

Changes in irradiance and soil properties explain why typical non-arboreal savanna species disappear under tree encroachment

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Abstract. Savanna vegetation maintains its openness and its diverse plant composition because of frequent fire events; however, when these are suppressed, encroachment is caused by increases in the tree density. In the neotropical savanna (cerrado of Brazil), typical forest trees are invading savanna areas, altering abiotic conditions and affecting the persistence of their exclusive species. Here we studied changes in abiotic conditions, species richness and diversity of a non-arboreal community (herbs, vines, grasses, subshrubs and shrubs) in a gradient of encroachment at a site where fire has been suppressed for more than 50 years in south-eastern Brazil. Encroached communities were more shaded and possessed a wetter and richer soil (higher contents of organic matter and P) compared with the typical savanna. These abiotic changes were related to a less rich and less diverse plant community in encroached savanna compared with typical savanna. The most important abiotic variables driving such changes were photosynthetically active radiation (PAR) reaching the understorey and soil P content: communities with lower species richness and diversity had lower PAR incidence and higher soil P content. Our results suggest that non-arboreal savanna species may be under serious threat of extinction given the expected ecological changes caused by the widespread expansion of forest on the savannas in the absence of fire.

Additional keywords: crowding, herbs, savanna, vegetation dynamics.

Received 12 December 2015, accepted 2 June 2016, published online 17 June 2016

Introduction

Tree encroachment is a worldwide phenomenon taking place in many tropical and temperate regions (Briggs *et al.* 2002; Wigley *et al.* 2010; Ratajczak *et al.* 2012; Murphy and Bowman 2012; Ascoli *et al.* 2013; Heijmans *et al.* 2013), altering ecosystem abiotic aspects and directly disturbing the biotic component (Eldridge *et al.* 2011). In tropical and subtropical regions it is taking place particularly in savanna ecosystems, where, for the past 3000 years, woody species has been increasing in density in more open physiognomies (Wiegand *et al.* 2006; Silva *et al.* 2008; Wigley *et al.* 2010; Bond and Midgley 2012; Buitenwerf *et al.* 2012; Murphy and Bowman 2012). In many tropical savanna sites, these changes are linked to the invasion of typical forest species, which can alter the savanna environment, changing its abiotic determinants and the availability of resources (McGuire *et al.* 2001; Hoffmann *et al.* 2012a).

Tropical savannas are environments structured by the presence of scattered trees over a very rich and diverse, continuous grassy herbaceous stratum (Scholes and Archer

1997). The components of arboreal and herbaceous layers possess mechanisms to adapt to the most important abiotic and biotic aspects of their systems; fire, light, water seasonality, soil resources and herbivory (Solbrig *et al.* 1996; Franco 2002; Staver *et al.* 2009, 2011). The capacity to deal with drought during the long dry season, low soil nutrient availability and high light irradiances are determinants of plant functioning in these ecosystems (Seghieri *et al.* 1995; Prior *et al.* 1997; Franco and Lüttge 2002; Goldstein *et al.* 2008; de Souza *et al.* 2016). Fire frequency is also a determinant of savanna physiognomies and their species composition (Sarmiento *et al.* 1985; Durigan and Ratter 2016), since it maintains the openness of the vegetation and decreases the amount of organic matter and nutrients in the soil surface (de Souza *et al.* 2016).

Under fire suppression regimes, typical forest tree species can establish in savanna environments promoting their encroachment (Hoffmann 2000; Ibanez *et al.* 2013). The high recruitment of forest species, with their denser and larger canopies (Rossatto *et al.* 2009; Hoffmann *et al.* 2012a), contributes to the closing of these environments. Additionally, their elevated leaf area index

and consequently higher capacity to deposit litter on the soil surface (Hoffmann *et al.* 2012a; Paiva *et al.* 2015; Rossatto and Rigobelo 2016) contributes to modifications to the savanna microclimate (Hoffmann *et al.* 2012b). Such changes provide the appearance of new niches in the encroached savanna environment, which are characterised by the reduced solar radiation reaching the understorey, followed by higher soil moisture (Hoffmann *et al.* 2012b) and nutrient availability (Hibbard *et al.* 2001; Blaser *et al.* 2014; Rossatto and Rigobelo 2016).

