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

Citation for published version:

Digital Object Identifier (DOI):
10.1111/jvs.12959

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Journal of Vegetation Science: Advances in plant community ecology

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Shade alters savanna grass layer structure and function along a gradient of canopy cover

Running title: Savanna grasses turnover across the light gradient

Natashi A.L. Pilon¹⁴ (ORCI ID: 0000-0001-7985-5842), Giselda Durigan¹² (ORCI ID: 0000-0003-0693-3154), Jess Rickenback³⁴, R. Toby Pennington⁴⁵ (ORCI ID: 0000-0002-1926-823X), Kyle G. Dexter³⁴, William A. Hoffmann⁶ (ORCI ID: 0000-0002-8797-4654), Caroline E. R. Lehmann³⁴ (ORCI ID: 0000-0002-6825-124X)

¹Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Caixa Postal 6109, Campinas, São Paulo 13083-865, Brazil.
²Laboratório de Ecologia e Hidrologia Florestal, Floresta Estadual de Assis, Instituto Florestal, Assis, São Paulo 19802-970, Brazil.
³School of GeoSciences, University of Edinburgh, Edinburgh, EH9 3FF, UK
⁴Royal Botanic Garden Edinburgh, Edinburgh, UK
⁵Department of Geography, The University of Exeter, Exeter, UK
⁶Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27695–7612, USA.
⁷Departamento de Ciências Ambientais, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, CEP 23897-000, Brasil.

*Corresponding author: natashipilon@gmail.com

Funding information
This study was supported by U.S. National Science Foundation – NSF (DEB1354943), grant conceded to WAH. NALP was funded by FAPESP (São Paulo Research Foundation) (process nº. 2016/17888-2) and (process nº. 2017/20897-6). GD was funded by CNPq (National Council for Scientific and Technological Development) (303179/2016-3). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.
Abstract

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

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

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

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

Conclusions. Woody encroachment changes diversity and structure of the grassy layer that is critical to the functioning of savanna ecosystems, highlighting the dependence of
the diverse grass layer on open and sunny conditions. Our results suggest a threshold of tree cover close to LAI ≈ 1 as being critical to cerrado grassy-layer conservation.

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

**Introduction**

Woody encroachment is one of the major threats to savanna conservation, a globally widespread phenomenon across the savanna biome (Stevens et al., 2017). As tree density increases, forming a closed-canopy system, the species adapted to open and sunny ecosystems are lost, leading to landscape homogenization and local extinction of species specialized to savanna (Pinheiro and Durigan, 2009; Parr et al., 2012; Abreu et al., 2017). Arguably, the most important impact of woody encroachment on savanna function is the elimination of C₄ grasses, and, consequently, of fire (Hoffmann et al., 2012a). Reduced burning further improves conditions for trees, leading to a cascade of events promoting further tree invasion (Hoffmann et al., 2012a). Therefore, clarifying how the grassy-layer responds to the increase of shade is crucial to management actions aimed at reestablishing fire feedbacks in landscapes under woody encroachment (Case and Staver, 2017). However, surprisingly, the relationship between tree cover and grass diversity and structure is poorly defined in savanna ecosystems.

Savannas are complex ecosystems with a continuous ground layer dominated by C₄ grasses and a discontinuous tree canopy (Lehmann et al., 2011; Ratnam et al., 2011). Ground layer light availability, that is, shading by trees canopies, is an environmental filter that impacts this grassy-layer structure and composition over just meters (Abdallah
et al., 2016). In turn, changes in the grassy layer alters ecosystem flammability, where fire acts to limit tree canopy cover (Hoffmann et al., 2012a, b). Examination of these feedbacks in savannas between trees and grasses, have tended to focus on grass biomass or woody plant recruitment (e.g., Belsky, 1994; Higgins et al., 2012; Hoffmann et al. 2012a; Vadigi and Ward, 2013), rather than grassy ground layer composition or the functional traits of the composing flora (Cardoso et al., 2018). However, differences in the light/shade tolerance of grass species are fundamental to unpacking the resilience of savanna ecosystem function to increases in tree cover.

