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Ground layer Cerrado plants sustain higher maximum photosynthetic rates after medium-term fire events☆

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ABSTRACT

Fire is one of the most important factors driving community assembly and ecosystem functioning in tropical savannas. However, few studies have evaluated the physiological responses of ground layer plant communities to fire disturbance. Here we used different fire regimes to investigate possible changes in leaf maximum gas exchange (A_{max} and g_s) and leaf nutritional content (N, P, K, Ca and Mg) among different plant growth forms in savanna ground layer communities. We compared responses of ground layer plant communities under two different fire regimes: (1) no recent fire occurrence; and (2) two recurrent fire events in the last 20 years. We estimated canopy cover, soil chemical properties and species abundance on burned and unburned plots in order to calculate abundance-weighted species average trait values for gas exchange and leaf nutrient content. We found that burned plots exhibited lower canopy cover and soil organic matter content, and an overall higher soil macronutrients availability compared to unburned plots. These environmental differences clearly influenced the ground layer plant communities, which depicted higher A_{max} and g_s in burned areas regardless of growth form. We found no significant differences among leaf nutrient traits, except for a lower Mg concentration in the burned site species. Our results support the hypothesis that distinct fire regimes select for a different set of leaf functional traits, with fire occurrence acting as an important driver increasing the maximum photosynthetic rate on the ground layer. While nutrient use seems not to be affected by medium-term recurrent fires, physiological plasticity on carbon and water use processes in response to changes in resource availability can promote the persistence of savanna species under frequent fire.

1. Introduction

Fire is one of the most important ecological disturbances in plant communities, particularly because of the partial or complete aboveground plant biomass removal (Beckage et al., 2009; Thonicke et al., 2001). Plant organs may be directly destroyed by fire (Hoffmann and Solbrig, 2003) or by the heat that can kills plants' living parts, especially the leaves (Beringer et al., 2015; Pivello et al., 2010), which are the most important organ performing photosynthesis (Simkin et al., 2020). Woody plants evolved a set of strategies to tolerate fire or escape its effects, such as investing in a thick outer bark and therefore insulating the living tissues of the wood; or growing taller and saving the canopy from flames and from the heat (Keeley et al., 2011; Scalon et al., 2020). However, savanna ground layer plants are shorter, and its different growth forms such as herbs, subshrubs and grasses are certainly the most affected functional groups within the plant community in terms of biomass loss (Kauffman et al., 1998; Pilon et al., 2021). Aboveground biomass of ground layer species is mainly composed by photosynthetic organs (Augustine et al., 2010), therefore these plants need to produce new leaves promptly after each fire event in order to maintain their acquisitive metabolism.

Many ground layer species are perennials and evolved under fire regimes that lead to the selection of specific traits to deal with the loss of aboveground biomass (McIntyre et al., 1995; Pilon et al., 2021). These fire-adapted traits are mainly located in their well-developed underground organs (Appezzato-da-Glória et al., 2008), which have a high number of protected buds (Fidelis et al., 2014; Filartiga et al., 2017) that result in the capacity to resprout after fire events. This type of

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resprouting strategy demands a large energy expenditure, supported by high amounts of carbohydrates, water and nutrients stored in underground organs (Clarke et al., 2013; da Silva and Rossatto, 2019; de Moraes et al., 2016) that can quickly translocate stored compounds, such as fructans, fructose and sucrose (de Carvalho and Dietrich, 1993; de Moraes et al., 2016; Souza et al., 2010) to produce new organs after fire. To successfully store a significant amount of carbohydrates, these plants depend on achieving high carbon assimilation rates in their leaves during favorable periods (i.e., during the wet season) when water, light and nutrients are available (Eamus et al., 1999; Rossatto et al., 2018). Thus, achieving high photosynthetic rates (A_{max}) during the wet season is of ultimate importance to refill carbon reserves spent during the regrowth of the aboveground biomass after disturbances (Okoro and Grace 1976; Niinemets 2007), a process that should be affected by differences in fire regimes (Beringer et al., 2015).

Although fire events have clear impacts on local plants communities, the burning of aboveground biomass can promote immediate increases in soil nutrient availability, especially for Ca, Mg and K (Pivello et al., 2010). The repeated removal of aboveground cover can also increase and maintain higher light availability in the understory (Miranda et al., 2009), which favours higher photosynthetic rates on ground layer plants (Ries and Shugart 2008; Rossatto et al., 2018). Thus, in a scenario of increased light availability, plants would be able to increase their photosynthetic rates after the disturbance (Goorman et al., 2011), favouring a more acquisitive strategy in their gas exchange traits (Ficken and Wright 2019).

Some studies showed that different fire regimes could select trees with dissimilar functional traits, especially trunk traits linked with persistence and resprouting ability (Stevens et al., 2020; Pellegrini et al., 2021; Scalon et al., 2020), but few studies analyzed gas exchange and leaf nutrient content under such a scenario. Dantas and Pausas (2013) showed that trees under frequent fire displayed resource-acquisitive strategies, such as higher specific leaf area and higher leaf nutrients content, even after a significant amount of time since the last disturbance event. Their findings provide evidence that trees should be able to increase and maintain higher photosynthetic rates given the increase on nutrient content in their leaves under intermediate fire regimes (Dantas and Pausas 2013). This expected pattern, however, remains not fully understood for tropical savanna ground layer plants. Available information on fire-prone environments in temperate regions (Diaz-Toribio et al., 2020; Férnandez-Gárcia et al. 2020) suggests that ground layer herbaceous species should probably be able to more rapidly respond to fire events in terms of photosynthetic rates and leaf nutrient, when compared to trees.

