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## Shade alters savanna grass layer structure and function along a gradient of canopy cover

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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**

4

5 Natashi A.L. Pilon<sup>1,4\*</sup> (ORCI ID: 0000-0001-7985-5842), Giselda Durigan<sup>1,2</sup> (ORCI ID:  
6 0000-0003-0693-3154), Jess Rickenback<sup>3,4</sup>, R. Toby Pennington<sup>4,5</sup> (ORCI ID: 0000-  
7 0002-8196-288X), Kyle G. Dexter<sup>3,4</sup>, William A. Hoffmann<sup>6</sup> (ORCI ID: 0000-0002-  
8 1926-823X), Rodolfo C.R. Abreu<sup>6,7</sup> (ORCI ID: 0000-0002-8797-4654), Caroline E. R.  
9 Lehmann<sup>3,4</sup> (ORCI ID: 0000-0002-6825-124X)

10

11 <sup>1</sup>Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Caixa Postal  
12 6109, Campinas, São Paulo 13083-865, Brazil.

13 <sup>2</sup>Laboratório de Ecologia e Hidrologia Florestal, Floresta Estadual de Assis, Instituto  
14 Florestal, Assis, São Paulo 19802-970, Brazil.

15 <sup>3</sup>School of GeoSciences, University of Edinburgh, Edinburgh, EH9 3FF, UK

16 <sup>4</sup>Royal Botanic Garden Edinburgh, Edinburgh, UK

17 <sup>5</sup>Department of Geography, The University of Exeter, Exeter, UK

18 <sup>6</sup>Department of Plant and Microbial Biology, North Carolina State University, Raleigh,  
19 NC 27695–7612, USA.

20 <sup>7</sup> Departamento de Ciências Ambientais, Universidade Federal Rural do Rio de Janeiro,  
21 Seropédica, Rio de Janeiro, CEP 23897-000, Brasil.

22 \*Corresponding author: [natashipilon@gmail.com](mailto:natashipilon@gmail.com)

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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 C<sub>4</sub> grass species  
48 abundances decreased with increasing shade, the opposite pattern was true for C<sub>3</sub>  
49 grasses. There were only small differences in light preferences among C<sub>4</sub> subtypes, with  
50 PCK species tolerating slightly more shaded conditions. Persistence of some C<sub>4</sub> 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 C<sub>4</sub> 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 C<sub>4</sub> 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 C<sub>4</sub> grasses (C<sub>4</sub> NADPme, C<sub>4</sub> NADme  
93 and C<sub>4</sub> 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 C<sub>3</sub>  
95 and C<sub>4</sub> 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 C<sub>4</sub> 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 C<sub>3</sub> and  
107 C<sub>4</sub> grass-communities sort along a gradient in tree cover?; iii) Is variability in grass  
108 species distributions associated with different C<sub>4</sub> 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 (1m<sup>2</sup>) 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 m<sup>2</sup>) 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 (C<sub>4</sub> NADPme, C<sub>4</sub>  
217 NADme, C<sub>4</sub> PCK and C<sub>3</sub>) (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  $\lambda$  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  $\lambda$ , 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  $\lambda$  was  
238 estimated for each tree, to account for phylogenetic uncertainty in the analysis. We  
239 report here the average  $\lambda$  values.

240 In addition, we investigated if the presence of C<sub>4</sub> 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 C<sub>4</sub> subtypes. We performed a phylogenetic logistic  
244 regression for the traits: presence of underground storage organs and bud protection. As  
245 all C<sub>4</sub> 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/m<sup>2</sup>.