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

In the Cerrado region (Brazilian savanna), we investigated how light availability can filter individual grass species distributions and whether different functional traits are associated with response to a shade gradient in a landscape experiencing woody encroachment (Abreu et al., 2017). We quantified grass composition, richness and ground cover related to Leaf Area Index (LAI) as a surrogate for ground layer light
availability. Specifically, we aimed to answer the following questions: i) How does grass cover and species richness change as tree canopy cover increases?; ii) Do C\textsubscript{3} and C\textsubscript{4} grass-communities sort along a gradient in tree cover?; iii) Is variability in grass species distributions associated with different C\textsubscript{4} photosynthetic sub-types or other grass functional traits?; and iv) What is the relative role of environmental filtering versus biotic interactions in shaping ground layer community composition across the light gradient? Because grass subfamilies have been shown to explain habitat preferences and morphological traits better than photosynthetic pathways, we also tested for phylogenetic signal in grass light preference (Liu et al., 2012; Ripley et al., 2015). We expected to find a decrease in grass cover and compositional changes as canopy cover increases and light availability decreases, with grass species more adapted to open conditions being gradually replaced by shade-adapted ones. At a fine scale, we also expected to detect competitive exclusion between grass species that occupy the same light range, and that consequently both environmental filtering and biotic interactions shape grass distribution and functional traits.

Methods

Study area

This study was conducted at Santa Barbara Ecological Station (SBES) (22°46’33” to 22°50’3” S, 49°10’27” to 49°15’36” W), São Paulo State, Brazil. The SBES lies near the southeastern edge of the Cerrado Domain, with dry winters and rainy summers. Monthly mean temperatures range from 16° to 24°C and the mean annual precipitation is about 1300 mm. The soils of the study area are deep oxisols with high
sand content, low nutrients and high aluminum saturation (Abreu et al., 2017). There were no exotic cattle or large native grazers in the study site, which could affect plant communities and species distribution. Native cervid species in the Cerrado are solitary or live in small groups. The vegetation in the SBES ranges from open cerrado grasslands to closed canopy forest (Abreu et al., 2017). Along this gradient, the open ecosystem, composed of a continuous grass layer with scattered savanna trees, is replaced by closed-canopy forests with the ground covered by a litter layer (Appendix S1). This vegetation gradient is a result of at least 30 years of woody encroachment due to fire suppression. Importantly, all the closed-canopy vegetation studied here was a savanna or grassland three decades ago (see Abreu et al., 2017 for details).

Experimental design and data collection

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

Cerrado grasses are essentially caespitose (Sarmiento, 1972), making it easy to distinguish individual tussocks above the soil surface. We considered individuals as
plants clearly separated from each other at soil surface. We acknowledge that in some cases these may not represent genetically or physiologically distinct individuals. Percent of ground cover by grasses was estimated visually (Wikum and Shanhnoltzer, 1978), with only living plant material included in cover estimation. Vegetation sampling took place in April 2015, at the end of the rainy season and peak greenness, before grasses cured.

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

Data analyses

The photosynthetic pathway of each species was classified based on the literature (Davidse, 1978; Sage and Monson, 1999; Giussani et al., 2001; Alvarez et al., 2005; Zuloaga et al., 2018) (Appendix S3). Further, species functional traits known to impact flammability, resilience to fire or to otherwise potentially play a role in distribution along a light gradient were compiled (Table 1) (Charles-Dominique et al., 2018; Archibald et al., 2019). We examined grass functional traits including: the
presence of underground storage organs with capacity to store nutrients and carbohydrates (rhizome, sensu Pausas et al., 2018); grass architecture (cespitose: growing in dense tufts versus mat-forming: horizontal and highly spread growth due to long rhizomes); leaf sheath persistence for protection of meristematic tissue (tunic grasses, sensu Rachid-Edwards, 1956); maximum height; maximum culm height; maximum leaf width and length. Data on these seven traits were compiled from the literature (Wanderley et al., 2001), herbarium specimens and field observations (Table 1, Appendix S3). Traits were classified as response traits and effect traits following Violle et al. (2007).

