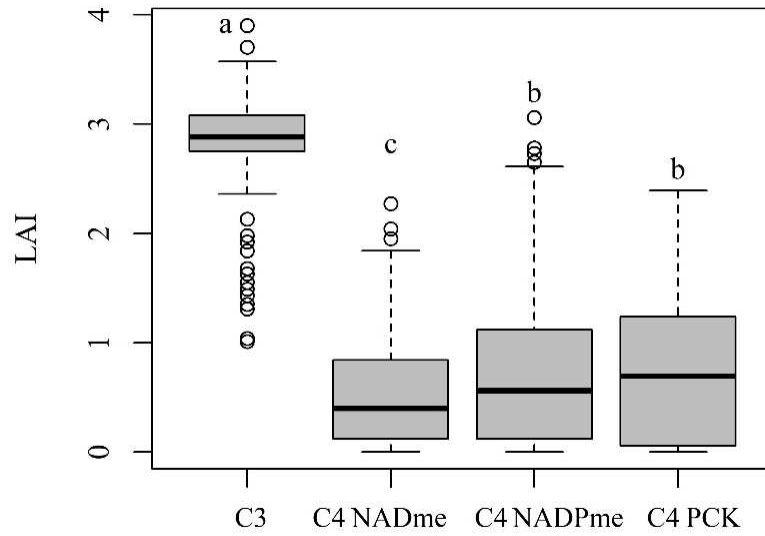
696

697 Figure 2. Density plot considering the frequency of species, categorized by
 698 photosynthetic pathway, along the light gradient in a neotropical savanna-forest mosaic.
 699 Total area of each curve represents the proportion of species frequency of a given
 700 photosynthetic type considering all plots sampled in the entire study site. Therefore, the
 701 sum of all areas beneath all four curves is 100%.

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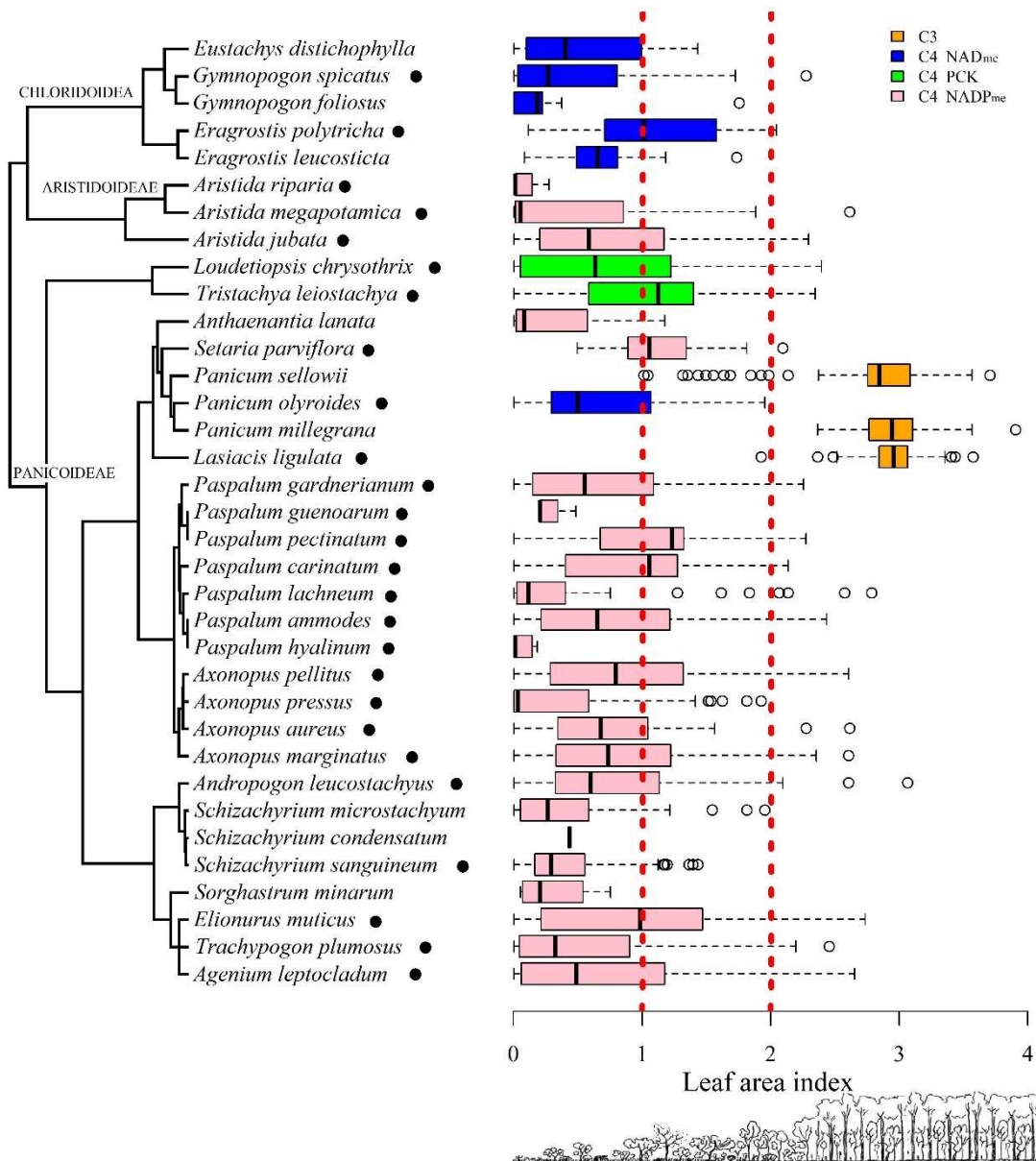
707 Figure 3. Frequency distribution of grass species of different photosynthetic pathways
 708 along the light gradient. Boxplots represent the median and quartiles, outlier values
 709 beyond the error bars are indicated with open circles, columns having a common letter
 710 are not significantly different (Tukey; $P \geq 0.05$).