Studies have reported changes in species composition and richness due to forest encroachment in typical savanna areas of Africa (Smit 2004; Mitchard *et al.* 2009), Australia (Bowman 1992; Bowman *et al.* 2001) and especially in central and south-eastern savanna areas of Brazil (Pinheiro and Monteiro 2006; Pinheiro and Durigan 2012; Almeida *et al.* 2014). However, these reports have mostly been based on the analysis of floristic and structural aspects of arboreal plant communities, not on relating them to changes in abiotic conditions. Further, studies evaluating the relationship between encroachment-driven environmental changes and the structure and composition of savanna plant communities are rare (Ludwig *et al.* 2004; Eldridge *et al.* 2011; Ratajczak *et al.* 2012). This is especially true when dealing with the savanna understorey component, because the literature is mainly focussed on the responses of trees or grasses to changes in abiotic aspects (Sankaran *et al.* 2004; Staver *et al.* 2009; Wigley *et al.* 2010). To comprehend the relationship between changes in abiotic aspects and plant communities is of considerable importance, particularly for tropical savannas of Brazil (the cerrado), since the composition of its non-arboreal layer is richer in terms of eudicot herbs, shrubs and subshrubs, in comparison with other savannas (Gottsberger and Silberbauer-Gottsberger 2006; Mendonça *et al.* 2008).

Typical savanna herbaceous and shrub species may be filtered in environments with low light and high water and nutrient availability (Ludwig *et al.* 2004; Bustamante *et al.* 2012; Rossatto *et al.* 2012, 2014). Changes in floristic composition and loss of typical and exclusive savanna non-arboreal species are then expected under encroached situations, being of extreme concern in terms of the conservation and maintenance of savanna ecosystems. Such species drive the most important ecosystemic aspects of these systems, as they are the primordial source of flammability (Hoffmann *et al.* 2012b) and provide essential resources for most of the invertebrate fauna (Gottsberger and Silberbauer-Gottsberger 2006).

In the present study, we investigated changes in abiotic factors caused by tree encroachment, and its effects on the number of species (species richness) and the Simpson's diversity index (diversity) of non-arboreal communities in a gradient of encroachment at a savanna in south-eastern Brazil. We hypothesised that communities differing in the degree of encroachment will show distinct values of species richness and diversity, derived from changes in the availability of light, water and soil resources. Specifically, we expected to find lower light availability, higher humidity and higher nutrient content in encroached conditions – situations where the number of typical and exclusive savanna species and diversity are low. In contrast, richness and diversity was expected to be higher in the savanna without encroachment, given its low

canopy cover, which would propitiate the typical high light intensities necessary for its species to grow and persist.

Materials and methods

Study area

This study was conducted at the Assis Ecological Station (AES), located at Assis municipality, São Paulo state, Brazil (22°35'51.36"S, 50°22'26.68"W), during October 2014. AES has elevations varying between 500 and 588 m above sea level. The climate is transitional between Cwa and Cfa according to Köppen classification, with a dry season between May and September, and a rainy season between October and April. Similar to many other conservation units throughout the São Paulo state, the AES reserve has experienced more than 50 years without fire (Durigan *et al.* 1999; Durigan and Ratter 2006; Pinheiro and Durigan 2009). This absence has caused the encroachment of many typical savanna sites due to the advance of the typical forest species over the savanna areas (Durigan and Ratter 2006; Pinheiro and Durigan 2009). We conducted our study in a typical savanna region that has experienced tree encroachment for the past 50 years (Durigan and Ratter 2006). The heterogeneity of tree invasion (Pinheiro and Durigan 2009) was utilised as a 'natural experiment' to evaluate the effect of forest encroachment into community structural aspects.

Sampling the non-arboreal community

To determine the floristic richness of the studied communities we adopted the transect and quadrat methods (Munhoz and Araújo 2013). We selected five sites at AES (22°35'53.47"S and 50°21'57.42"S) where it was possible to found a gradient between typical savanna (yet under initial process of encroachment) and forested savanna (totally encroached), following the commonly used approach to study forest–savanna boundaries dynamics (Geiger *et al.* 2011). We positioned a transect in each of these sites, such that the transect was perpendicular to the encroached savanna edge and centred at the intermediate encroachment condition (dense savanna). The edge in our study was considered the region in where typical savanna becomes a denser savanna (increase in the tree density, but still with the presence of herbaceous species). We selected these sites based on maps published by Pinheiro and Durigan (2009), which provided aerial photographs of the tree encroachment in the AES in a period of 40 years (1962–2006).