Although the Neotropical savanna ground-layer is mostly composed by grasses, small eudicot herbs and subshrubs can also be important components of such communities (Ribeiro and Walter 2008). These different growth forms can be considered different functional groups based on their leaf physiological traits: graminoids show higher photosynthetic capacity and low leaf nutrient content, while herbs show higher leaf nutrient content, but lower photosynthetic rates, and subshrubs show intermediate photosynthetic rates and leaf nutrient concentration (Rossatto and Franco 2017). Since these different growth forms may differ in carbon, water and nutrient use, response to fire may change among such distinct functional groups, with grasses being favoured under recent fire events, given the high growth rates of these plants after fire (Pilon et al., 2018, Zirondi et al. 2021).

Here, we investigated how maximum gas-exchange rates and leaf nutrient concentrations in different functional groups of ground layer species from the Brazilian Neotropical savanna, the Cerrado, would vary under two different fire regimes: no recent fire event (i.e., > 20 years without fire) and two recurrent burning events over a 20 years period. We measured leaf maximum gas exchange rates and leaf nutrient concentrations in a total of 60 ground layer species (graminoids, herbs, subshrubs and palms) three years after an accidental fire in the burned area, and did parallel measurements in an unburned typical savanna site (IBGE 2020). Since fire decreases canopy cover and increases soil fertility (Pivello et al., 2010; Veenendaal et al. 2018), generally providing higher resource availability, we expected to detect these effects even after a medium-term period (3 years) since the last disturbance event. Therefore, we expected that all species, regardless of growth form, would be capable to sustain higher nutrient concentration in their leaves and depict a higher capacity to assimilate carbon in more disturbed and open environments i.e., the burned site, compared to more stable and closed environments (i.e., 20 years without fire). However, we also expected that the magnitude of these responses would differ according with species growth form, irrespective of fire occurrence, with graminoids showing higher photosynthetic rates and lower leaf nutrient content in comparison to other functional groups, since they are known to have higher productivity under frequent fire regimes (Ripley et al., 2015). To test our hypotheses, we first tested if resource availability (light and nutrients) would in fact differ between the studied sites by comparing soil macronutrients (P, K, Ca and Mg), organic matter content, and light availability (canopy cover) between the two studied conditions. We expected that burned areas would show higher soil macronutrients and soil organic matter content, but lower canopy cover compared to the unburned area. Furthermore, we hypothesize that species in burned sites would have higher leaf nutrient concentration and higher maximum photosynthetic capacity, as a direct response to environmental changes and as a strategy to enhance carbohydrate storage refill and secure individual persistence in face of a future fire event. We also expected that trait-trait relationships (i.e., scaling relationships among SLA, leaf N and P, Mg and Ca concentrations, photosynthetic rates and stomatal conductance according to Wright et al. (2001)), would display similar scaling slopes (i.e., a similar pattern where SLA, N, P, A_{max} and g_s would scale positively with one another across the two plant communities) except for plants in the burned site, with more resource access, these relationships would be shifted (Reich et al., 1999), since trait ranges would differ (i.e., they will show higher A_{max}, g_s and leaf nutrient concentrations).

2. Material and methods

2.1. Study site and species selection

We performed this study at the IBGE Ecological Reserve (Instituto Brasileiro de Geografia e Estatística) in the surroundings of Brasília, Federal District, Brazil (15°56′41''S and 47°52′48.36″W). The site is approximately 1100 m above sea level and, according to IBGE's meteorological station (1992–2012), the average annual rainfall is 1462 mm, with an annual mean temperature of 22.5 °C, and a distinct dry season spanning from May to September.

In the previous 20 years before this study (1994–2014 period), there were two accidental fires which respectively consumed 50% (2005) and 90% (2011) of the reserve vegetation (IBGE 2020). We took the opportunity to analyze ecophysiological responses of ground layer plants after the occurrence of these accidental fires, sampling plants in areas that were burned twice, in 2005 and 2011, and in areas that remained unburned (the last recorded fire for the 'unburnt' area was in 1994, and no previous records exist since the reserve was established in 1975). Sampling was performed 3 years after the 2011 fire, during the wet season (December of 2014). The burned site is located between the coordinates 15°56′29.39″S and 47°53′06.53″W, and 15°56′39.96″S and 47°53′12.94″W at an altitude of 1100 m, while the unburnt site is located between 15°55′59.97'’S and 47°52′26.91″W and 15°56′06.83″S and 47°52′20.36″W at an altitude of 1128 m. We assumed that, given the close proximity of the sites, they should be equivalent in terms of environmental conditions prior to the 2005/2011 fire events.

2.2. Floristic survey

During the 2014 wet season (November-December) we performed a floristic survey of the ground layer vegetation by placing ten 20 m^2 (10 m x 2 m) plots along a line consisting of 300 m between the coordinates cited above (five plots for burned and five plots for the unburned area). In each plot, we sampled all herbaceous, subshrubs, graminoids and palm species (excluding woody plants seedlings) higher than 10 cm in height. Species were identified according to specialized literature (Medeiros, 2011; Proença et al., 2000) and by comparison with vouchers deposited at the IBGE Herbarium (IBGE). We classified all sampled species according to growth form (i.e., graminoids, herbs, subshrubs or palms) based on field observations using the criteria adopted by Rossatto and Franco (2017). In this study, we assumed a plant-level approach (focusing on functional traits) rather than site-level, and sampled a total of 76 species (Supplementary Material, Table S1). We measured gas exchange traits in 42 species (6 graminoids, 19 herbs and 17 subshrubs) given limitations of leaf size to cover the IRGA chamber – 6.5 cm^2. Leaf nutrient content was measured in 60 species (8 graminoids, 2 palms, 19 subshrubs and 31 herbs) (Supplementary Material, Table S1). The relative abundance of each species (Table S1) was estimated as the number of individuals in relation to the total number of individuals from all species at each site, expressed as a percentage.

