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REGULAR ARTICLE

Influence of long-term nutrient manipulation on specific leaf area and leaf nutrient concentrations in savanna woody species of contrasting leaf phenologies

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Abstract
Background and aims The Neotropical Savanna in Central Brazil (Cerrado) is characterized by periodic fires and soils with extremely low nutrient levels. We used long-term field experiments to investigate how frequent fires and long-term changes in soil nutrient availability would affect leaf structure and leaf nutrient status of Cerrado trees.
Methods We measured specific leaf area (SLA, the ratio of leaf area per leaf dry mass) and leaf nutrient concentrations in 15 tree species of distinct leaf phenologies subjected to eight treatments, including control. Treatments comprised various conditions of nutrient availability (fertilization, addition and removal of litter) and fire regime (controlled biennial fires). The control consisted of undisturbed natural vegetation.
Results Leaf traits generally varied among species. Species responded to fertilization, exhibiting higher Ca and Mg leaf concentrations, while SLA, and leaf N, P and K concentrations did not differ across treatments. We found significant differences reflecting contrasting ecological strategies among phenological groups: deciduous species had higher nutrient leaf concentrations and SLA, while evergreen species showed the lowest values and briefly deciduous species showed intermediate values.
Conclusions We found low leaf-level responses to nutrient manipulations, probably reflecting the conservative resource-use strategy typical of nutrient-poor environments.

Keywords Leaf phenology · Fertilization · Fire · Litter manipulation · Phenotypic plasticity · Cerrado

Introduction

Adaptive hypotheses for the functional significance of plant ecological strategies have been commonly tested by studying quantitative variations in plant traits along natural environmental gradients. However, studies to evaluate the effects of long-term resource manipulation in natural systems are few, particularly in tropical systems (Grime 2006; Reich et al. 2003; Santiago 2015). Among all variables that might influence plant ecological strategies, fire and nutrient availability are important

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environmental factors shaping vegetation structure and composition. These factors are especially important in fire-prone vegetation, such as savannas (Medina and Silva 1990; Lehmann et al. 2011, 2014). Here, we availed of a unique opportunity to test variations in key leaf traits across different long-term resource manipulation experiments in a neotropical savanna in Central Brazil.

The savannas of Central Brazil (known as Cerrado) are characterized by high species richness and diversity due to a strong landscape heterogeneity that varies in response to climatic and edaphic factors (Haridasan 2000), and frequent fires (Coutinho 1990; Eiten 1972). The soils of the Cerrado are old and were exposed to a long period of intense leaching, which led to soil nutrient depletion (Furley 1999; Silva et al. 2013), reflecting in plants with low leaf nutrient concentrations (Rossatto and Franco 2017) and litter of relatively low nutritional quality (Kozovits et al. 2007). The climatic conditions, with a strong rainfall seasonality, also contribute to a very slow decomposition rate, averaging 15% per year in a cerrado sensu stricto (Peres et al. 1983), though plant nutrient losses are kept to a minimum through a high rate of re-translocation (Nardoto et al. 2006) and low residual leaf nutrient concentrations (Miatto et al. 2016), favouring nutrient retention within the living tissues.

Besides soil nutrient limitations, the Cerrado vegetation is subjected to frequent fires in the dry season. While the Cerrado vegetation has evolved several adaptive features to endure this factor (Eiten 1972), particularly the tree layer may still be negatively affected, e.g. decreasing species diversity and higher mortality rates, depending on fire frequency and intensity (Miranda et al. 2002). More broadly, fire also affects nutrient cycling, by reducing the amount and turnover of litter and by removing nutrients from the above-ground biomass (Kauffman et al. 1994).

Nutrients can be lost or added to the soil by various natural or human processes. The limiting nutrients in the Cerrado are most noticeably macronutrients, such as nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) (Furley and Ratter 1988). Higher soil fertility and lower soil pH are associated with differences in floristic composition, density and relative dominance of species, and greater concentrations of nutrients in the leaves (Haridasan 2000, 2008; Viani et al. 2014). The addition of nutrients by fertilization or litter manipulations in field trials may result in an increase in plant productivity, higher diameter growth and greater leaf area, in addition to changes in leaf phenology patterns (Poorter and Nagel 2000), although the magnitude of the response in tropical trees were dependent on the element in question and which ones were applied in combination (Santiago 2015). In the Cerrado, addition of N and P resulted in a faster litter decomposition rate (Kozovits et al. 2007) and altered several components of the hydraulic architecture of five dominant woody species (Bucci et al. 2006).

