Functional antagonism between nitrogen-fixing leguminous trees and calcicole-drought-tolerant trees in the Cerrado

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ABSTRACT

The Cerrado is the largest savanna of South America and its physiognomy varies from savanna to woodlands. There are two main types of woodlands in the Cerrado: dystrophic woodlands, dominated by N-fixing leguminous trees (LEG), and mesotrophic woodlands dominated by non-leguminous drought-tolerant trees (DRY), which are calcicoles and sensitive to Al^{3+}. The working hypothesis is that LEG and DRY are functional antagonists in terms of the acidification/alkalization processes involving different forms of inorganic nitrogen and pH, Ca^{2+} and Al^{3+} in soil. Tree species basal area and soil properties were used to investigate the antagonism between LEG and DRY using generalized linear models. The results suggest that LEG and DRY are antagonists. The LEG were positively associated with Al^{3+}, NO_3^- and NH_4^+ content and negatively related to increasing Ca^{2+} content, whereas the DRY were negatively associated with Al^{3+}, NO_3^- and NH_4^+ and positively associated with increasing Ca^{2+} content. The upper soil layer in plots dominated by LEG species became more acidic and the upper soil layer in plots dominated by DRY species became more alkaline. The results suggest that LEG and DRY are functional antagonists and their preferences for NH_4^+ or NO_3^- might influence the way the Cerrado woodland changes.

Keywords: aluminum toxicity, calcicole species, dry forests, ecosystem function, nitrogen fixing, tropical savanna, woodlands

Introduction

The Cerrado domain is a tropical savanna that varies from savannic physiognomies to woodlands (Oliveira-Filho & Ratter 2002). It is considered the richest tropical savanna in the world, with more than 12,000 plant species (Mendonça *et al*. 2008). It encompasses the largest area of arable land in South America; more than 50 % of its 2 million km² area is suitable for crops and cattle ranching (Lopes & Daher 2008). Nevertheless, its acidic soils are arable and productive only with the implementation of practices such as liming because

of aluminum (Al^{3+}) toxicity and calcium deficiency (Caires *et al*. 2006). Yet, the occurrence of Cerrado woodlands (also called "cerradões" in Portuguese) is not clearly understood (Júnior *et al*. 2005). Recently, water retention, rather than the nutrient or aluminum content, has been reported as the most important soil factor determining the occurrence of Cerrado woodlands (Assis *et al*. 2011). However, different studies in Cerrado's savannas and woodlands found a significant positive relationship between the basal area or canopy area and soil P, K and Mg (Neri *et al*. 2012; 2013; Lloyd *et al*. 2015). These findings, along with many others, have helped explain some of the physiognomic variations of the Cerrado, but there is

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still a lot remaining to be understood.

Two types of woodlands have been distinguished in the Cerrado: dystrophic and mesotrophic. The dystrophic woodlands (DWs), found on strongly acidic soils with a high Al^{3+} content, are very often dominated by leguminous trees, many of them nitrogen fixers (hereafter LEG, only for potential nitrogen fixers) (Sprent 2009; Araújo *et al.* 2011), whereas the mesotrophic woodlands (MWs), found on soils with a pH close to 7.0 (Neri *et al.* 2012), are dominated by non-leguminous and drought-tolerant tree species typical of seasonally dry forests (DRY), despite many leguminous trees occurring in MW, and vice-versa. The traditional method to distinguish the two types of woodlands is based on mesotrophic indicator species (Ratter *et al.* 2003; Durigan & Ratter 2006), which are calcicole and aluminum-intolerant trees (Ratter *et al.* 1977; Ratter *et al.* 2003).

Although the Al-Ca gradient has been reported as an ubiquitous selective pressure in ecosystems with acidic soils (Jansen *et al.* 2002), many findings have encouraged investigations concerning uptake preferences for different forms of N in those soils (Bustamante *et al.* 2004; Andrews *et al.* 2011). Since nitrogen is the main taken-up mineral, nitrogen cycling may cause soil changes. For instance, NH^{4+} preference may induce soil acidification whereas NO^{3-} preference may induce soil alkalization (Haynes 1990; Bravin *et al.* 2008). Thus, LEG and DRY preferences on the Al-Ca gradient might reflect uptake preferences for different forms of nitrogen.