Each transect was 165 m long and 4 m wide, representing three plant communities differing in their tree density and leaf area index (LAI). In each of these transects, the first studied community was a typical savanna (TS): a vegetation in initial stages of encroachment, possessing discontinuous tree layer over a continuous herbaceous-grass stratum, with 785 trees ha⁻¹ and LAI of 0.96 ± 0.049. The second was a dense savanna (DS): primary a TS site at intermediate stage of encroachment, which possesses a denser tree layer (1050 trees ha⁻¹) and a LAI of 1.22 ± 0.13, given the invasion of typical forest species. Finally, we had a forested savanna (FS): previously a TS vegetation, which now possesses a continuous tree layer (1605 trees ha⁻¹) and elevated LAI (1.90 ± 0.12), with few herbaceous species and where grasses are absent. In each transect, we randomly placed one 2 × 10 m plot at the TS, one plot at the

DS and one plot for FS, totalling three plots per transect, and five plots in each community (total of 15 plots).

All non-arboreal individuals with more than 10 cm in height were counted in each plot. Samples were collected and identified through the specialised literature (Durigan *et al.* 2004) and with the help of experts. The names of the species were confirmed as validation and synonymy in The Plant List (<http://www.theplantlist.org/>, accessed 15 October 2015) and Flora do Brasil (<http://www.floradobrasil.jbrj.gov.br/>, accessed 15 October 2015). Plant material samples were herborised according to usual procedures, and deposited in the herbarium 'Hassi' Unesp-Assis. The species were grouped in families recognised by the Angiosperm Phylogeny Group III. The species classification in growth forms was performed according to Durigan *et al.* (1999, 2004) and Rossatto *et al.* (2008).

To describe biodiversity aspects of the studied plant communities, we evaluated two measurements; number of species recorded at each studied condition (species richness), and the Simpson's diversity index (Bock *et al.* 2007). Species richness was obtained after performing rarefaction analysis in the EcoSim7.00 software (Gotelli and Entsminger 2004) to compare the species richness between the three different degrees of tree density (Magurran 2004). We compared the richness achieved by a similar number of individuals in each studied plot. We also calculated the Simpson's diversity index (D) in the Past 2.0 software (Hammer *et al.* 2001), because D is highly recommended for differentiating sites and is more discriminating when dominant species are present in different communities (Morris *et al.* 2014).

Abiotic aspects

To characterise the light environment in each studied community, we measured the photosynthetic active radiation (PAR) reaching the plants in the understorey of our plots during the period of 1000 to 1200 hours in a typical sunny day without clouds (10 measurements per hour per plot). The measurements were performed using a CI-110-24P-ID (CID Bioscience Inc., Camas, WA, USA) at 70 cm above the soil surface (enough to encompass the majority of our studied species).

To evaluate the quantity of litter deposited in each plot, we measured the thickness of the litter layer in five points (one at the centre and one at each corner of the plot) for each of the 15 plots studied using a precision ruler (mm). The mean value was used to calculate the volume of litter deposited in each plot (m^3) as the area of the plot ($20 m^2$) multiplied by the thickness of the litter layer.

We collected samples at the top 0–20 cm soil depth, after removing the litter layer, to characterise the physical and chemical properties of the surface soil of each site (TS, DS and FT) at each transect. Just after sampling, the plot subsamples were mixed and placed in plastic bags, quickly sealed and then taken to the laboratory. For physical analysis, we determined the particle size distribution (fine and coarse sand, silt, and clay soil fractions) using the pipette method (Camargo *et al.* 1986). For chemical analysis, pH was determined under $CaCl_2$ following Raij *et al.* (1987). Soil P was determined by spectrophotometry after anion exchange resin extraction (Raij *et al.* 1987). Soil K,

Ca and Mg were determined by flame spectrophotometry (Allen *et al.* 1974). Organic matter was determined after the addition of potassium dichromate-sulfuric acid to the soil samples (Sims and Harby 1971). Soil moisture was measured at 0–20 cm depth, in the same plots where the floristic and soil analysis were performed. We measured soil moisture using a volumetric soil moisture instrument (TRIME-PICO IPG, Imko micromodultechnik, GMBH). Five measurements were performed per plot in a sunny day of October 2014, where the last rainfall (60 mm) occurred 2 weeks before our measurements.