2.3. *Leaf functional traits*

In December 2014, during the wet season, we measured key functional leaf traits related to leaf gas exchange and nutritional status. We followed the most appropriate approach reported for ground layer species (Rossatto and Franco, 2017 and Rossatto et al., 2018). For gas exchange traits, we measured the maximum CO_2 assimilation in an area basis (A_{max}), leaf transpiration (E) and stomatal conductance (g_s) under ambient conditions with a portable open photosynthesis system (LCpro, Analytical Development Co., Hoddesdon, U.K.) coupled with a led light source providing a light intensity between 1300 and 1400 umol.m^{-2}.s^{-1} (enough to saturate the photosynthetic apparatus of herbaceous plants according to Rossatto et al. (2018)); for grasses, we used 2000 umol. m^{-2}.s^{-1}, since most of the studied species were C4 (Rossatto and Franco, 2017). Leaf temperature was maintained around $27 \pm 0.54\ ^{\circ}C$. The measurements were performed in one sunlit leaf of five pre-marked individuals during a 2- to 4-h period between 08:00 h and 12:00 h. After gas exchange measurements, each leaf was collected and scanned on a flatbed scanner and its area determined using the free software Area (Caldas et al., 1992). After drying at 70 °C for three days and weighting, we calculated the specific leaf area (SLA, $cm^2\ g^{-1}$). We also calculated the intrinsic water use efficiency (IWUE) as the ratio of A_{max} and g_s.

The dried leaves used for SLA measurement were finely ground and a subsample was taken for total N, P, K, Ca and Mg determinations on the species level at each study site. N was determined in diluted acid digests according to the micro-Kjeldhal procedure (Allen et al., 1974). Total extractable P was determined colorimetrically by complex formation with molibdovanadate according to Allen et al. (1974). Other elements were determined by combustion in a flame spectrophotometry (Allen et al., 1974).

2.4. *Canopy cover and soil properties*

We measured the leaf area index (LAI) to characterize canopy cover in each plot by taking hemispheric photographs (four photographs per plot) using a CI-110–24P-ID plant canopy imager (CID Bioscience, Camas, WA, USA). Measurements were performed during the end of the day, when light was diffuse. To characterize soil chemical properties, we collected four samples at the top 0–20 cm soil depth in each studied plot using soil corers. Samples were mixed and only one composite sample was analyzed per plot. For chemical analysis, pH was determined in $CaCl_2$ (10 mmol.l^{-1}) with a ratio of 1:2.5 (solution to soil sample) (Raij et al., 1987). Soil P was determined by spectrophotometry after anion exchange resin extraction (Raij et al., 1987). Soil K, Ca, Mg and Al were determined by flame spectrophotometry (Allen et al., 1974). Organic matter was determined after the addition of potassium dichromate-sulphuric acid to the soil samples following Sims and Haby (1971).

2.5. *Data analysis*

We used *t-tests* to compare environmental differences in light and soil chemical properties between burned and unburned sites. Since species composition varied between burned and unburned sites, and we wanted to account for the ground layer community responses as a whole, we used abundance-weighted leaf traits, which accounts for relative abundance of each species within the community. Hence, we calculated abundance-weighted species mean trait values (Ackerly and Cornwell, 2007). When data did not meet normality assumptions it was log-10 transformed prior to analyses. We used multivariate analysis of variance (MANOVA) followed by two-way ANOVAs to compare abundance-weighted leaf traits differences between ground layer species community in burned and unburned sites, accounting for the effect of species functional groups (i.e., the growth forms: herbs, graminoids, palms and subshrubs). Within functional groups, we compared means using post-hoc Tukey-Kramer tests for unequal sample sizes, for the traits that showed differences. To test for leaf traits bi-variate relationships differences in the distinct sites, we used the *smatr* package (Warton et al., 2012), by $\log_{10}$-transforming all variables. To investigate the direct influence of soil and light availability on herbaceous plant performance we also created a correlation matrix between community-weighted mean trait values and environmental variables for each plot ($n = 10$), using the *corrplot* package (Wei et al., 2017). We used R 4.0.1 environment (R Core Team, 2019) for all analyses and significant differences were considered when P-value < 0.05.

3. Results

As expected, we found a significant difference between burned and unburned plots for LAI (Fig. 1A) and most of the soil chemical properties analyzed (Fig. 1B-H). Burned sites showed lower LAI, lower organic matter content and Al, but higher soil K, Ca and Mg (Fig. 1). Soil pH and P concentration were markedly stable and similar between burned and unburned plots (t-tests, $P > 0.05$).

Within the ground layer plant community, we found differences between the four functional groups (MANOVA: $F = 3.597$, $P < 0.001$, Table 1), but only for A_{max}, SLA, and leaf Ca and Mg concentrations (Fig. 2, Table S2). We found that graminoids showed higher A_{max} and SLA (Fig. 2A-B), but lower leaf Ca and Mg (Fig. 2C-D) compared to herbs and subshrubs. Palms, herbs and subshubs showed similar SLA, but palms had lower leaf Ca and Mg (Fig. 2A-D).