We postulate that LEG are crucial to the functioning of Cerrado woodlands because N-fixing bacteria associated with LEG roots are widely known to obtain NH^{4+} from N_2 molecules in the air, causing an unbalance of cation over anion uptake, and acidifying their rhizosphere (e.g., Bolan *et al.* 1991; Zahran 1999). Furthermore, NH^{4+} has been related to aluminum resistance in crops that have NH^{4+} uptake preference over NO^{3-} in acidic soils, accumulating NH^{4+} and preventing Al^{3+} toxicity, an ubiquitous stress factor of acidic soils (Jansen *et al.* 2002; Gastauer & Meira-Neto 2013a) that causes roots damage (Zhao *et al.* 2008; Zhao & Shen 2010). We postulate that DRY are crucial as well because calcicole plants may alkalize their rhizosphere since they have high exudation rates of organic acids for P uptake (Ström *et al.* 2005). As DRY are calcicole (Ratter *et al.* 1977; Durigan & Ratter 2006), an alkalization of soil upper layer might be expected where DRY dominate. Therefore, the working hypothesis is that LEG and DRY respond in opposite way to the soil pH as well as to contents of NH^{4+}, NO^{3-}, Ca^{2+}, and Al^{3+} and are functional antagonists in terms of the acidification and alkalization of the uppermost layers of soil.

Materials and methods

Study area

The present study was conducted in the Paraopeba Reserve (Floresta Nacional de Paraopeba or FLONA de Paraopeba), Brazil (19°20'S; 44°20'W), which is located at 730-760 m above sea level (ASL) and covers an area of 200 ha. The climate of the region is classified as Aw (tropical humid) by the Koeppen system, with a rainy summer from September to April and a dry season from April to September. According to personal statements from former employees, the vegetation in the Paraopeba Reserve regenerated after clear-cutting in 1952. There are records of fires in 1960 and 1963, but since then the area has been protected from fire. Moreover, the Cerrado of the reserve is at an appropriate altitudinal range, small enough to have the same climate throughout required to test our hypothesis. The selection of the studied areas was based on the classification of the soils and physiognomies. The soil types where the woodlands occur are red-yellow oxisols and red oxisols (Neri *et al.* 2012; Neri *et al.* 2013).

Vegetation, soil and functional groups

To investigate the relationship between spatial variation in vegetation and soil, forty-five plots were grouped into nine transects (20 m × 100 m) spaced at least 100 meters from each other. Each transect contained five plots (20 m × 20 m each), comprising a total area of 1.8 ha. Three transects were allocated per woodland type: dystrophic, mesotrophic and transitional. The dystrophic woodland (DW) is on a dystrophic red latosol, the transitional woodland (TW) is on a dystrophic red-yellow latosol and the mesotrophic woodland (MW) on a mesotrophic red latosol. All woody plants with a stem diameter at ground level (DGL) ≥ 3 cm were sampled. Species abundance and basal area were calculated for each plot. The basal areas were converted into relative basal areas for each plot (values between 0 and 1).

During 2005, one soil profile at five to ten meters from one transect in each woodland type was sampled at depths of 0 - 10 cm, 10 - 20 cm, 20 - 30 cm, 30 - 50 cm, 50 - 70 cm, 70 - 90 cm, 90 - 110 cm, 110 - 130 cm and 130 - 150 cm.

From each 20 m × 20 m plot, three mixed soil samples were collected from a depth of 0 to 10 cm in January. All samples were stored in polyethylene bags and analyzed by the Soil Analysis Laboratory at the Universidade Federal de Viçosa. The mixed soil was then air-dried and analyzed for: pH (H_2O); extractable phosphorus (P), sodium (Na), potassium (K) and iron (Fe) (extracted by Mehlich 1extract); exchangeable calcium (Ca^{2+}) magnesium (Mg), and exchangeable aluminium (Al^{3+}) (extracted in KCl); potential acidity (H+Al) (calcium acetate at pH 7.0); organic matter (OM) (Walkley-Black method); bases sum (Ca+Mg+K+Na), Cation Exchange Capacity (CEC), bases saturation (V), aluminium saturation (m) (standard international procedures). The Kjeldahl method was used for measurements of total soil N (Raveh and Avnimelech 1979). The NO^{3-} and NH^{4+} concentrations were determined using colorimetric analyses. For each variable, the mean was calculated for each plot from the three values from

the three soil samples.

The functional groups were based on species identification, and the plants were partitioned into LEG and DRY species. The other species were considered as a third group: OTHER (Tabs. S1, S2 in supplementary material). For LEG delimitation, we considered only leguminous species from the genera that are nitrogen fixers (Sprent 2009). We considered *Senegalia polyphylla* as OTHER because it is a non-nodulating reference among leguminous trees for experiments with 15N stable isotope (Soares 2007). For DRY delimitation, we considered all non-leguminous tree species reported as the most frequent ($\geq$ 70 % of checklists) in the tropical dry forests of the eastern and central western regions of South America (Oliveira-Filho *et al*. 2006). We used the lists from both regions because Paraopeba lies near their borders. In addition, we used the lists of the low and high altitude tropical dry forests from both regions because the altitude of the Paraopeba Reserve (730-760 m ASL) is near the limit (700 m ASL) of both categories (Oliveira-Filho *et al*. 2006). The functional trait measured was basal area, a proxy of biomass (Baraloto *et al*. 2010).