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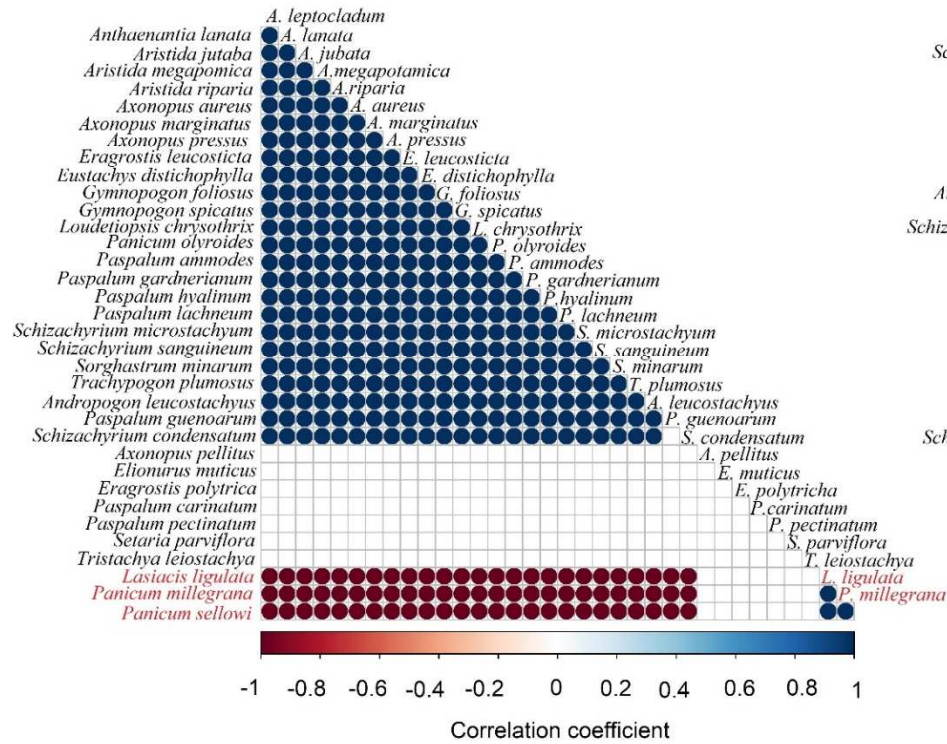
715