Cerrado climate is strongly seasonal, and precipitation is almost completely restricted to the wet season. During the dry season, plants experience a very high evaporative demand, developing an array of different strategies to deal with water deficit, which are associated to distinct patterns of leaf phenology (Bucci et al. 2005; Franco et al. 2005). Generally, Cerrado woody species falls into three broad phenological groups, according to the length of time that they remain without leaves during the year. While evergreen trees have a green canopy throughout the year, exerting strong stomatal control over transpiration to sustain plant water balance, deciduous and briefly deciduous species remain leafless for varied periods of time during the dry season (Franco et al. 2005). Most of these differences among plant species can be related to differences in the ecological strategies of use and acquisition of resources and expressed by the so-called ‘functional traits’. The concept of ‘ecological strategy’ adopted here, as defined by Westoby (1998), expresses the opportunities and selective forces shaping the ecology of a plant species to sustain a population. Economic analogies are often used in plant ecology in order to help understanding the trade-offs that shape ecological strategies, and especially to describe the trade-offs inherent to physiological processes; i.e., to identify the costs, benefits and evolutionary constraints that underpin functional traits and trait relationships (Bloom et al. 1985; Wright et al. 2004).

Specific leaf area (SLA) and leaf nutrient concentrations are important leaf-level traits to ascertain resource use strategies and the extent that these can be modified by changes in resource availability. SLA, which expresses the ratio of leaf area and leaf dry mass, describes the allocation of biomass per unit of leaf area. It is an important physiological trait to evaluate the trade-off between rapid production of biomass and efficient conservation of nutrients, and to report strategies of plant growth (Poorter and Garnier 1999). It also reflects the investment return for a given captured resource, correlating globally to photosynthetic rate, nitrogen concentration and leaf lifespan (Wright et al. 2004). Leaf nutrient concentration indicates the state of mineral nutrition of the species, and reflects soil fertility (Chapin III 1980). It is an important determinant of the species performance in their habitats (Aerts and Chapin 1999) and a metric widely used to study nutritional limitations, reflecting physiological needs, growth potential and nutrient availability in the environment (Field and Mooney 1986).

Assuming that leaves are plastic in physiology and morphology and, therefore, able to respond to changes in the surrounding environment, the aim of this study was to evaluate leaf-level functional responses of the Cerrado woody plants of contrasting leaf phenologies to fire and nutrient availability. We quantified variations in specific leaf area and leaf macronutrients concentration within and among 15 co-existing tree species, belonging to three phenological groups and subjected to distinct environmental conditions by long-term manipulation of fire regime and soil nutrients, investigating community, functional groups and species-level responses separately. We hypothesised that phenotypic plasticity (i.e. leaf trait variation) would play an important role in woody plant community response to differences in soil resource availability and stress conditions. Specifically, we expected that, compared to control (undisturbed) sites, woody species would show (1) higher leaf nutrient concentrations and higher SLA in fertilization and litter addition treatments and (2) lower leaf nutrient concentration and lower SLA in biennial fire regime and litter removal treatments. Secondly, we hypothesised that species from different phenological groups would respond differently, indicating that differences in leaf habit are associated to distinct ecological strategies of resource use within cerrado woody plant communities. Because evergreens exhibit a more conservative strategy of resource use, we expected that the effect of nutrient manipulation would be less pronounced in evergreen species compared to deciduous and briefly deciduous species (Franco et al. 2005). Thirdly, we hypothesised that intraspecific trait variability (the variation between individuals within species), would be an important component explaining trait variation in response to changes in abiotic conditions.

Material and methods

The study was conducted at the Ecological Reserve of the Brazilian Institute of Geography and Statistics (RECOR / IBGE), which is located 35 km south of Brasilia-DF, Brazil (15° 56′ 41″S, 47°53′07″ W). The site is part of the Environmental Protection Area of “Gama-Cabeça de Veado”, which has a total of 10,000 ha of continuous protected area.

In order to have a broad range of nutrient availability we selected nutrient manipulative treatments based on three ongoing long-term experiments at RECOR in 2006. These encompassed a classic nutrient addition experiment that utilized commercial fertilizers to change the availability of N, P, N + P or Ca + Mg and one experiment where the major natural source of nutrients (litter) to the soil was manipulated either by litter addition or litter removal. As recurrent fires can change nutrient availability (Kauffman et al. 1994), the third manipulative experiment was a cerrado sensu stricto site undergoing biennial fires. We elected as the, control an unaltered cerrado sensu stricto site that was protected from fire since 1992.

The fire treatment consisted of a 200 m × 200 m plot under controlled biennial fires occurring in the middle of the dry season (i.e., August) since 1992. The last controlled fire was three months before the leaves were collected for analyses during November–December, 2006. Our control site was also part of this experiment. Further details can be found in Pivello et al. (2010) and Oliveras et al. (2013). Soil pH and availability of Ca, Mg and K were shown to be higher in the burned plot compared to the unburned plot (Pivello et al. 2010).