Soil chemical composition

To document vegetation and soil changes due to the relationship of LEG and DRY species to soil properties, five plots (20 m × 20 m) of each woodland type were sampled in January 2005, in the Paraopeba Reserve (Neri *et al*. 2012; Neri *et al*. 2013). The same plots were resampled in January 2009. The same information for the vegetation and soil (depth of 0 to 10 cm) described above was acquired, except for the N content (total N, NO_3^- and NH_4^+), which was not evaluated in 2005. Variation in soil pH between 2005 and 2009 was calculated by subtracting the pH in 2005 from the pH in 2009 (pH2009-2005).

Phylogenetic structure

To detect environmental filtering, we used phylogenetic structure as a tool, assuming that phylogenetic clustering occurs where environmental filtering (i.e., drought caused by contrasting soil pH where DRY species dominate) acts as the predominant assembly rule (Cavender-Bares *et al*. 2009). To evaluate the community composition for phylogenetic clustering or overdispersion, the net relatedness index (NRI) between individuals was calculated for the 20 m × 20 m spatial scale. All species from the phylogenies were inserted into APG III megatree R20100701.nMW using the phylomatic function of Phylocom 4.2 (Webb & Donoghue 2005). This tree was dated using the Bladj algorithm (Webb *et al*. 2008), which is based on the fossil dating proposed by Wikström *et al*. (2001) in combination with the agescl3 file for precision improvement (Gastauer & Meira-Neto 2013b). A checklist from a wider sampling that also included species from the savannic physiognomies of the Paraopeba Reserve was used as a metacommunity. For community partitioning, individuals were split into LEG (creating samples only with LEG) and all non-leguminous species (DRY + OTHER) to run in Phylocom 4.2.

Statistical analyses

To test the relationships between vegetation and soil properties, we used generalized linear models (GLM) with the following variables: the functional groups (LEG and DRY) and the relative basal areas of LEG and DRY; soil Al^{3+}, NO_3^-, NH_4^+, Ca^{2+}, Ca^{2+} variation (Ca^{2+} 2009-2005), pH and pH variation (pH2009-2005) and community NRI. The dredge function from the MuMIn package was used to test all combinations of variables included in the models. An information theoretical approach based on the Akaike Information Criterion of the Second Order (AICc) was used to determine which variables were more important. The best model was indicated by the lowest AICc value (Burnham *et al*. 2010). All analyses were performed using R version 2.15.1 (R Development Core Team 2012).

Results

The results from the GLM analyses indicated antagonistic relationships between LEG and DRY (as functional groups), and the relative basal areas of the LEG and DRY species interacted strongly with NH_4^+, NO_3^-, Ca^{2+}, pH and Al^{3+} (Figs. 1, 2, Tabs. 1 and S1 in supplementary material). The LEG and DRY were negatively related to each other whereas LEG and OTHER were not related (Fig. 1A). The relative basal areas of the LEG species were positively related to the inorganic soil N (NO_3^- and NH_4^+) concentrations, whereas those of the DRY species were negatively related (Fig. 1). However, we did not find a significant relationship between total N and the basal area of LEG or DRY species (Tab. S2 in supplementary material). The relative basal area of LEG species was negatively related to soil pH and positively related to soil Al^{3+} content, which was inversely related to basal area of DRY species. The basal area of DRY species was positively related to pH and negatively related to soil Al^{3+} content (Fig. 1, Tabs. 1 and S1, S2 in supplementary material).

The NRI of the entire community was not related to the NRI of the LEG community; however, the NRI of the entire community was strongly related to the NRI of the non-leguminous trees (DRY + OTHER; Fig. 1, Tab. 1).