278 A total of 8110 individual plants from 35 grass species were sampled across the  
279 1200 1 m<sup>2</sup> 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 C<sub>4</sub>, of which 75% were  
284 classified as C<sub>4</sub> 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 C<sub>4</sub> species were negatively affected by shading (see negative  $\beta_1$  estimates for  
288 models in Appendix S3). In contrast, the frequency of the three C<sub>3</sub> 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 C<sub>4</sub> 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 C<sub>4</sub> species decreasing,  
294 regardless of the photosynthetic subtype, and C<sub>3</sub> species increasing in frequency with an  
295 increase in shade (Figure 2). All C<sub>4</sub> subtypes exhibited a preference for greater light  
296 availability, with median LAI values of 0.45 (C<sub>4</sub>-NADme), 0.64 (C<sub>4</sub>-NADPme) and  
297 0.78 (C<sub>4</sub>-PCK), compared to C<sub>3</sub> species in which median LAI was 2.88 ( $F= 12160$ ,  
298  $P<0.001$ ,  $df= 3$ , Figure 3). Among C<sub>4</sub> subtypes, C<sub>4</sub>-NADme were restricted to sites with  
299 lower tree cover than C<sub>4</sub>-PCK and C<sub>4</sub>-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<sub>4</sub> species typical of open cerrado vegetation, has distribution mainly in LAI < 1 that  
308 differs from other *Panicum* C<sub>3</sub> 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<sub>4</sub> 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<sub>4</sub> 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<sub>3</sub> and C<sub>4</sub> species (Figure 5a). With few exceptions, all C<sub>4</sub> species were  
322 positively associated, therefore occurring in similar environments. The same pattern was  
323 found for C<sub>3</sub> species (Figure 5a). The negative correlations in Figure 5a occur only in  
324 comparisons between C<sub>3</sub> and C<sub>4</sub> 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 C<sub>4</sub> versus C<sub>3</sub>  
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 C<sub>4</sub>  
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 C<sub>4</sub> 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 C<sub>4</sub> 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 C<sub>4</sub> 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 C<sub>4</sub> grasses under shaded conditions cannot replenish the stored reserves  
379 (Zimmermann et al., 2010).

380         Among the C<sub>4</sub> 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 C<sub>4</sub> 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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#### 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
<b>Photosynthetic pathway</b>	C <sub>3</sub> , C <sub>4</sub> NADme, C <sub>4</sub> NADPme and C <sub>4</sub> PCK	Effect trait: The flammability associated to the high C:N ratio in C <sub>4</sub> 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
<b>Underground storage organs</b>	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
<b>Bud protection</b>	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)
<b>Maximum height</b>	Continuous (cm)	Response trait: Light capture efficiency	Lebrija-Trejos et al. 2010
<b>Maximum culm height</b>	Continuous (cm)	Response trait: Higher culms may represent advantages in seed dispersal in post-fire environment	Thomson et al. 2011, Geissler et al. 2019
<b>Maximum leaf width</b>	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
<b>Maximum leaf length</b>	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	
<b>Architecture</b>	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

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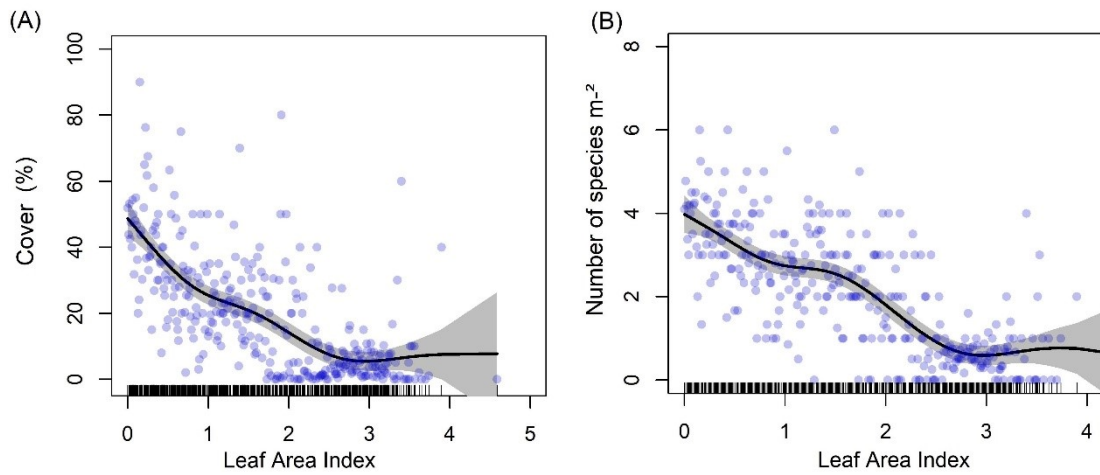
684 **Table 2:** Phylogenetic regression parameters for correlations of species' traits with  
 685 shade tolerance (maximum Leaf Area Index values). (\*Z-value for logistic regression, t-  
 686 value for linear regression)  
 687