The fertilization experiment consisted of four 15 m × 15 m replicate plots per treatment (N, P, N + P and Ca + Mg, Table 1) separated by at least 10 m from each other to avoid contamination among different treatments. In order to reduce the border effect, fertilization was also applied to a 1 m area surrounding the plots. The plots were fertilized twice a year since 1998 (Table 1), at the beginning and the end of the rainy season (April and September, respectively) to allow nutrients to be absorbed by the soil and avoid losses from the system by leaching. The plots in which lime (Ca + Mg) was added were fertilized only once a year, at the beginning of the rainy season. The whole study area accidentally burned in 2005. Details on this experiment and differences in soil properties as a result of the fertilization can be found in Scholz et al. (2007), Kozovits et al. (2007),

Table 1 Fertilizers used and the amount of nutrients added to the system in the long-term experiment in the cerrado sensu stricto experimental plots

Treatment	Fertilizer	Nutrient (kg ha^{-1} year $^{-1}$)
N	Ammonium sulphate ($(NH_4)_2SO_4$)	100 (N)
P	Simple superphosphate ($Ca(H_2PO_4)_2 + CaSO_4.2H_2O$)	100 (P)
N + P	Ammonium sulphate and simple superphosphate	100 (N) and 100 (P)
Ca + Mg	60% Dolomitic lime* 40% Calcium sulphate	4000 (Ca + Mg)

*consisting of approximately 50% calcium carbonate and 40% magnesium carbonate

Jacobson et al. (2011), and Bustamante et al. (2012). Briefly, there was an increase in soil pH, available P and inorganic N in the N, N + P and P treatments and an increase in Ca and Mg concentrations and decrease in soil pH in the Ca + Mg plot, but not a significant difference in total N in soil top layers.

The litter manipulation experiment consisted of ten 20 m × 20 m plots, where five had the litter removed every two months (litter removal treatment), and five had the removed litter added (litter addition treatment). The litter removal and addition were carried out manually since January 2005, including a 5 m border outside each plot (30 m × 30 m), aimed at minimizing the border effect. Details of this experiment can be found in Villalobos-Vega et al. (2011) and Scalon et al. (2014). Villalobos-Vega et al. (2011) reported that litter addition plots showed higher N, P, C, Ca, Mg, and Mn levels than litter removal plots, but soil pH was marginally lower.

Species

The 15 species were chosen based on their abundance in the experimental areas and classified according to their leaf phenology (da Silva Júnior and dos Santos 2005; Franco et al. 2005) as evergreen, briefly deciduous or deciduous (Table 2). In this study, species were considered evergreen if they maintained a photosynthetic canopy all year around. Briefly-deciduous species lost all their leaves and remained leafless for a period of about 21 days or less, while deciduous species remained without leaves for a period longer than 21 days. For each treatment, we sampled up to five and a minimum of three species from each leaf phenological group, according to their occurrence in each experiment. Three individuals of each species were selected in each treatment, depending on the species occurrence (see Supplementary Material for complete data set).

Leaf measurements

Five mature, fully expanded sun leaves were collected from each individual of each species and taken immediately to the laboratory, where they were scanned, and leaf area calculated using the software AREA version 2.1 (Caldas et al. 1992). The leaves were washed with distilled water and dried at 70 °C until constant mass. Dried leaves were weighed and the specific leaf area was calculated as the ratio between the fresh leaf area

Table 2 Phenological classification of the Cerrado woody species used in this study according to the duration they remain leafless during the dry season

Phenological group/ Species	Family
Evergreen	
Schefflera macrocarpa (Cham. & Schltdl) Frodin	Araliaceae
Tachigali vulgaris L.F. Gomes da Silva & H.C. Lima	Fabaceae
Miconia pohliana Cogn.	Melastomataceae
Ouratea hexasperma (A.St.-Hil.) Baill.	Ochnaceae
Vochysia elliptica Mart.	Vochysiaceae
Briefly deciduous	
Caryocar brasiliense A.St.-Hil.	Caryocaraceae
Dalbergia miscolobium Benth.	Fabaceae
Byrsonima pachyphylla A. Juss.	Malpighiaceae
Blepharocalyx salicifolius (Humb., Bompl. & Kunth) Berg	Myrtaceae
Stryphnodendron adstringens (Mart.) Coville	Fabaceae
Deciduous	
Aspidosperma tomentosum Mart.	Apocynaceae
Kielmeyera coriacea (Spreng.) Mart.	Calophyllaceae
Guapira noxia (Netto) Lundell	Nyctaginaceae
Qualea grandiflora Mart.	Vochysiaceae
Qualea parviflora Mart.	Vochysiaceae

and dry mass of the leaves (or leaflets) of each individual. The dry material was grounded in a Wiley mill and leaf concentrations of P, K, Ca, Mg and Al were determined after wet digestion with a tri-acid mixture (nitric, sulfuric and perchloric acid - 10:1:2). P was determinated by colorimetry and Ca, Mg and Al by atomic absorption or emission spectrophotometry. N was determined by micro-Kjeldahl digestion and distillation. Leaf samples were collected during the rainy season (November and December 2006).