716 Figure 4. Phylogenetic tree for savanna grass species and their respective distribution
 717 along the light gradient in the savanna-forest mosaic at our study site. Black dots
 718 represent the presence of underground storage organs. Red dashed lines represent three
 719 light classes based on previous investigation of the general species distribution and the
 720 literature (Scholes & Archer, 1997), as follows: LAI ≤ 1 (open physiognomies), $1 < \text{LAI}$
 721 < 2 (intermediate conditions, with a large proportion of the soil surface with shade
 722 projected by trees, but still with gaps in the canopy) and LAI > 2 (forest, with soil
 723 surface completely shaded by tree canopies).

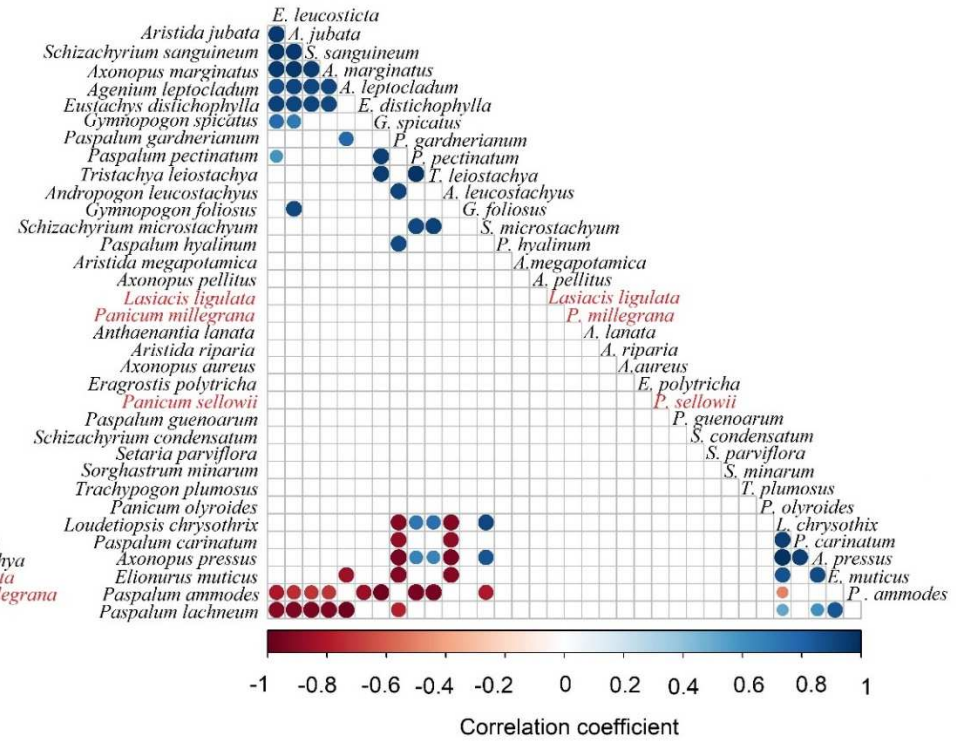
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(A)



(B)



726

727 Figure 5. Correlations in species abundance. Correlation due to covariates (a) and correlations of residuals, after accounting for overstorey LAI
 728 and site, performed to quantify species interactions (b). Significant correlations, based on the 95% credible intervals, are represented by colored
 729 dots. Different colors in the circles represent the sign of the Pearson correlations that ranges from -1 (red tones) to 1 (blue tones). Names in red
 730 indicate C₃ species.