Species in the burned plots, irrespectively of growth form (MANOVA: $F = 0.604$, $P = 0.168$, Table 1), showed marked differences in physiological traits compared to species in the unburned plots (MANOVA: $F = 8.576$, $P < 0.001$, Table 1). In the burned area, species showed ~20% higher A_{max} and ~60% higher g_s, resulting in a significant lower iWUE, and slightly lower SLA (Fig. 3). At a given photosynthetic rate, species from the burned plot exhibited 1.5-fold higher g_s (Fig. 4A, Table 2).

Despite differences in nutrient availability between the two different areas (Fig. 1), there was generally no difference in leaf nutrient concentration between species in burned and unburned sites (Fig. 3), except for leaf Mg concentration, which was lower for species in burned site. Leaf N and P also scaled positively and similarly between sites (Fig. 4B), showing that fire, at least in a medium term, did not change major nutrient stoichiometry in herbaceous plants. However, leaf Ca and Mg relationship was shifted in elevation, meaning that at a given leaf Ca, species from the unburned area showed ~1.4-fold higher Mg (Fig. 4C, Table 2). N and P did not scale with SLA or A_{max} for any of the sites (Table 2), and SLA and A_{max} were only positively associated for species from the unburned site (Table 2).

Community-weighted mean A_{max} was weakly negatively correlated

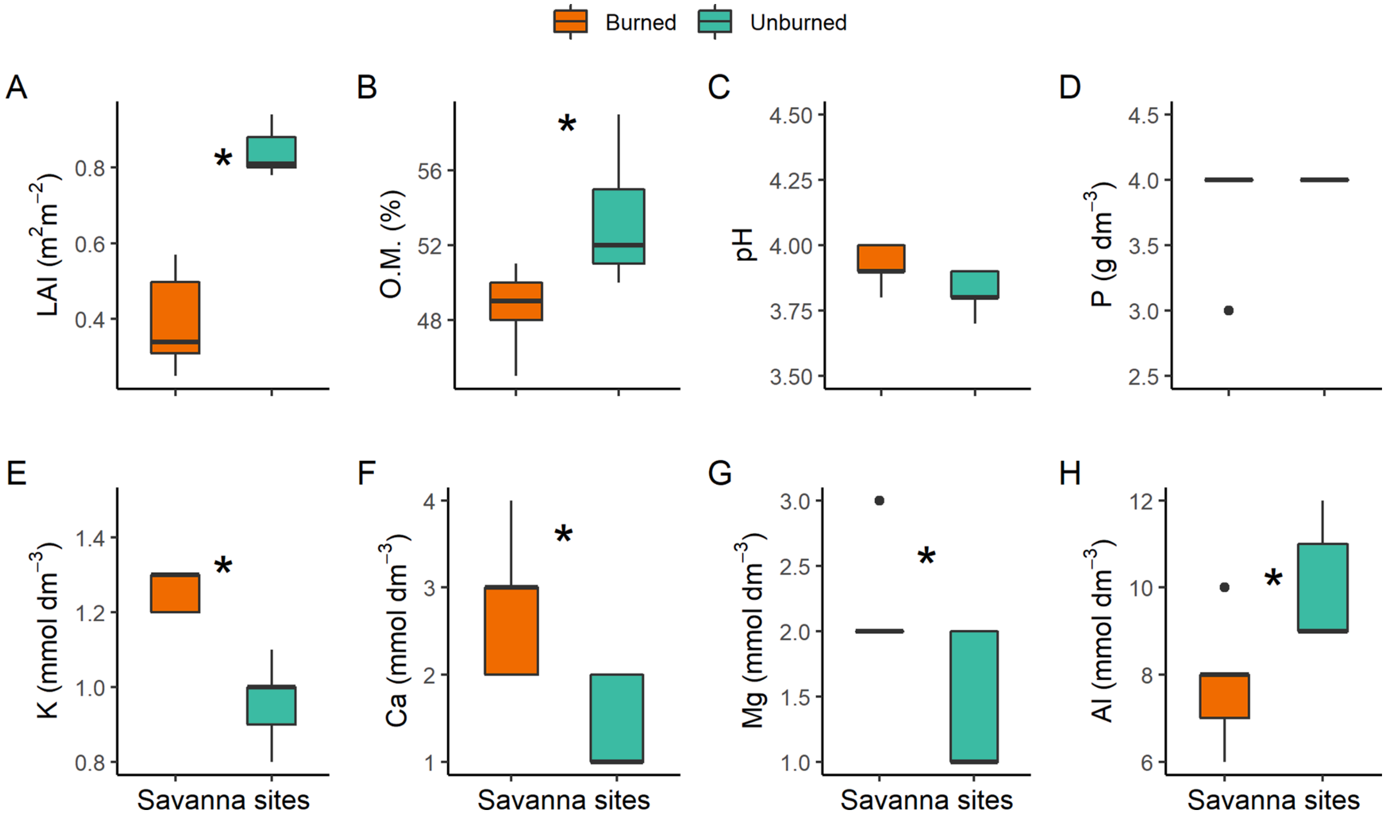

Fig. 1. Pairwise comparison between burned (orange boxes) and unburned (green boxes) savanna sites in relation to light availability and nutritional status. (A) Leaf area index (LAI); (B) organic matter content (OM); (C) pH; (D) P concentration; (E) K concentration; (F) Ca concentration; (G) Mg concentration and (H) Al concentration. Boxplots showing median (thick horizontal line), error bars with 10 and 90 percentiles. The small solid circles represent outliers. The symbol * show significant differences when $P < 0.05$ (t-tests).