Data analyses

All analyses were performed using software R version 3.1.0 (R Development Core Team 2014). The mean value for each species for each measured variable was used for all statistical analyses. Data were $\log_{10}$-transformed to restore normality and checked with the Shapiro-Wilk test (Quinn and Keough 2002). The effects of treatments and phenological groups on SLA and nutrient concentration were determined by multivariate analysis of variance (MANOVA), followed by univariate ANOVAs to test for individual effects of each of the six response~~s~~ variables. We performed Tukey's test for a post-hoc mean comparison and differences were considered to be significant at $P < 0.05$. Pearson correlations were used to assess bivariate relationships. For bivariate analysis and comparison between leaf phenological groups, results of experiments were lumped. All other analysis were done separately for each experiment.

To examine the amount of trait variation explained by differences across treatments, we partitioned the variance of $\log_{10}$-transformed traits within and among species and phenological groups. The decomposition of variance on restricted maximum likelihood method (REML) was applied according to Messier et al. (2010), using the package 'cati' version 0.99 (Taudiere and Violle 2015).

Results

Multivariate analysis of variance showed significant differences among the phenological groups and among treatments (Table 3). The interaction between these two factors was not significant, suggesting that the three phenological groups responded similarly to the environmental differences among sites.

Table 3 Multivariate analysis of variance (MANOVA) for the effects of phenology (deciduous, evergreen and briefly deciduous) and treatments (biennial fire, fertilization and litter removal and addition) on specific leaf area (SLA) and leaf nutrient concentrations (N, P, Ca, Mg and K) of Cerrado woody plants

Source of variation	df	*F*-value	*P*-value
Phenological groups	2	20.94	< 0.001
Treatments	7	8.19	< 0.001
Phenological groups x Treatments	14	0.68	0.985
Residuals	84		

Specific leaf area ranged from 70.9 ± 21.0 $\mathrm{cm}^2\ \mathrm{g}^{-1}$ (mean ± sd) in the nitrogen fertilization treatment to 90.5 ± 27.4 $\mathrm{cm}^2\ \mathrm{g}^{-1}$ in the control plot, and did not vary significantly among treatments (Table 4; Fig. 1a, $P = 0.843$). Considering the different phenological

Table 4 Univariate analysis of variance (ANOVA) for each of the response variables tested: leaf concentrations of N, P, K, Ca and Mg, and specific leaf area (SLA) among phenological groups (deciduous, evergreen and briefly deciduous), treatments (biennial fire, fertilization and litter removal and addition) and the interaction between phenology and treatments