Structural changes and species distribution through the light gradient

To characterize the general patterns in grass community changes along the woody encroachment gradient, total grass cover and grass species richness per subplot along the light gradient were modeled using generalized additive models (GAM). The models were fitted using the ‘mgvc’ package (Wood, 2017). For both response variables (cover and richness) we modeled the average values sampled at each LAI value registered (predictor variable). For example, for all subplots with overstory LAI values equal to zero, we have taken the average richness and grass cover. This method reduces the extremely high variances inherent to the numerous small subplots (1m²) and is a common procedure in nested designs (Quinn and Keough, 2002). It is important to clarify that we did not group subplots within a plot (20 m x 50 m plot) due to the large variation in canopy cover among subplots. Within a plot, it was possible to find subplots (1 m²) with very low overstory LAI and also high LAI values (mainly due to the nucleation process that is particular to cerrado landscapes in woody encroachment). To analyze average subplot-values from a big plot would obscure the ecological patterns.
In addition, in these models we deleted subplots with grass richness and cover equal to zero due to the monodominance of shrubs (e.g., \textit{Campomanesia adamantium} or \textit{Psidium australie}), which eliminated the noise related to open plots (low overstory LAI) without grass species or cover.

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

Permutational analysis of variance (PERMNOVA) was conducted to test for differences in the light distribution among photosynthetic pathways, with overstory LAI as the dependent variable, and photosynthetic pathway as the factor ($C_4$ NADPme, $C_4$ NADme, $C_4$ PCK and $C_3$) (Legendre and Legendre, 1998).

\textit{Evolutionary patterns in light regime preference}

To investigate the possibility of niche conservatism in light regime preference we calculated phylogenetic signal using Pagel’s $\lambda$ to estimate the degree that LAI maximum, median and minimum values (for each species occurrence) show “niche similarity” depending on phylogeny, following the prediction of a simple Brownian model of trait evolution (Pagel, 1999; Münkemüller et al., 2012). In the estimation of Pagel’s $\lambda$, a value of $\lambda=0$ means that there is no phylogenetic dependence in the trait evaluated, while $\lambda=1$ indicate a pattern of phylogenetic dependence expected under Brownian motion evolution of the trait. The phylogenetic tree for the grass species studied was generated using the Angiosperm megaphylogeny of Zanne et al. (2014) updated and corrected by Quian and Jin (2016). We used the “\textit{SPhyloMaker}” function to
prune our species list from the megatree, which includes the updated version of the Zanne’s tree (Quian and Jin, 2016) and phytools package (Revell, 2012). For the species not present in the original megatree, their phylogenetic relationships were resolved using taxon-specific molecular phylogenies (Soreng, 2015; Saarela et al., 2018). This approach reduces the bias in tree building and gives a more precise calculation of phylogenetic indices (Vega-Álvarez et al., 2019). As the tree was constructed based on the “Scenario 2” of Quian and Jin (2016) (i.e. polytomies are randomly resolved inside the specific genera), 100 phylogenetic trees were generated and the Pagel’s λ was estimated for each tree, to account for phylogenetic uncertainty in the analysis. We report here the average λ values.

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

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

Results

Structural changes and species distribution through the light gradient

Across all plots, decreasing light availability corresponded to an overall decrease in both grass cover (F= 54.62, P<0.001, R²(adj)= 0.50, Deviance explained= 50.6%) and grass species richness (F= 62.73, P<0.001, R²(adj)= 0.57, Deviance explained= 57.5%) and these relationships are non-linear (Figure 1). Grass cover was reduced by 48% as tree LAI reached 1, and by 70% at LAI = 2. Grass-species richness reduced by 30% at LAI =1 and 45% as LAI reaches 2. At overstory LAI = 0, the most frequent grass cover is 70 to 90% and 4 to 6 species/m².
A total of 8110 individual plants from 35 grass species were sampled across the
1200 m² plots (nested within the 30 larger 0.1 ha plots). These 35 species were from
the subfamilies Aristidoideae (9%), Chloridoideae (14%) and Panicoideae (77%), from
the PACMAD clade. Considering the number of individuals sampled across the entire
study, 95% belong to Panicoideae, with 40% from tribe Paniceae, 33% Paspaleae and
19% Andropogoneae. Considering all species, 91% were C₄, of which 75% were
classified as C₄ NADPme, 19% NADme and 6% PCK (Appendix S3).

Among the 28 species that had distributions analyzed by generalized mixed
models, 71% (20 species) were significantly negatively related to increasing overstory
LAI. All C₄ species were negatively affected by shading (see negative β₁ estimates for
models in Appendix S3). In contrast, the frequency of the three C₃ species (Lasiacis
ligulata, Panicum millegrana and Panicum sellowii) was positively related to shading
(β₁=2.5, Z=4.13, P<0.001; β₁=1.89, Z= 2.48, P<0.001, β₁=2.32, Z= 7.07, P<0.001,
respectively). The frequency occurrences of only five C₄ species were unrelated to LAI
(Appendix S3). Parameters from all logistic regressions are reported in Appendix S3.