Trait	$\beta_1$	Phylogenetic correlation parameter	Statistic*	P-value
<b>Underground storage organ</b>	1.10	0.4	2.15	0.03
<b>Tunic Grass</b>	0.97	5.6	0.56	ns
<b>Maximum height</b>	1.76	< 0.000	0.48	ns
<b>Maximum culm height</b>	-9.46	< 0.001	-0.89	ns
<b>Maximum leaf width</b>	-0.16	< 0.001	-1.95	ns
<b>Maximum leaf length</b>	-0.14	0.1	-0.03	ns

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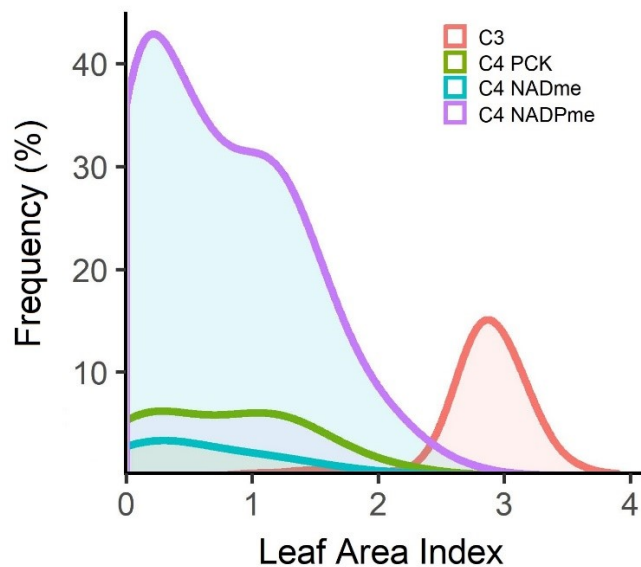
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692 Figure 1. Changes in grass cover (a) and grass species richness (b) in the light gradient  
693 in a savanna-forest mosaic. Both C<sub>3</sub> and C<sub>4</sub> species are included. Solid lines represent  
694 the predicted distribution, with the grey shaded area giving the 95% confidence interval.

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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.

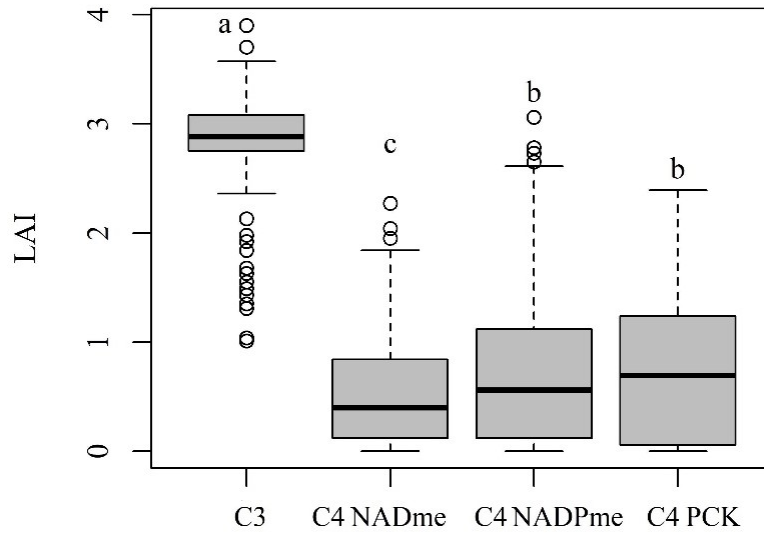
699 Total area of each curve represents the proportion of species frequency of a given  
 700 photosynthetic type considering all plots sampled in the entire study site. Therefore, the  
 701 sum of all areas beneath all four curves is 100%.