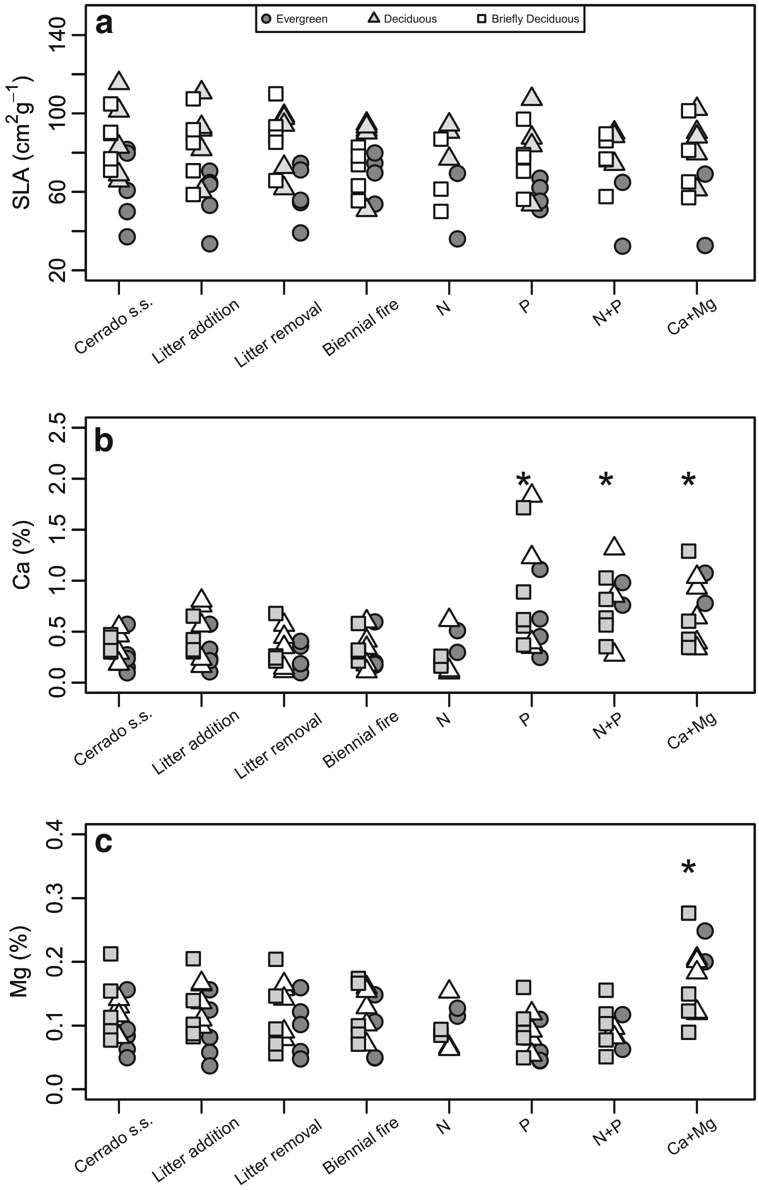
Source of variation	df	*F*-value	*P*-value
Phenological groups	2,12		
N		4.85	0.010
P		11.84	< 0.001
K		12.78	< 0.001
Ca		0.84	0.413
Mg		1.95	0.149
SLA		21.50	< 0.001
Treatments	7,42		
N		0.53	0.807
P		2.06	0.058
K		0.89	0.517
Ca		7.02	< 0.001
Mg		4.07	< 0.001
SLA		0.48	0.843
Phenological groups x Treatments	14,84		
N		0.27	0.995
P		0.61	0.847
K		0.18	0.999
Ca		0.46	0.948
Mg		0.70	0.764
SLA		0.48	0.937

groups, SLA of briefly deciduous (87.42 ± 17.46 cm^2 g^{-1}) and deciduous (81.04 ± 20.52 cm^2 g^{-1}) species were significantly higher than evergreen species (60.51 ± 17.92 cm^2 g^{-1}; $P < 0.001$; Fig. 2).

Leaf nutrient concentration varied with fertilization treatment, resulting in significant increases in Ca leaf concentration for the two treatments of simple superphosphate addition, and Ca and Mg leaf concentrations in the treatment with dolomitic lime and calcium sulphate addition (Table 4, Fig. 1b and c). In contrast, phenological groups differed in leaf N, P and K concentrations but not in Ca and Mg (Table 4). The highest leaf concentrations of N, P and K were measured in deciduous species (Fig. 2, all $P < 0.05$) while briefly deciduous species showed higher N leaf concentrations compared to evergreen species, but did not differ in P and K concentrations (Fig. 2).

For most traits, more of the overall variance was attributable to differences among species, ranging from 36.5% of the variation explained by species differences for P leaf concentration, to

Fig. 1 Specific leaf area (SLA) and foliar concentrations of Ca and Mg of evergreen (black circles), deciduous (grey circles) and briefly deciduous (white circles) species in different environmental treatments and control (Cerrado s.s.): litter addition; litter removal; biennial fire; fertilization with ammonium sulphate (N); fertilization with simple superphosphate (P); fertilization with ammonium sulphate and simple superphosphate (N + P); fertilization with dolomitic lime and calcium sulphate (Ca + Mg). The symbol * represents significant differences among treatments (ANOVA, $P < 0.05$)