Table 1

Multivariate analysis of variance for the effects of site (burned and unburned areas) and functional group (FG; graminoids, herbs, subshrubs and palms) on the studied traits (A_{max}: maximum CO_2 assimilation rate; g_s: stomata conductance; IWUE: intrinsic water use efficiency; SLA: specific leaf area; and leaf N, P, K, Ca and Mg concentrations).

Source of variation	*df*	*F* value	P-value
Site	1, 9	6.396	< 0.0001
FG	2, 18	3.665	< 0.0001
Site*FG	2, 18	1.365	0.185
Residuals	36		

with MO and LAI, but positively correlated with soil Al concentration (Fig. 5). SLA was strongly correlated with leaf K concentration (negatively) and LAI (positively) (Fig. 5). Leaf Ca and Mg were highly influenced by LAI, OM and soil nutrient concentrations, whereas leaf P and K concentrations relationships with environmental variables were less clear (Fig. 5). P and N leaf concentration was also positively correlated with soil Ca and Mg and leaf P concentration was weakly negatively correlated with LAI (Fig. 5).

4. Discussion

We found that the ground-layer plant community showed higher maximum gas exchange rates at periodically burned sites compared with unburned sites, corroborating our initial hypothesis. However, despite a significant effect of functional group alone, the responses of individual growth forms were not determined by the occurrence of fire (i.e., there was not a significant effect in the interaction between functional group and fire regime). As also found in previous studies (Rossatto and Franco

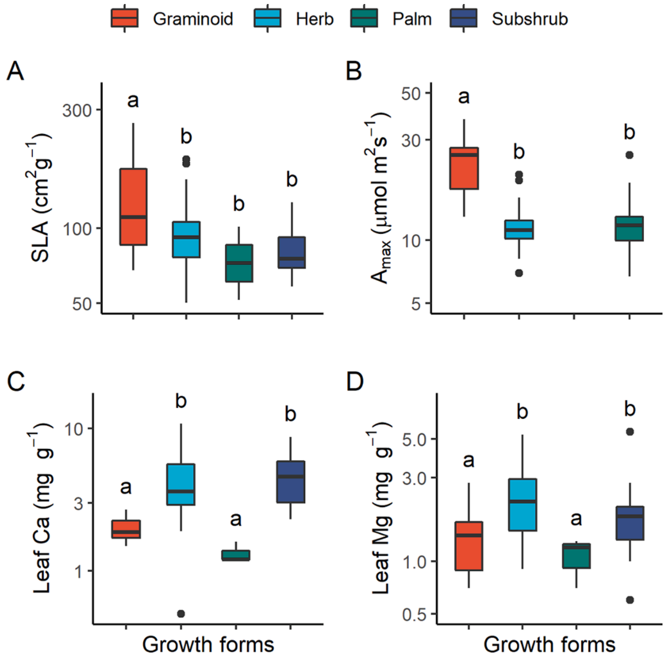

Fig. 2. Comparison between growth forms (graminoids: red boxes, herbs: light blue boxes, palms: green boxes, and subshubs: dark blue boxes) in savanna sites for (A) specific leaf area (SLA); (B) maximum carbon assimilation rate (A_{max}); (C) leaf Ca concentration and (D) leaf Mg concentration. Boxplots showing median (thick horizontal line), error bars with 10 and 90 percentiles. The small solid circles represent outliers. Different letters indicate significant differences when $P < 0.05$ (Tukey-Kramer test). A_{max} was not measured for palms.