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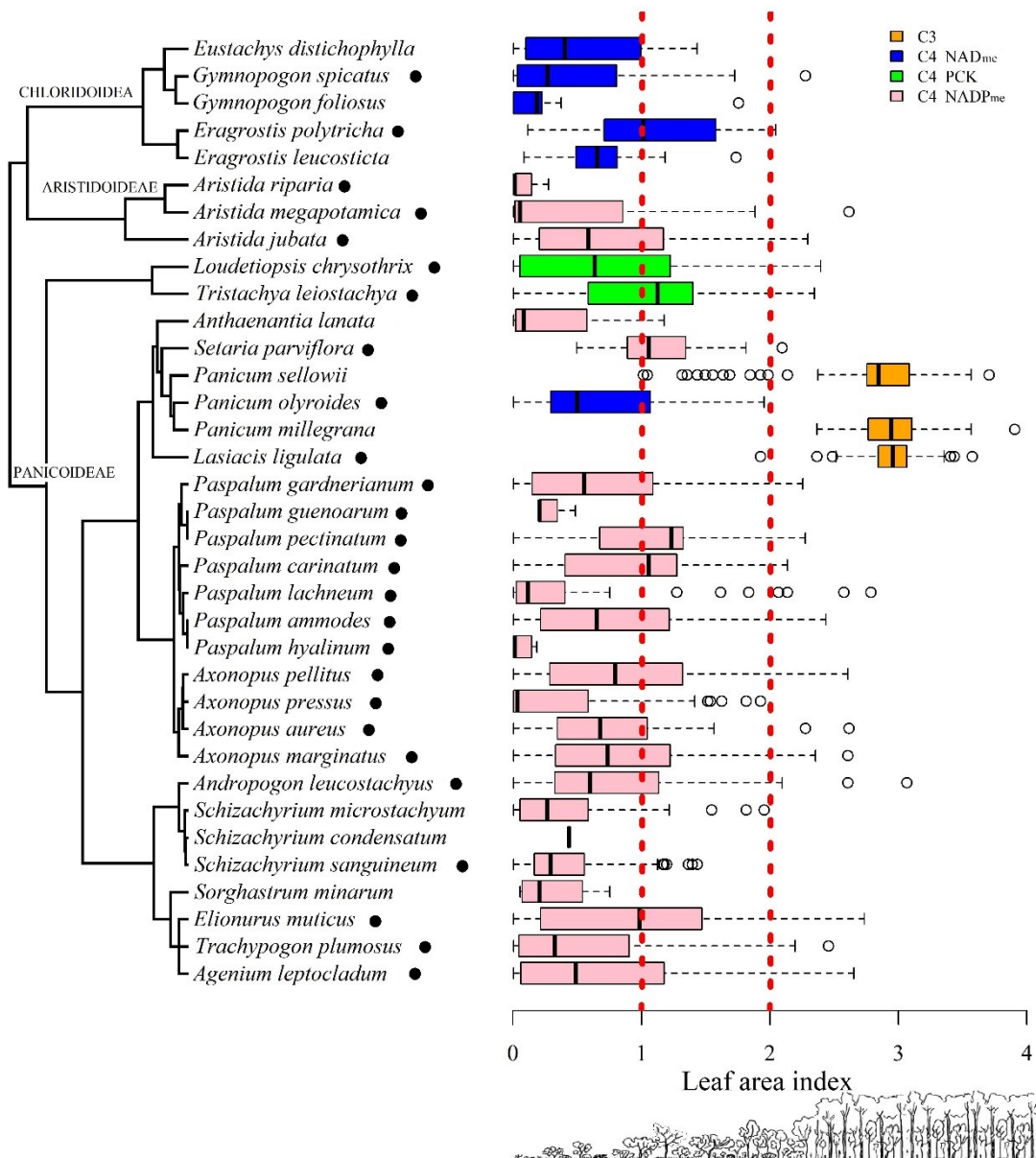
707 Figure 3. Frequency distribution of grass species of different photosynthetic pathways  
 708 along the light gradient. Boxplots represent the median and quartiles, outlier values  
 709 beyond the error bars are indicated with open circles, columns having a common letter  
 710 are not significantly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