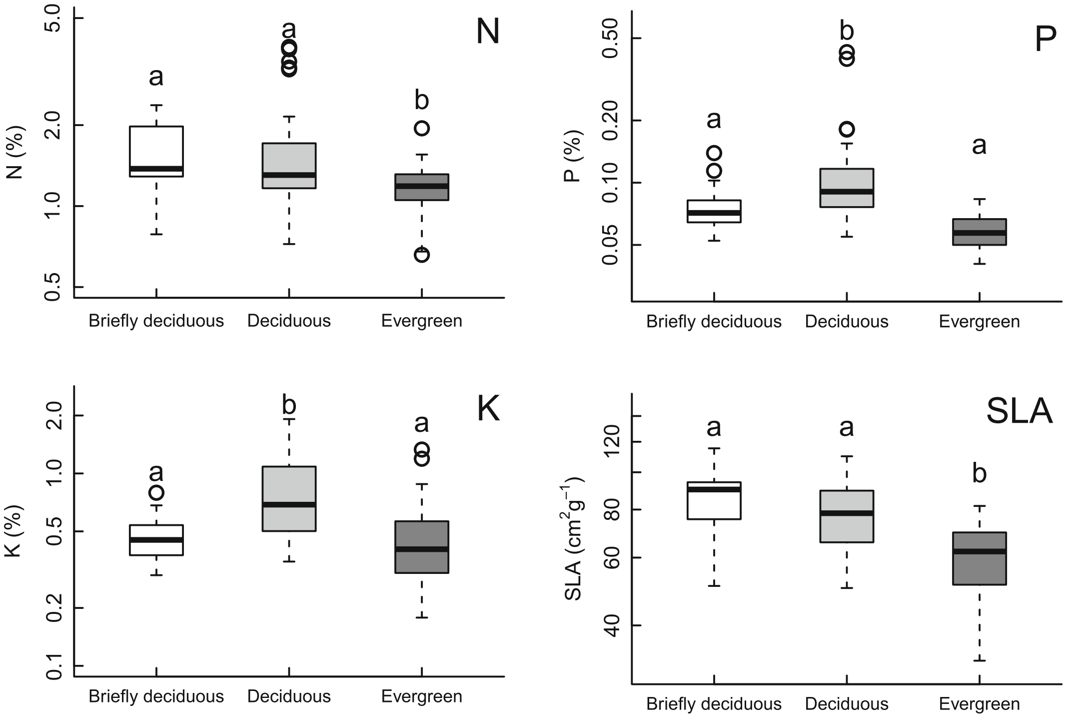


Fig. 2 Boxplots of foliar concentrations of N, P and K, and specific leaf area (SLA) among the three different phenological groups of Cerrado woody species. The continuous line within the box shows the median, and error bars show 10 and 90 percentiles ($n = 5$ species per phenological group). Different letters indicate differences according to Tukey's test ($P < 0.05$)

61.9% and 78.8% for N and Al leaf concentration, respectively (Fig. 3). Phenology explained between <0.01% of Ca leaf concentration to 38.1% of the variability in P concentration (Fig. 3). Within species variation ranged from 11.4% for Al to 42.7% for Ca leaf concentration (Fig. 3).

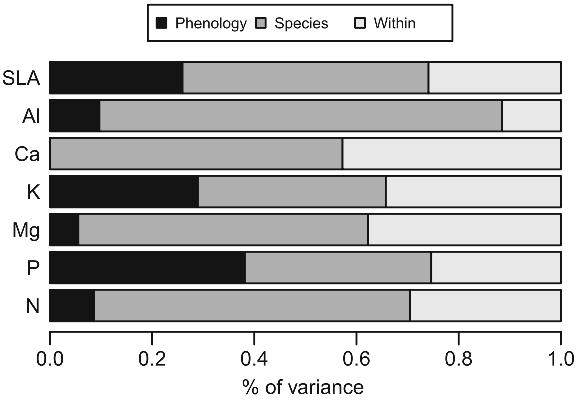


Fig. 3 Variance partitioning of the nested linear models on specific leaf area (SLA), Al, and leaf nutrient concentrations (Ca, K, Mg, P, N) within and between species and phenological groups. All data were log-10 transformed prior to analysis

Despite the large variability in nutrient concentration among species, N and P, Ca and Mg concentration in leaves were positively and significantly correlated (Figs. 4a and b). As found in Araújo and Haridasan (2007) and in this study, *Guapira noxia* had much higher concentrations of N and P, showing disparate (outlier) values. When this species was disregarded from the correlation analysis between SLA and leaf concentrations of these nutrients SLA was positively correlated with N and P leaf concentrations (Fig. 4c and d). There was no significant correlation between SLA and other nutrients.

Discussion

Nutrient manipulation responses

Leaf responses to nutrient manipulation were limited for the functional traits we analysed in this study. With the reduction of nutritional limitation in fertilizer and litter addition treatments, we expected to observe a greater investment in leaf area and a reduction in leaf mass,