Statistical analysis

To verify differences in the mean values of environmental variables, we performed a MANOVA followed by individual ANOVAs to test the significance of each variable. The majority of the data possessed normality (Kolmogorov–Smirnov test, $D < 0.15$ and $P > 0.20$) and homogeneity of variances (Levene's test $F < 2.00$ and $P > 0.11$). When these assumptions were not met, data were transformed using \log_{10} . Differences in richness and diversity were also assessed with an ANOVA.

To test the effects of the abiotic variables on the species richness and diversity, we proposed an *a priori* model using structure equation modelling (Fig. 1). Structure equation models are normally used to unravel the linking structure of variables that are correlated in a multivariate way, based on previous knowledge of variables relationships (Shipley 2002). In our model, we considered LAI as a significant descriptor variable of the tree encroachment (Hoffmann *et al.* 2012a). Based on this assumption, LAI caused changes in the light environment (affecting PAR values) and soil aspects (affecting litter quantity, organic matter, P content and humidity). We assumed that these variables (PAR and litter quantity) were the drivers of changes in the species richness and diversity in the studied communities (Fig. 1). Tests of significance and the creation of a diagram of relationships were performed in the Ω nyx free software. For all analysis $\alpha = 0.05$.

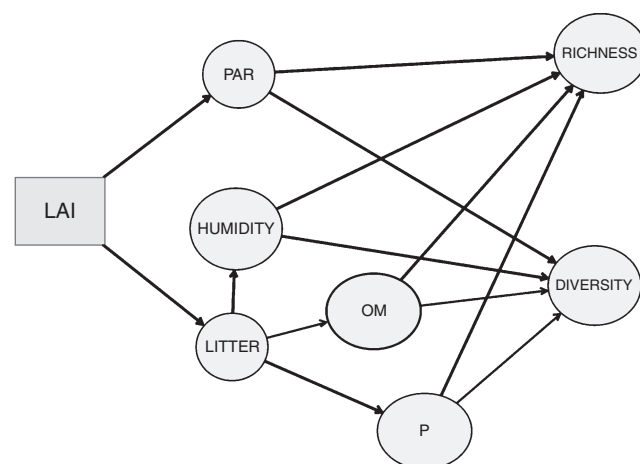


Fig. 1. A priori model of the causal relationships between abiotic and biotic characteristics measured in this study. Leaf area index (LAI) was the descriptor variable of the tree encroachment. LAI affects availability of light (PAR) and litter quantity in the soil surface, which are the major drivers of changes in the community richness and diversity. OM, organic matter.

Results

Changes in abiotic factors

We found clear differences in the availability of light resource between encroached conditions (Fig. 2), once these changes were derived from the distinct leaf area indexes of the studied communities ($r^2=0.84$, $P<0.01$) (Fig. 2a). Differences in encroachment led to differences in photosynthetic active radiation reaching the plants ($F_{2,12}=142.79$, $P<0.01$), where forested savanna had less than 10% of the total PAR incident in the typical savanna plants (Fig. 2b).

Soil physical properties did not differ between the studied conditions (MANOVA Wilk's $s=0.650$, $F=0.082$, $P=0.58$), as the studied communities possessed similar values of coarse sand, fine sand, silt and clay (Table 1). Soil pH, K, Ca and Mg content also did not differ ($F_{2,12}<2.10$, $P>0.05$) between the studied conditions (Table 1). We found significant differences for litter quantity (Table 1, $F_{2,12}=10.32$, $P<0.05$), where typical savanna had lower volume of deposited litter, whereas forested savanna had higher amounts of deposited litter. These differences were related with differences in LAI (Fig. 3a). Soil P content (Table 1, $F_{2,12}=4.80$, $P=0.029$), soil humidity (Fig. 3b, $F_{2,12}=8.45$, $P<0.01$) and total soil organic matter (Fig. 3c, $F_{2,12}=3.99$, $P<0.05$) also differed between encroached

situations. Typical savanna showed lower soil humidity, P and organic matter content in relation to forested savanna (Fig. 3; Table 1). Values for the dense savanna sites varied, with some sites showing similar values found to that of typical savanna and other sites with values similar to that found for forested savanna (Fig. 3; Table 1).