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

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

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

**Environmental filtering and species interaction**

The correlation between species due to plot and LAI showed a clear habitat separation for $C_3$ and $C_4$ species (Figure 5a). With few exceptions, all $C_4$ species were positively associated, therefore occurring in similar environments. The same pattern was found for $C_3$ species (Figure 5a). The negative correlations in Figure 5a occur only in comparisons between $C_3$ and $C_4$ species. These results highlight the effect of the environmental filtering in the grass-distribution through the gradient studied. There was some evidence for competitive exclusion among grass species in some cases, based on patterns of co-occurrence (Figure 5b). The big-tussock species *Elionurus muticus,*
*Axonopus pressus*, *Loudetiopsis chrysanthrix* and *Paspalum lachneum* were negatively correlated with some short species (see species functional traits in Appendix S3). We do not find a pattern in the grass traits that is correlated with the positive associations in Figure 5b.

**Discussion**

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

The results indicate that an increase in tree canopy cover over open cerrado vegetation will systematically change the grass community structure and composition. The total grass cover decreases sharply to less than ~50% when LAI surpasses 1. Meanwhile, it is still possible to find grass diversity in the understory, with more than half the species remaining when LAI reaches 2, which corresponds to an almost closed-canopy vegetation. In terms of function, changes in grass cover may be paramount when
considering the maintenance of the whole savanna system. Frequent, cool ground fire conserves savannas as species-rich systems (Durigan and Ratter, 2016; Abreu et al., 2017). For operational fire feedbacks, a contiguous grass-layer is necessary (De Castro and Kauffman, 1998). Consequently, despite C₄ grass species being able to maintain small populations in shaded conditions, the reduction of grass cover when LAI > 1 will likely affect fire feedbacks (Hoffmann et al., 2012b).

Changes in structure of the grass layer were accompanied by changes in floristic and functional composition. While the majority of C₄ grasses decreased in frequency as shade increased, some species were able to maintain a reduced population at intermediate light levels, with LAI between 1 and 2 (e.g. Tristachya leiostachya, Paspalum pectinatum, Paspalum carinatum, Elionurus muticus) (Fig. 4). These species extend their distributions into shadier conditions, but are not truly adapted to shade (Rossato et al., 2018). Our results showed that underground storage organs were associated with shade tolerance (Table 2) and it may be that these species are relicts from the open savanna existing 30 years before sampling (Abreu et al., 2017). Storing resources can represent an adaptation to fire, by facilitating rapid regrowth, and which can also help species to survive for a limited time period in shaded understories (Dong and Kroon, 1994; Cruz, 1997; De Moraes et al., 2016; Pinheiro et al., 2016). Savanna grasses under dense canopy, however, are usually not able to reproduce, germinate or propagate (Gardener et al., 2001; Kolb et al., 2016). Consequently, populations of these species are likely to contract over time (Cruz, 1997). Essentially, these shaded grasses are likely ‘zombies’, still living but likely dependent on stored reserves. These results raise potential questions for future research, such as: for how long can C₄ grass species survive under shaded conditions using their reserves? If fire returns to the system, will these shaded species be able to respout or produce seeds? Fire could also increase
mortality, since C₄ grasses under shaded conditions cannot replenish the stored reserves (Zimmermann et al., 2010).

Among the C₄ subtypes, we found a narrow gradient of light preferences where NADme presents a median distribution in more open conditions, followed by NADPme, and PCK in shadier but still open conditions (LAI<1). This partially corroborates the pattern observed in a savanna-forest mosaic in South Africa (Charles-Dominique et al., 2018). Contrasting with results from Charles-Dominique et al. (2018), NADme species presented less tolerance to shade, being more frequent in open and sunny patches of the mosaic studied. This difference could be a product of the distinct data collection procedures, since we sampled the entire grass community and not only the most abundant species. However, even if we had considered only the most abundant species in the subplots the results still would be different as only NADPme and PCK species would be registered in this hypothetical scenario. The NADPme subtype was the most abundant in terms of number of species and individuals in the study site, compared with the other C₄ subtypes. Regarding the PCK subtype, one species - *Loudetiopsis chrysothrix*, can be also quite abundant in some plots. In addition, the NADPme subtype is generally associated with wetter environments (Veenendaal et al., 1993; Cabido et al., 2008), while the NADme subtype increases under drier conditions (Cabido et al., 2008).