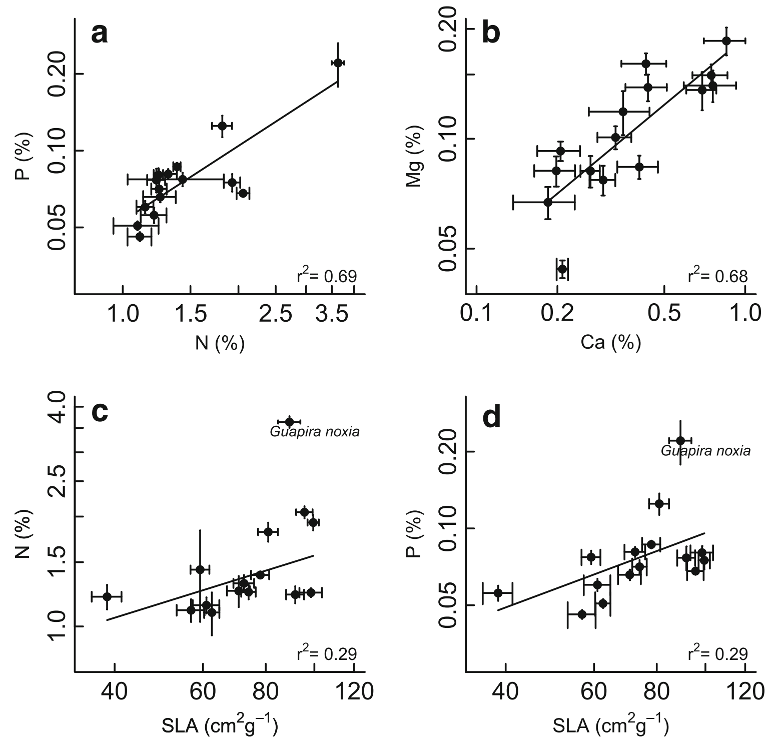


Fig. 4 Correlations between mean values for species of **a** N and P, **b** Ca and Mg, **c** specific leaf area (SLA) and N, **d** SLA and P. The bars represent standard deviation ($P < 0.05$)

resulting in decreasing leaf sclerophylly (higher SLA), which was not observed (Fig. 1a). Other studies also did not find changes in SLA with nutrient addition either for woody species in Cerrado (Bucci et al. 2006; Kozovits et al. 2007) or in other nutrient-poor systems (Harrington et al. 2001; Valladares et al. 2000a). Because the activity of leaf meristematic tissues also depends on carbohydrate supply (Sugiyama and Gotoh 2010), the limited response of SLA, holding fairly constant across the different nutrient treatments, may be associated with additional environmental filters that limit carbon uptake. Irrespective of nutrient availability, leaf carbon balance of cerrado trees is constrained by the high atmospheric evaporative demand and hydraulic limitations on the amount of water that can be extracted and transpired daily, which imposes strong stomatal limitation of transpiration and a prolonged midday depression of photosynthetic rates during both the wet and dry seasons (Franco 1998; Meinzer et al. 1999; Franco et al. 2007; Goldstein et al. 2008). Alternatively, it is well known that species from abiotic stressful environments tend to be less plastic than species from milder environments (Valladares et al. 2000a; b). Thus, conservative traits that minimize nutrient losses would be strongly selected in cerrado woody species at the expense of traits that give a rapid response to a transient increase in the supply of nutrients that may not be able to be sustained once these more favourable conditions are over (Chapin 1991; Chapin III et al. 1993). Therefore, the addition of nutrients would not be sufficient to cause significant structural changes in the leaves on an ecological (short) timescale, such as few years or decades.

The treatments of litter addition or removal may not have had significant effect on the traits studied here because, in the Cerrado, litter is characterised by low levels of macronutrients and a low and seasonal rate of decomposition (Peres et al. 1983). Because of its low nutrient levels, litter is not the main source of nutrients for vegetation, and plants depend heavily on an internal transfer cycle, or nutrient resorption (Kozovits et al. 2007). However, Villalobos-Vega et al. (2011) and Scalon et al. (2014) have shown that litter manipulation affected soil temperature, water availability, and leaf traits linked to the water status of the plants. Removing the litter resulted in higher leaf water use efficiency in woody plants, suggesting a rapid response by adjusting leaf water loss (Scalon et al. 2014), even though these changes were probably not strong enough to cause any significant difference in the traits we evaluated here.

Leaf nutrient concentration and SLA of woody Cerrado trees were also not affected by frequent fires. Indeed, while Kauffman et al. (1994) and Pivello et al. (2010) found significant amount of nutrient losses associated with fire, it is suggested that Cerrado nutrient pools are little affected by fire since most of plant biomass is located below-ground (Kauffman et al. 1994). Moreover, dynamics between nutrient input by litterfall enrichment after fire, and nutrient output due to depleted nutrient stocks (Nardoto et al. 2006) may compensate each other so that leaf nutrient concentration is not affected.