Community composition and structure

A total of 52 species in 44 genera and 23 families was registered in the three studied conditions (see Table S1, available as Supplementary Material to this paper). Families with the highest number of species were Myrtaceae (six), Poaceae (six), Fabaceae (four) and Rubiaceae (four). There were few species and individuals of herbaceous species in the forested condition, with few typical herb species of this environment, as, for example, *Coccocypselum lanceolatum*. There was a greater presence of climbing plants (both in number of species and individuals) in the more densely populated formations, especially *Serjania lethalis*. Besides having highest number of species and individual herbs, the typical savanna also possessed a greater number of subshrubs and shrubs. Among the species, we noted the grassy *Axonopus pressus*, which was very abundant in the typical savanna, and the sedge *Rhynchospora*

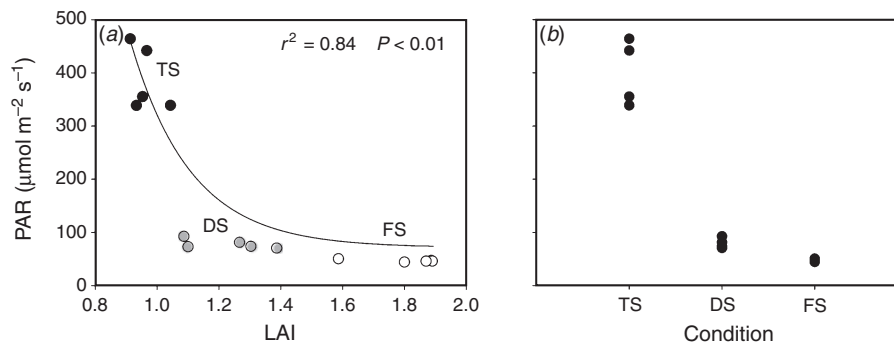


Fig. 2. Relationship between leaf area index (LAI, a proxy for encroachment) and photosynthetic active radiation (PAR) reaching the understorey plants (a) and values of PAR in the savanna plant communities with distinct structures (b). All points were shown. Data differed according with Fisher's l.s.d. test. Abbreviations: TS, typical savanna; DS, dense savanna; FS, forested savanna.

Table 1. Soil physical and chemical aspects (0–20 cm) in the sampled plots at the Assis Ecological Station, Assis, São Paulo state, Brazil

Data are average values of five samples followed by standard deviation. Abbreviations: TS, typical savanna; DS, dense savanna; FS, forested savanna; BS, basis saturation. Different letters in lines indicate significant differences between plots according to Fisher's l.s.d. test ($\alpha=0.05$)

Soil property	Condition		
	TS	DS	FS
Coarse sand (g kg^{-1})	402.6 ± 33.62a	371.00 ± 13.61a	375.25 ± 29.34a
Fine sand (g kg^{-1})	470.40 ± 19.41a	443.60 ± 39.25a	493.40 ± 19.90a
Silt (g kg^{-1})	27.80 ± 4.92a	54.20 ± 16.42a	32.00 ± 3.44a
Clay (g kg^{-1})	102.6 ± 10.32a	131.20 ± 14.69a	114.40 ± 7.19a
Leaf litter volume (m^3)	0.265 ± 0.04a	0.425 ± 0.04b	0.59 ± 0.06c
pH	3.77 ± 0.08a	3.69 ± 0.08a	3.74 ± 0.05a
P (g dm^{-3})	3.01 ± 0.11a	3.79 ± 0.28b	4.77 ± 0.10c
K ($\text{mmol}_c \text{dm}^{-3}$)	0.76 ± 0.31a	0.64 ± 0.15a	0.72 ± 0.15a
Ca ($\text{mmol}_c \text{dm}^{-3}$)	3.10 ± 0.41a	3.05 ± 0.37a	2.98 ± 0.24a
Mg ($\text{mmol}_c \text{dm}^{-3}$)	1.01 ± 0.12a	1.22 ± 0.34a	1.20 ± 0.25a