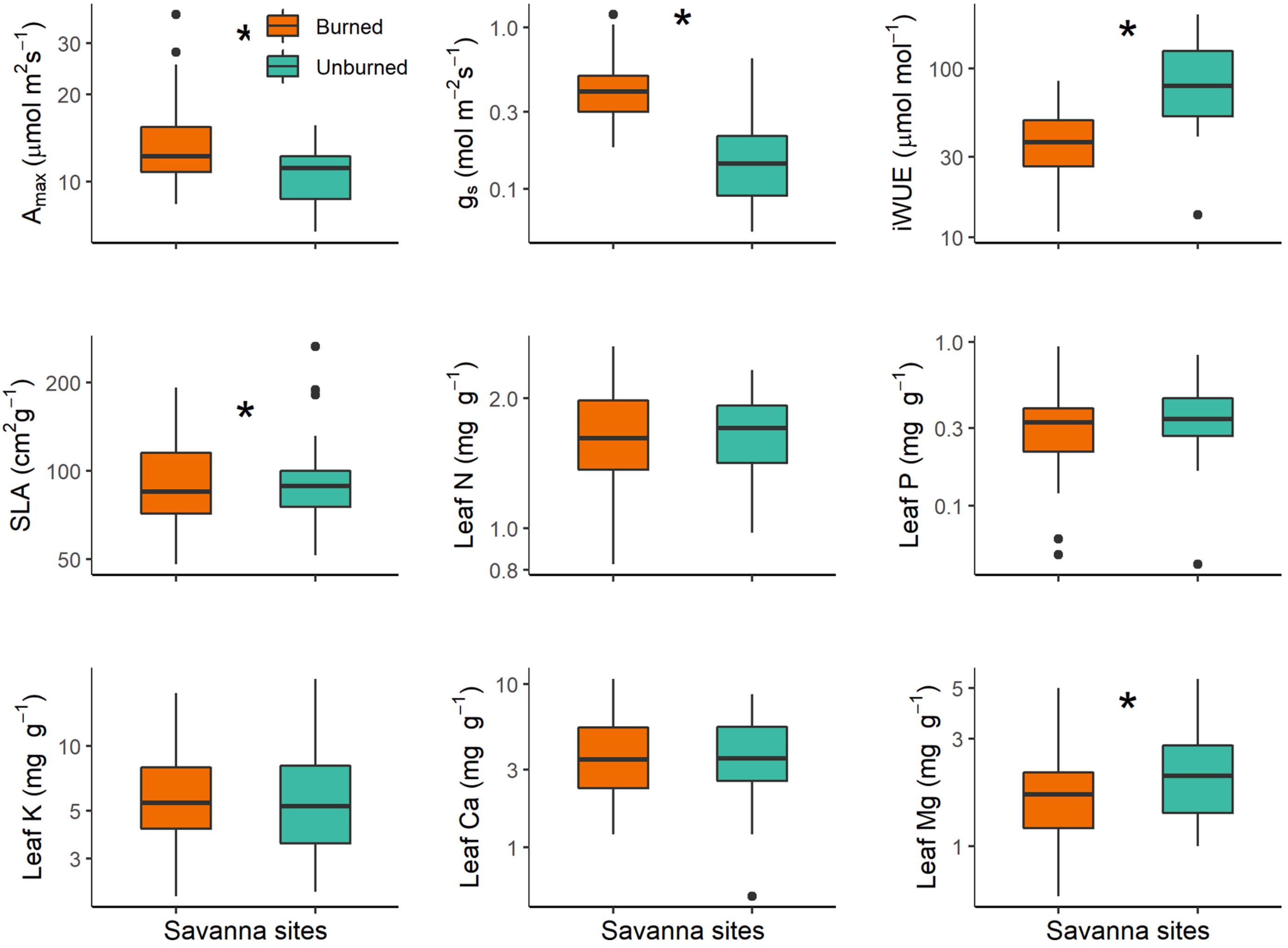

Fig. 3. Pairwise comparison between ground layer species in burned (orange boxes) and unburned (green boxes) savanna sites for maximum carbon assimilation (A_{max}); stomatal conductance (g_s); intrinsic water use efficiency (iWUE); specific leaf area (SLA); leaf N concentration; leaf P concentration; leaf K concentration; leaf Ca concentration and leaf Mg concentration. Boxplots showing median (thick horizontal line), error bars with 10 and 90 percentiles. The small solid circles represent outliers. The symbol * show significant differences when $P < 0.05$ (t-tests).

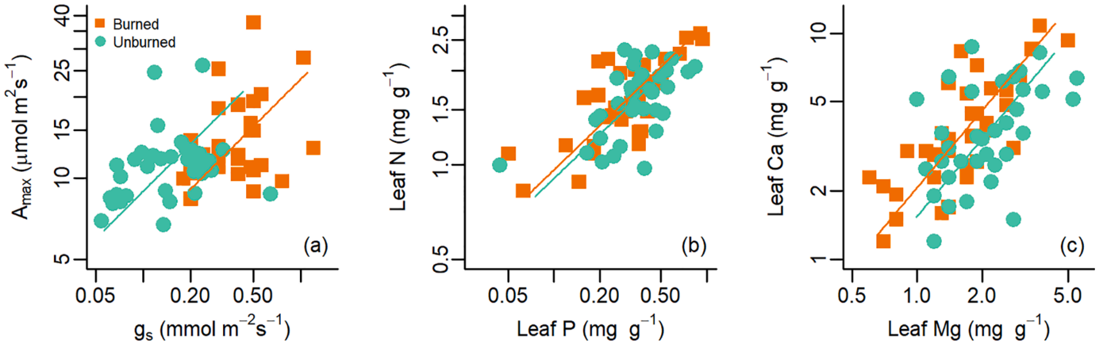

Fig. 4. Bi-variate relationships for ground layer species in burned (orange symbols and lines) and unburned (green symbols and lines) savanna sites between (a) maximum carbon assimilation (A_{max}) and stomatal conductance (g_s) (similar slopes: Test = 2.043, P = 0.153 with difference in elevation: Wald-test = 10.16, P = 0.001); (b) leaf N and P concentrations (similar slopes: Test = 0.10, P = 0.751 and similar elevation: Wald-test: 1.44, P = 0.230); (c) leaf Ca and Mg concentrations (similar slopes: Test = 0.06, P = 0.809 and difference in elevation: Wald-test: 6.65, $P < 0.001$). Slopes and confidence intervals are shown in Table 2.

2017), functional groups differed in leaf traits, with graminoids showing the higher SLA and A_{max}, but lower leaf nutrients. The community-level functional responses were related to the differences in environmental variables, mostly light, since no correlation with soil nutrients or differences between leaf nutrient concentration was found. We demonstrated that, even after a medium-term fire occurrence (i.e., 3 years after fire), the ground layer plant community responded to the environmental differences by maintaining a higher carbon assimilation at a cost of higher stomatal conductance.