Thus, the remarkable differences in edaphoclimatic conditions between the two savannas (southeastern Cerrado and South Africa savanna) could better explain the differences than the data collection procedures. Our samples were taken in an area with lower fertility soils and higher annual precipitation than the area studied by Charles-Dominique et al. (2018). Further investigation is necessary to elucidate whether the patterns for the photosynthetic pathways found here differ from drier savannas of the Neotropics.
Within savanna ecology, investigation of plant competition has focused on grasses versus trees to explain coexistence (Scholes and Archer, 1997). Much research has examined the feedbacks between functional traits and disturbance to describe how grasses “win” the competition via flammability promoting fire that negatively affects the growth of sapling trees in the “fire-trap” (Hoffman et al., 2012a). But, is there competition among grass species? In humid and productive savanna ecosystems, where resources are abundant (e.g. water), species are prone to compete (Liu et al., 2012). Most species documented here are caespitose, some of which do not seem to invest in large root biomass (Sarmiento, 1992; Taylor et al., 2010-for Paniceae; Aires et al., 2014) and rapidly senesce aboveground biomass in the dry season (Sarmiento, 1992). Consequently, these grass species are unlikely to compete for water later into the dry season. Rather, competition is likely to be for space and light through times of high-water availability, and both tall and large grasses with dense canopies and high annual production of biomass (such as *Paspalum lachneum*) can exclude smaller and more slender grasses (such as *Agenium leptocladum* or *Eragrostis leucosticta*) in the absence of fire (Figure 5b). The higher diversity observed in high light conditions may well be mediated by fire, which reduces the overall grass biomass creating space and conditions for the small grasses to propagate (Coutinho, 1990, Fidelis et al., 2012; Pilon et al., 2019). We do not interpret the positive associations between species (Figure 5b) as a trend towards facilitation processes. In our interpretation, the positive association is a strong signal for the same habitat preferences, and absence of competitive exclusion, in most cases.

There is a turnover of grass species at the landscape and community level in a savanna-forest mosaic in the Neotropics, driven by light availability. As the ecology and plant community dynamics of the savanna ground-layer are a “black-box” in the
knowledge of savanna ecosystem processes, our results highlight the importance of
habitat filtering as a major force shaping the assemblage of grasses in savanna-forest
mosaics. In addition, considering the increase in canopy cover and landscape
homogenization due to the woody encroachment process, the reduction in grass cover as
tree LAI surpasses a value of 1 has strong implications for fire management and
savanna conservation, an important threshold with regards to grass cover and diversity.

Acknowledgments

We thank Instituto Florestal do Estado de São Paulo for the permission to develop this
scientific research (SMA#26108-008.476/2014). NALP thank the colleagues at Royal
Botanic Garden Edinburgh for the valuable discussions, in special the PhD office.

Authors’ Contributions

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

Data Availability statement

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

References

Abdallah, F., Michalet, R., Maalouf, J.P., Ouled-Dhaou, S., Touzard, B., Noumi, Z. and
Chaieb, M. (2016) Disentangling canopy and soil effects of a savanna tree species on its