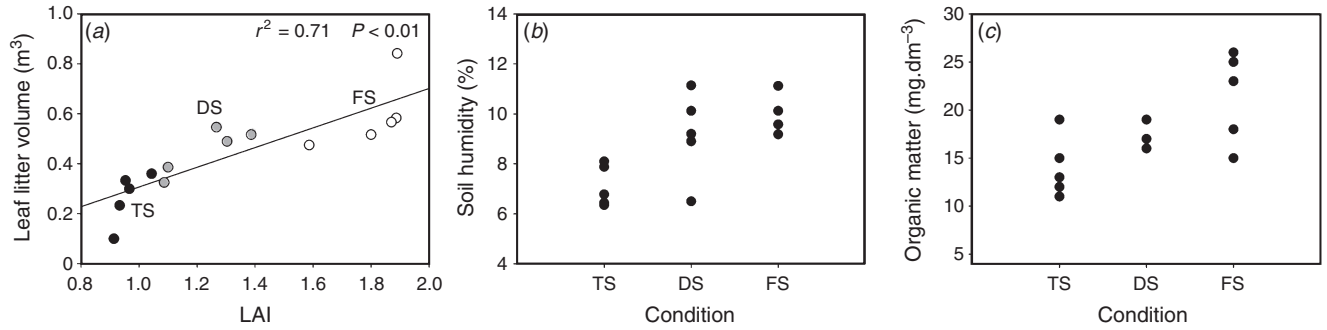


Fig. 3. Relationship between leaf area index (LAI) and leaf litter volume (a), soil humidity (b) and soil organic matter (c) in the different vegetation types. All points were shown. Data differed among vegetation types according to Fisher l.s.d. test. Abbreviations: TS, typical savanna; DS, dense savanna; FS, forested savanna.

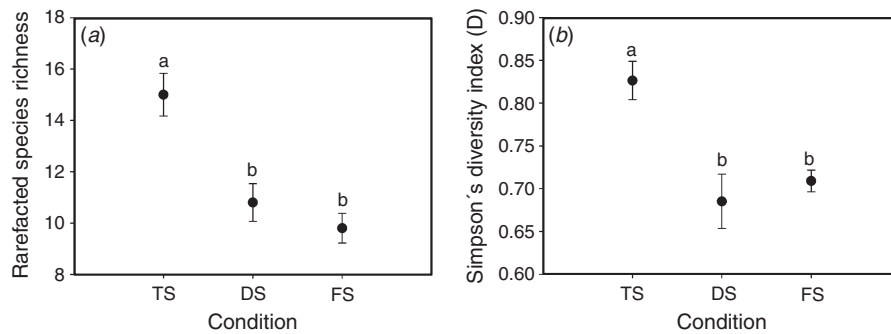


Fig. 4. Average species richness (after rarefaction) (a) and Simpson's diversity index (b). Vertical bars indicate s.e. Data differed according to Fisher l.s.d. test. Abbreviations: TS, typical savanna; DS, dense savanna; FS, forested savanna.

albiceps. This latter species occurs in greater abundance in the dense areas as well as bromeliad species (*Bromelia balansae*) in the dense and forested areas.

Rarefaction curves (see Fig. S1, available as Supplementary Material to this paper) demonstrated different species richness for the studied communities: typical savanna contained the largest number of species (33), followed by dense savanna (26) and forested savanna (19). Considering a similar number of individuals (500), the average species richness differed between the studied vegetation ($F_{2,12} = 14.46$, $P < 0.01$), with typical savanna possessing the higher value (Fig. 4a). Simpson's diversity index also differed between the studied communities ($F_{2,12} = 10.32$, $P < 0.01$), with typical savanna showing higher values of diversity than dense and forested areas (Fig. 4b).

The influence of abiotic factors on non-arboreal communities

After performing a series of regressions and using the structure equation modelling, we found that the LAI caused significant changes in PAR and litter in the studied communities (Fig. 5), negatively affecting PAR ($r^2 = 0.84$) and positively ($r^2 = 0.69$) affecting the litter quantity (Fig. 5). PAR had a positive influence on richness ($r^2 = 0.58$) and on diversity of species ($r^2 = 0.51$). Litter quantity was positively related to the soil organic matter ($r^2 = 0.30$) and P content ($r^2 = 0.31$), however, it was not related to soil humidity ($r^2 = 0.18$). P was negatively related to both, diversity ($r^2 = 0.20$) and species richness ($r^2 = 0.21$).