Burned and unburned plots showed markedly differences in canopy

Table 2
Standardized major-axis (SMA) slopes (95% confidence intervals in parentheses), correlation r, and P-values for burned and unburned areas. P-values lower than 0.05 are shown in bold.

Y	X	Burned slope (95% CI), r, P-value	Unburned slope (95% CI), r, P-value
A_{max}	g_s	0.73 (0.51, 1.04), 0.11, **0.041**	0.51 (0.36, 0.72), 0.08, **0.046**
A_{max}	N	−1.28 (−1.02, −0.82), 0.01, 0.685	−1.06 (−1.65, −0.68), 0.05, 0.218
A_{max}	P	0.62 (0.96, 0.40), 0.04, 0.376	0.48 (0.30, 0.76), 0.00, 0.867
A_{max}	SLA	1.16 (0.79, 1.69), 0.03, 0.363	0.86 (0.63, 1.18), 0.24, **0.003**
N	SLA	0.85 (0.58, 1.24), 0.00, 0.983	−0.76 (−1.09, −0.52), 0.00, 0.756
P	SLA	−1.91 (−2.78, −1.31) 0.00, 0.826	1.68 (1.17, 2.41), 0.01, 0.579
N	P	0.45 (0.36, 0.58), 0.55, **<0.001**	0.48 (0.37, 0.65), 0.34, **< 0.001**
Ca	Mg	1.15 (0.89, 1.39), 0.58, **<0.001**	1.17 (0.86, 1.59), 0.23, **0.004**

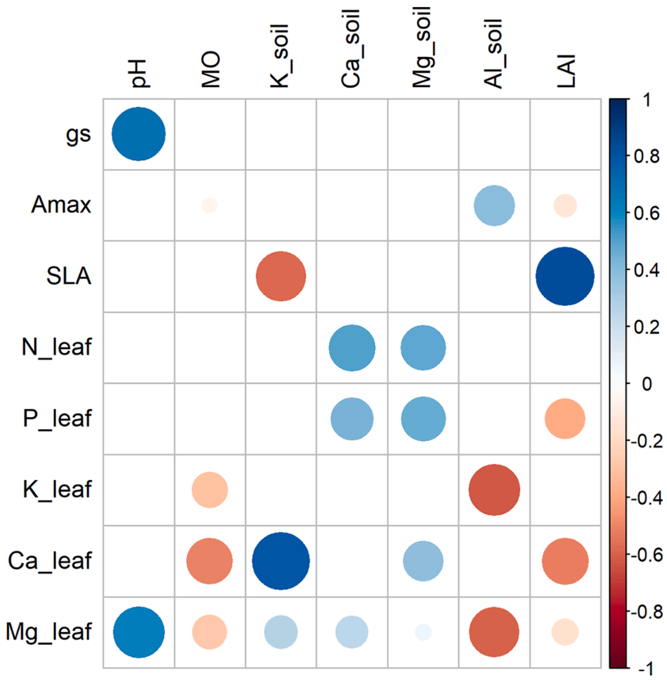

Fig. 5. Correlation matrix between ground layer plants community weighted means traits (gs: stomatal conductance; Amax: carbon assimilation; SLA: specific leaf area; N, P, K, Ca and Mg leaf concentrations) and environmental properties (pH, OM: organic matter; K, Ca, Mg and Al coil concentrations; LAI: leaf area index) in burned and unburned sites ($n = 10$). Correlation strength are represented by the size of the circle and values are displayed in a scale color going from −1 (dark red) to +1 (dark blue). Only significant correlations are shown ($P < 0.05$).

cover and soil chemical properties. As expected, plots where fire was excluded in the previous 20 years showed higher canopy cover, which may have been achieved by woody savanna species encroachment, or even by the invasion of gallery forest woody species, a common phenomenon observed in different sites at the same Ecological Reserve (Silva et al., 2008; Hoffmann et al., 2012). The relative high canopy cover lead to a higher soil organic matter content in the unburned sites, an effect caused by higher deposition of leaves on the soil surface (Rossatto and Rigobelo, 2016; Utaile et al., 2020). Although we did not measure soil water availability, we suggest that canopy cover and higher tree density in unburned sites may lead to higher soil water use compared to the burned plots, resulting in higher competition for water under vegetation encroachment (Quesada et al., 2008) and absence of fire (Sankaran et al., 2004). These responses, however, could obviously change according to the type of soil where the savanna vegetation is growing (Juhász et al., 2007).

Under frequent fire, woody species canopy and grass cover tends to decrease (Hoffmann et al., 2012; Pilon et al., 2020), releasing light competition. This process provides higher radiation levels, which may explain the observed increase in the photosynthetic activity of herbaceous plants (Ludwig et al., 2004; Rossatto et al., 2018). In fact, higher light availability may be the most important factor explaining the higher A_{max}, g_s and lower SLA of plants in burned plots, since leaf nutrient concentration did not differ, and N and P relationship scaled similarly between burned and unburned plots. These results are also supported by the positive correlation between LAI and SLA and the negative relationship between LAI and A_{max}. Indeed, light availability might be the immediate factor affecting species persistence under savanna sites with different degrees of canopy closure (Pinheiro et al., 2016), mainly because it can select for specific physiological performances regarding light use (Rossatto et al., 2018). Under high LAI, plants that exhibit traits linked with the capacity to explore low/diffuse light levels would be favoured by natural selection, and therefore it is expected to find species with lower maximum photosynthetic rates and high SLA (Carlos and Rossatto 2017).