https://doi.org/10.1111/jvs.12402


Supporting information

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

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

Appendix S3. Grass species and functional traits sampled in Brazilian savanna.
Table 1: Description and predicted environmental role of traits associated to fire and shade tolerance for savanna grass species.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Attribute</th>
<th>Description and ecological meaning</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthetic pathway</td>
<td>C₃, C₄</td>
<td>Effect trait: The flammability associated to the high C:N ratio in C₄ species that can act in the fire feedbacks</td>
<td>Archibald et al. 2019</td>
</tr>
<tr>
<td></td>
<td>NADme, C₄</td>
<td>Response trait: Shade and drought tolerance may be associated to different photosynthetic pathways</td>
<td>Charles-Dominique et al. 2018, Veenendaal et al. 1993, Cabido et al. 2008</td>
</tr>
<tr>
<td></td>
<td>NADPme and C₄ PCK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bud protection</td>
<td>Presence vs. absence</td>
<td>Response trait: Persistent old leaf-sheath at the plant base, protecting the meristematic tissue during fire passage</td>
<td>Rachid-Edwards 1956, Warming (1892)</td>
</tr>
<tr>
<td>Maximum height</td>
<td>Continuous (cm)</td>
<td>Response trait: Light capture efficiency</td>
<td>Lebrija-Trejos et al. 2010</td>
</tr>
<tr>
<td>Maximum culm height</td>
<td>Continuous (cm)</td>
<td>Response trait: Higher culms may represent advantages in seed dispersal in post-fire environment</td>
<td>Thomson et al. 2011, Geissler et al. 2019</td>
</tr>
<tr>
<td>Maximum leaf width</td>
<td>Continuous (cm)</td>
<td>Response trait: When light is a scarce resource, e.g. 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</td>
<td>Lebrija-Trejos et al. 2010, Solofondranohatra et al. 2018</td>
</tr>
<tr>
<td>Maximum leaf length</td>
<td>Continuous (cm)</td>
<td>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</td>
<td>Hoffmann et al. 2012b, Solofondranohatra et al. 2018, Archibald et al. 2019</td>
</tr>
<tr>
<td>Architecture</td>
<td>Caespitose or mat forming</td>
<td>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</td>
<td>Solofondranohatra et al. 2018, Archibald et al. 2019</td>
</tr>
</tbody>
</table>
Table 2: Phylogenetic regression parameters for correlations of species’ traits with shade tolerance (maximum Leaf Area Index values). (*Z-value for logistic regression, t-value for linear regression)

<table>
<thead>
<tr>
<th>Trait</th>
<th>β1</th>
<th>Phylogenetic correlation parameter</th>
<th>Statistic*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underground storage organ</td>
<td>1.10</td>
<td>0.4</td>
<td>2.15</td>
<td>0.03</td>
</tr>
<tr>
<td>Tunic Grass</td>
<td>0.97</td>
<td>5.6</td>
<td>0.56</td>
<td>ns</td>
</tr>
<tr>
<td>Maximum height</td>
<td>1.76</td>
<td>&lt; 0.000</td>
<td>0.48</td>
<td>ns</td>
</tr>
<tr>
<td>Maximum culm height</td>
<td>-9.46</td>
<td>&lt; 0.001</td>
<td>-0.89</td>
<td>ns</td>
</tr>
<tr>
<td>Maximum leaf width</td>
<td>-0.16</td>
<td>&lt; 0.001</td>
<td>-1.95</td>
<td>ns</td>
</tr>
<tr>
<td>Maximum leaf length</td>
<td>-0.14</td>
<td>0.1</td>
<td>-0.03</td>
<td>ns</td>
</tr>
</tbody>
</table>
Figure 1. Changes in grass cover (a) and grass species richness (b) in the light gradient in a savanna-forest mosaic. Both C$_3$ and C$_4$ species are included. Solid lines represent the predicted distribution, with the grey shaded area giving the 95% confidence interval.
Figure 2. Density plot considering the frequency of species, categorized by photosynthetic pathway, along the light gradient in a neotropical savanna-forest mosaic. Total area of each curve represents the proportion of species frequency of a given photosynthetic type considering all plots sampled in the entire study site. Therefore, the sum of all areas beneath all four curves is 100%.
Figure 3. Frequency distribution of grass species of different photosynthetic pathways along the light gradient. Boxplots represent the median and quartiles, outlier values beyond the error bars are indicated with open circles, columns having a common letter are not significantly different (Tukey; $P \geq 0.05$).
Figure 4. Phylogenetic tree for savanna grass species and their respective distribution along the light gradient in the savanna-forest mosaic at our study site. Black dots represent the presence of underground storage organs. Red dashed lines represent three light classes based on previous investigation of the general species distribution and the literature (Scholes & Archer, 1997), as follows: LAI ≤ 1 (open physiognomies), 1 < LAI < 2 (intermediate conditions, with a large proportion of the soil surface with shade projected by trees, but still with gaps in the canopy) and LAI > 2 (forest, with soil surface completely shaded by tree canopies).
Figure 5. Correlations in species abundance. Correlation due to covariates (a) and correlations of residuals, after accounting for overstory LAI and site, performed to quantify species interactions (b). Significant correlations, based on the 95% credible intervals, are represented by colored 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 indicate C₃ species.