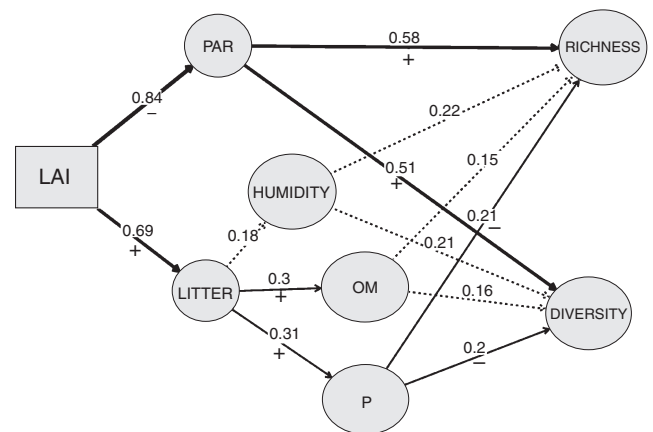


Fig. 5. Causal relationships among abiotic and biotic characteristics measured in this study. Values above lines indicates the value of r. Dotted lines demonstrate non-significant relationships ($P > 0.05$). Thick bold lines indicate strong and significant relationships ($P < 0.01$), other lines indicate only significant relationships ($P < 0.05$). Abbreviations: PAR, photosynthetically active radiation; LAI, leaf area index; OM, organic matter.

Discussion

We found clear differences in abiotic features of the studied communities, as well as in the richness and diversity of species between the distinct situations in the studied area. These results support our hypothesis that tree encroachment changes

the environmental conditions and availability of resources (especially light and nutrients) of typical savanna, affecting the composition and diversity of the non-arboreal plants. Forested savanna vegetation resulted in a species poor community, composed mainly of small shrubs and vines, whereas the typical savanna maintained its richest and diverse community, composed of many growth forms (grasses, herbs, subshrubs and shrubs). This result is the opposite of that found to the arboreal community species at the same studied area – for trees, forested savanna possessed a richer community in comparison with the typical savanna (Pinheiro and Durigan 2012). Photosynthetic active radiation and soil P content were the significant variables in explaining changes of the non-arboreal community richness and diversity. Changes in these variables were proven to be directly or indirectly related to differences in the LAI of tree species in the studied area.

Changes in crowding drastically affected most of the abundant and typical savanna species, such as *Axonopus pressus*, *Ananas annasoides* and *Campomanesia adamantium*, which disappeared completely in the forested condition. Other typical sclerophyllous species such as *Anacardium humile* and *Andira humilis* were also absent in denser and forested savanna areas. However, changes in crowding seemed to propitiate adequate niches that allow an increase in the number of individuals of *Bromelia balansae*, *Rhynchospora albiceps* and *Psychotria hoffmannseggiana* in denser conditions. These species may accelerate the disappearance of typical savanna species and provide a more homogenous habitat, especially in the case of *B. balansae*, which may affect the composition of grasses, herbs and subshrubs species given its great size and aggregate pattern of distribution (Durigan *et al.* 2002).

Encroached sites showed reduced richness and low diversity of non-arboreal species compared with typical savanna sites. Based on our analysis and previous studies (Hoffmann *et al.* 2012a; Pinheiro and Durigan 2012), we proposed a model based on structural equation modelling to explain our results. In this model, the most important abiotic factor influencing the community composition, richness and diversity is the availability of PAR. Changes in PAR are created given the variations in LAI, which increases as woody encroachment progresses.

The lower LAI values in the typical savanna provide a great variety of luminous niches to be explored by non-arboreal plants, resulting in the appearance of a great number of species, in comparison with more closed physiognomies (Batalha *et al.* 2001). Many non-arboreal savanna species may be more sensitive to disturbances in their light niche than the woody component of the typical savanna, given that woody species are reported to be very plastic in traits related to light availability (Rossatto and Kolb 2010; Bedetti *et al.* 2011). Additionally, some herbaceous species may require greater amounts of light to perform elevated rates of photosynthesis, enough to produce carbohydrates to be stored in their roots (Apezatto-da-Glória *et al.* 2008; Almeida *et al.* 2015), since these species normally resprout every year and after fire events. Although further studies are necessary, disappearance of typical non-arboreal savanna species under tree encroachment may be due to depletion of their subterranean reserves. This may be happening because available light in forested conditions is not sufficient to allow high rates of photosynthesis, so carbon

must come from reserve mobilisation, which maintains plant survival under shaded conditions only until reserves are depleted (de Moraes *et al.* 2016).