Altought light may be an important factor affectng A_{max}, we cannot exclude the effects of soil water availability, which may directly affect stomatal conductance and water use efficiency. At frequently burned areas, competition for soil water tend to be lower in comparison with denser sites, which shows higher demand for soil water (Quesada et al., 2008). In fact, plants in the burned sites showed lower iWUE, which explains that their higher photosynthetic rates where achieved under higher stomatal conductance, only possible by either increasing soil water availability (Medrano et al., 2009), or by decreasing soil water competition (Sankaran et al., 2004).

When fire was absent for the past 20 years, herbaceous species showed lower A_{max} and g_s, suggesting a more conservative strategy in resource use. In fact, the possible woody encroachment, indicated by higher LAI and elevated organic matter in the soil, was shown to significantly decrease the richness and diversity of herbaceous plants in other Cerrado sites (Abreu et al., 2017; Pinheiro et al., 2016), decreasing also functional community metrics (Pilon et al., 2020). Under a closed-canopy system, species adapted to open environment and high light availability can be lost (Abreu et al., 2017; Rossatto et al., 2018), which may lead to the homogenization of the functionalities. This functional homogenization may be particularly noticeable in photosynthetic strategies, such as the elimination or significant decrease in C4 grasses abundance (Hoffmann et al., 2012). In such conditions, C4 grasses, which are critically limited by low irradiances, cannot reach adequate carbon assimilation rates to maintain their leaf costs (Rossatto and Franco 2017; Rossatto et al., 2018). The maintenance of a higher photosynthetic potential, even after medium term fire, may play important role for species persistence, mainly because the loss of the aboveground part caused by fire directly influences carbon balance of plants. Under frequent fire, herbaceous plants have to repeatedly rely on their underground organs to resprout and recover the aboveground biomass, which are destroyed during fire passage (Oliveras et al., 2013). In fact, in a recent before-after-control-impact study conducted in the South of the Cerrado, Pilon et al. (2020) found that ~79% of the herbaceous species resprouted basally or from below ground, while only 2% resprouted aerially, demonstrating the importance of the maintenance of the underground bud bank coupled with carbohydrate and nutrient storage in underground organs. The capacity to show higher maximum photosynthetic activity during the wet season may guarantee the rapid replacement of the used reserves for plants under fire regimes (de Moraes et al., 2016).

Although there is a vast literature reporting the effects of fire in different environmental conditions (Govender et al., 2006; Newberry et al., 2020; Sankaran et al., 2008; Souza et al., 2016) and resource availability (Pellegrini et al., 2015; Pivello et al., 2010), few studies have analysed its effects on leaf functional traits of tropical savanna ground layer plants. Additionally, most of these studies focused on a few species (Rossatto and Franco 2017; Carlos and Rossatto 2017; Rossatto et al., 2018), and they do not consider the community level. In one of these few studies found for herbaceous layer, Loiola et al. (2010) analysed plant individual traits and showed no changes for herbaceous functional diversity along different fire regimes. Our results, however, showed that, at least after a medium-term and at a community scale, herbaceous plants are capable to shift to a more resource-acquisitive strategy, exhibiting higher maximum photosynthetic capacity at the cost of higher stomatal conductance, regardless their growth form.

Sites that burned regularly generally showed higher soil nutrient availability and lower organic matter content, an expected effect caused by ash deposition, which was also reported for a nearby site (Pivello et al., 2010). Although it is expected that these deposited ashes release nutrients that are promptly absorbed by herbs and grasses, which have abundant shallow roots (Pivello and Coutinho, 1992; Pivello et al., 2010), we did not find differences in leaf nutrient concentration at the community level, except for lower Mg leaf concentration in burned plots. It might be that these responses, however, may only occur in the short-term immediately after fire, not persisting after three years. Similar unexpected results were also found in an experimental approach for frequently burnt plots in the same study area (Oliveras et al., 2013). Increases in nutrient availability in the soil of burned plots may lead to higher assimilation and allocation of such nutrients to the underground organs, so that leaf nutrient concentration does not indicate the nutritional status of the whole plant. Indeed, da Silva and Rossatto (2019) showed evidence that herbaceous and subshrub Cerrado species had higher macronutrient contents, such as K and Ca, in their subterranean organs.

In this study, we only compared two contrasting fire regimes, and therefore our results may be context-specific with limitations to extrapolate our findings to other sites under different fire regimes or with different edaphic conditions. Nonetheless, our study provides evidence that the impacts of fire at the community level of ground layer plants can be found even after 3 years of its occurrence. These impacts reflected in community-level changes in key functional traits related to the potential of carbon acquisition and water use. While ground layer plants nutrient use seems to be more resilient and constrained, maximum gas exchange rates are capable to adjust to changes in resource availability, which could guarantee the refill and accumulation of soluble carbohydrate to enable the persistence of these species under frequent fire. Although differences were found for some of the studied traits between growth forms, these effects did not interact with fire presence, showing a common physiological response of different growth forms to changes in environmental factors. Further studies should take into account different fire regimes and fire frequencies, as well as perform analyses of long-time series measuring photosynthetic capacity and water use in different functional groups to better understand how different disturbance regimes can affect plants in their shifting between an acquisitive and conservative strategy of resource use, and for how long such changes can last.

CRediT authorship contribution statement

Marina Corrêa Scalon: Data curation, Formal analysis, Writing – review & editing. **Davi Rodrigo Rossatto:** Conceptualization, Methodology, Writing – original draft.

Declaration of Competing Interest

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

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