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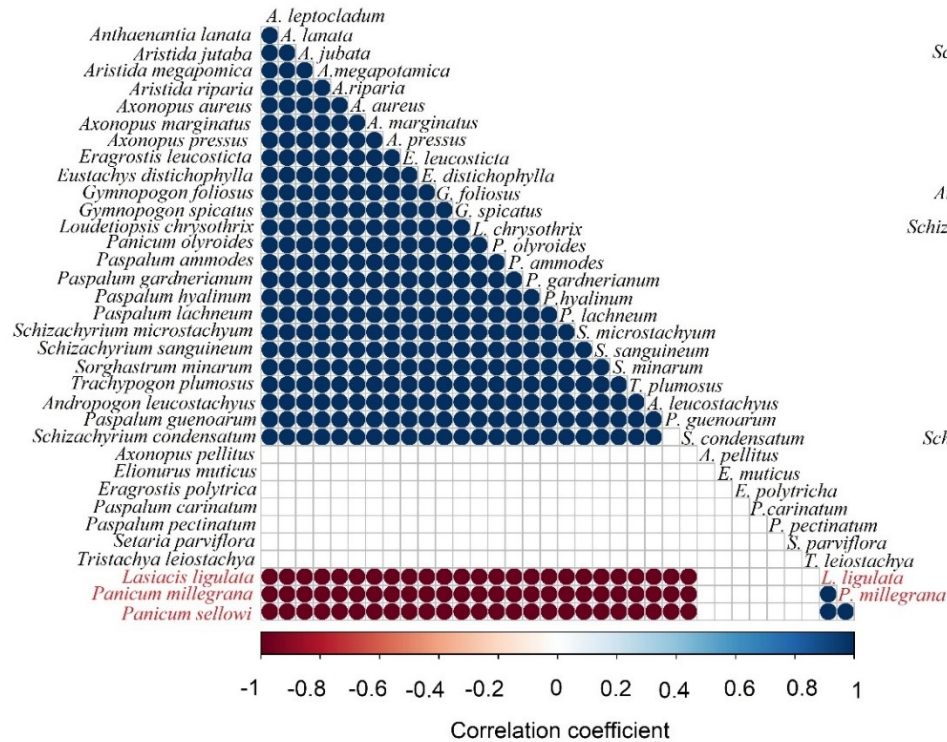
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716 Figure 4. Phylogenetic tree for savanna grass species and their respective distribution  
 717 along the light gradient in the savanna-forest mosaic at our study site. Black dots  
 718 represent the presence of underground storage organs. Red 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  $\leq 1$  (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).

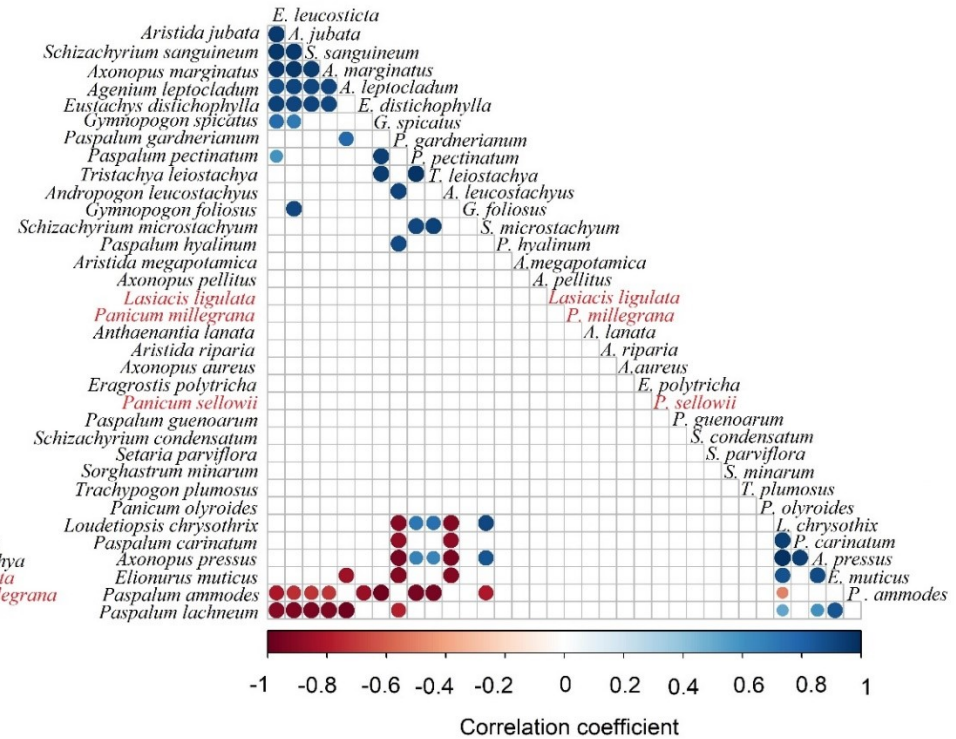
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(A)



(B)



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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 C<sub>3</sub> species.