Aspects related to germination niches can also affect the presence of grasses and other species under forested savanna. Many savanna grasses, herbs and shrubs need alternating temperatures and light to germinate and establish with success in their environment (Zaidan and Carreira 2008; Musso *et al.* 2015). The higher soil moisture and the lower radiant energy can reduce soil temperature in the forested savanna. Furthermore, soil temperature would become more stable, hindering the germination of species that need fluctuations in temperature to break the dormancy of their seeds (Kolb *et al.* 2016). These aspects can lead to a reduction in germination success of cerrado understorey plants and, thus, compromise the persistence of these species in the encroached community. These ecophysiological aspects may explain why many of the typical savanna non-arboreal species were not sampled at the forested savanna, and are also becoming more scarce in the areas that are becoming more dense (dense savanna). In turn, the homogenisation in light, temperature and humidity conditions of the forested savanna propitiated an adequate environment for the establishment and growth of vine species, which showed the highest abundance in this situation. Climbers have great importance in floristic, functional and biomass terms in tropical forests (Schnitzer and Bongers 2011), despite being also commonly found at some typical savanna sites (Mendonça *et al.* 2008).

Together with the changes in light niches, nutritional niches were also modified: the elevated LAI increased the quantity of litter deposited on the soil surface, which, in turn, created a more humid environment, with a higher organic matter content. The presence of a higher quantity of dead organic matter probably increased the content of P in the forested savanna soil. Changes in soil nutrients, especially N and P, can reduce species diversity because faster growing species can outcompete species adapted to low nutrient availability (Bobbink *et al.* 2010; Bustamante *et al.* 2012). This seems to be the case at the study site, especially for *Rhynchospora albiceps* (a sedge), which drastically increased its number and dominance in the forested condition.

In a scenario where forests have naturally expanded in the last 3000 years (Silva *et al.* 2008), allied with continuous human interventions such as the absence of fire in protected areas, pronounced ecological changes are expected in savanna systems (Higgins *et al.* 2007), especially due to the lack of reversibility of this encroachment without human intervention (Durigan and Ratter 2016). These changes are relatively quick and closely linked with the extinction of light niches that are necessary for the survival of typical non-arboreal savanna species (Bond *et al.* 2005; Ratajczak *et al.* 2012). Additionally, such changes may promote a homogenisation of the floristic component, causing drastic losses in the savanna biodiversity (Durigan and Ratter 2016). For this reason greater effort needs to be directed towards development of conservation strategies for the typical savanna vegetation, especially because of its specific non-arboreal component and possible endemic distribution of its species (Durigan and Ratter 2016). Despite the prescription of fire as a means of species conservation and maintenance of ecosystem processes in the management plans of some fire-prone ecosystems worldwide (Keeley 2002), fire has been not widely

used by the governmental agencies in Brazil (Ramos-Neto and Pivello 2000; Durigan and Ratter 2016). Indeed, few Brazilian conservation units have fire cited in their management plans (Melo and Durigan 2011; Durigan and Ratter 2016).

In conclusion, we have shown that tree encroachment drastically changed the light and nutritional niches of typical savanna vegetation, creating new conditions in which distinct non-arboreal species richness and diversity patterns were found. Thus, it is extremely important to highlight strategies for the conservation of grasses, herbs, shrubs and subshrubs species of the typical savanna, which may be under serious threat of extinction given the expected ecological changes caused by the widespread expansion of the forest on the savannas in the absence of fire.

Acknowledgements

We thank Dr Giselda Durigan (Instituto Florestal – Assis – SP) for valuable comments on the early version of this manuscript, as well for help in identifying the species. We also thank Dra Renata Udulutsch (UNESP – Assis – SP) for identifying the climber species. This study was funded by FAPESP-BIOTA (Proc. 2013/18049–6, Proc. 2015/19771–2) and Pq-CNPq (Grant 301589/2015–1).

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