In addition to the lack of response of SLA to changes in nutrient availability, there was no significant response in N and P leaf concentrations in the N, N + P and P fertilization treatments. Even though the fertilization experiment area was accidentally burned one year before leaves were collected, we do not believe this affected our results since there were different leaf-level responses according to the nutrient considered. However, the lack of response at the leaf level does not rule out that those nutrients are limiting growth of cerrado trees. For example, Bucci et al. (2006) studying five species in the same fertilized area, found an increase in total leaf area in the N addition treatment, and a greater basal area increment under both N and P fertilizations compared to the control. Traits such as above and belowground biomass ratio, leaf lifespan, and nutrient concentration responded to N and P fertilization in other nutrient-poor systems (Barger et al. 2002; Cordell et al. 2001; Harrington et al. 2001). Therefore, our results suggest that leaf traits, such as SLA and leaf N and P concentrations, are conserved in Cerrado woody trees, but other traits might be more plastic and useful to investigate plant ecological strategies under environmental changes in this system. On the other hand, the significant increase in Ca and Mg leaf concentrations in treatments where these nutrients were added (Fig. 1b and c) suggests that the available quantity of these nutrients in the soil was probably limiting leaf level processes.

Phenology, inter- and intraspecific variability

While intraspecific variability was lower than interspecific variability, it was not negligible, reaching over 25% of the total variability for most traits (Fig. 3). The influence of the individual trait value variability in species responses to environmental changes is still poorly tested. It has been argued that, when studying ecosystem functioning, using species mean trait values due to its low sensitivity to intraspecific variation is acceptable (Lavorel et al. 2008). However, intraspecific variability is an important adaptive mechanism, enabling plant species to survive under changing environmental conditions and, therefore, should be accounted for in such studies (Albert et al. 2010a, b).

The differences observed among the phenological groups in SLA reflect the differences in strategies of resource use and carbon balance. SLA can be used as a proxy for leaf sclerophylly (Edwards et al. 2000), being correlated to leaf lifespan, leaf N concentration and photosynthetic rates (Reich et al. 1997). All these traits and trade-offs are powerful indicators of plant resource use strategies, described as the ‘worldwide leaf economics spectrum’ (Wright et al. 2004). At one end of the spectrum, representing the resource conservation strategy, there are species with low values of SLA and nutrient leaf concentrations, such as the evergreen species (Fig. 2), investing more in the construction of leaves with greater longevity, prioritising resource conservation (Reich et al. 1997). Low SLA is considered to be advantageous in nutrient poor habitats with seasonal water availability, promoting nutrient retention by extending leaf lifespan and reducing water loss by thickening lamina cuticle (Chapin III 1980; Cunningham et al. 1999). At the other end of the spectrum, representing rapid resource acquisition strategy, there are species such as deciduous species with higher SLA, and higher leaf N and P concentrations (Fig. 2) that invest more in productivity and, consequently, show lower leaf longevity and greater herbivore vulnerability (Coley et al. 1985; Grime et al. 1996). Briefly deciduous species showed intermediate behaviour, positioning somewhere in the middle of the spectrum, showing SLA and N concentrations similar to deciduous species and P and K concentrations similar to evergreen species (Fig. 2). It is important to note, however, that all three phenological groups adopt different strategies but coexist in the same environment, dealing with similar constraints in distinct ways. While evergreen species have a higher cost of maintenance and construction of the leaves, deciduous species face a higher belowground cost, associated with the maintenance of deeper root systems to access year-around moist soil layers and therefore allowing recovery of plant water status which is essential to leaf resprouting at the end of the dry period (Franco et al. 2005). Indeed, in a recent study exploring plant water use strategies and phenological variations in response to climatic variability, Vico et al. (2015) have developed a model that emphasises the importance of root depth variation in predicting leaf abscission and duration.

Despite interspecific differences in leaf nutrients, reflecting the intrinsic characteristics of each species, there was a positive correlation between Mg and Ca, and N and P, related to the biochemical balance of the cells, maximizing the synthesis of proteins and the production of plant tissue in natural environments (Garten 1976). Ca and Mg are associated in the metabolism and photosynthesis, both acting as activators of metabolic reaction enzymes (Epstein and Bloom 2005) and with structural functions, while N and P are closely associated in cytoplasmic and nuclear material in the plant cell, both being involved in metabolic reaction for photosynthesis, light capture and growth (Niklas et al. 2005).

Conclusions

The fertilization treatments increased leaf Ca and Mg concentrations in Cerrado trees, but did not influence specific leaf area or N, P or K concentrations. The low leaf-level responses to nutrient manipulations and fire are indicative of resource-use strategies for enhancing nutrient conservation as expected in nutrient-poor environments, such as the Cerrado. Contrasting strategies among the phenological groups were reflected both in leaf nutrient concentrations and specific leaf area. For most nutrients, the highest leaf concentrations were measured in deciduous species, while the evergreen species showed the lowest values. Briefly deciduous species had intermediate values, similar to deciduous species in terms of SLA and Mg concentrations, and to the evergreens in K and P concentrations. Intraspecific variability was not negligible for most traits, reiterating the importance to account for quantitative variation at the population level in plant trait-based approaches.

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