Changes of soil chemical properties represented by pH variation (i.e., the difference in the 2009 and 2005 soil pH (pH2009-2005, Fig. 2)), suggests that the acidic soils became more acidic and that the alkaline soils became more alkaline. In four years, the soil pH changed negatively (down to 0.81) in DW, positively (up to 0.830) in the MW,
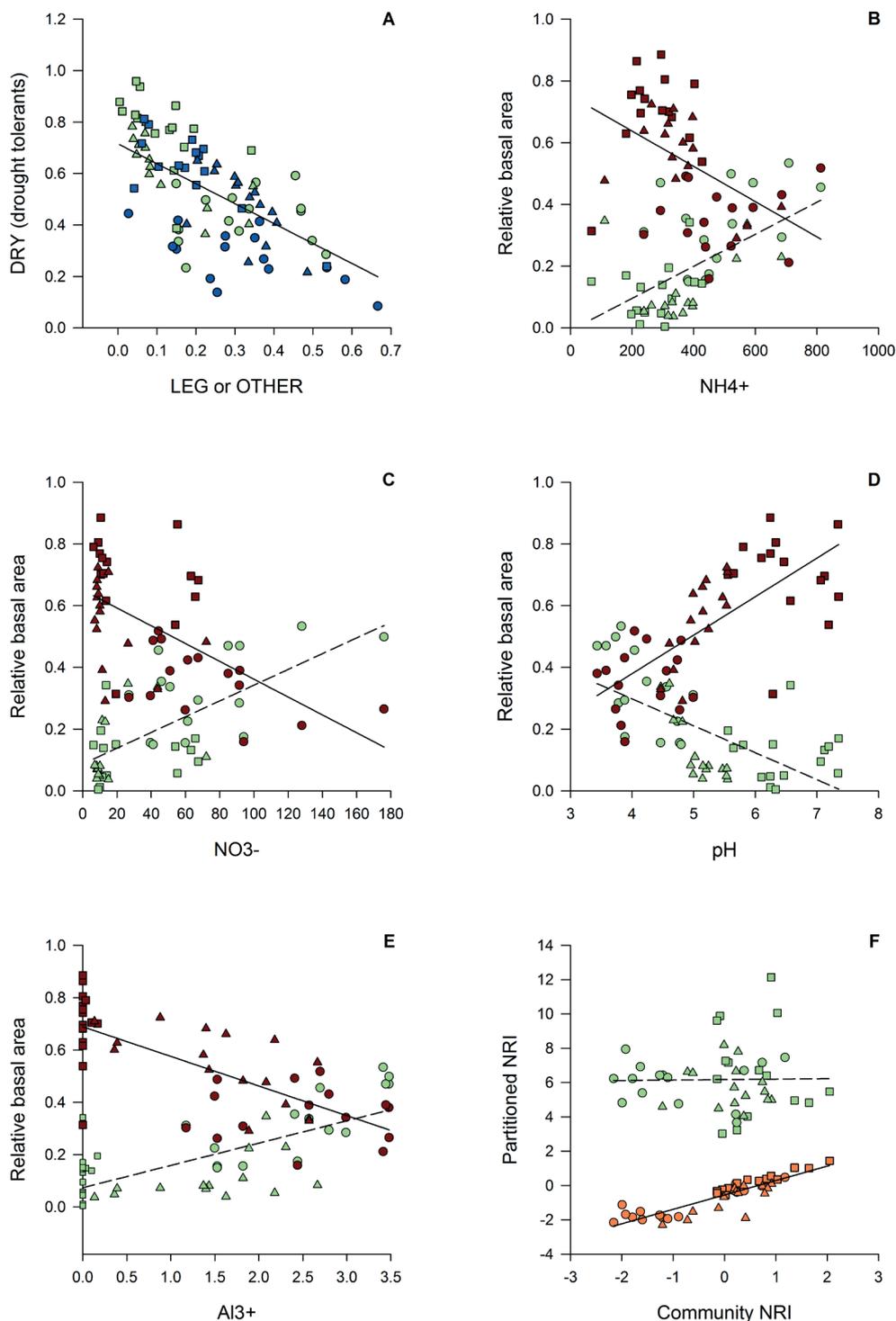
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Figure 1. A – Generalized linear model (GLM) of the relative basal area of nitrogen-fixing leguminous trees (LEG, light green polygons, dashed line) or other species (OTHER, blue polygons, solid line) and the relative basal area of drought-tolerant tree species typical of seasonally dry forests (DRY) in Cerrado woodlands. B – GLM of the soil ammonium content (NH^{4+}) and relative basal area per plot (cm^2) of LEG (light green polygons, dashed line) and DRY (red polygons, solid line); P=0.004 and P=0.00002, respectively. C – GLM of the soil nitrate content (NO^{3-}) and basal area per plot (cm^2) of LEG (light green polygons, dashed line) and DRY (red polygons, solid line). D – GLM of the soil pH and basal area per plot (cm^2) of LEG species (light green polygons, dashed line) and DRY species (red polygons, solid line). E – GLM of the soil Al^{3+} content and basal area per plot (cm^2) of LEG (light green polygons, dashed line) and DRY (red polygons, solid line). F - GLM of the community net relatedness index (NRI) and the NRIs of the LEG community (light green polygons, dashed line) and all non-leguminous communities (orange polygons, solid line). Circles – dystrophic woodlands, squares – mesotrophic woodlands, triangles – transitional woodlands. P values are presented in Table 1. Please see the PDF version for color reference.
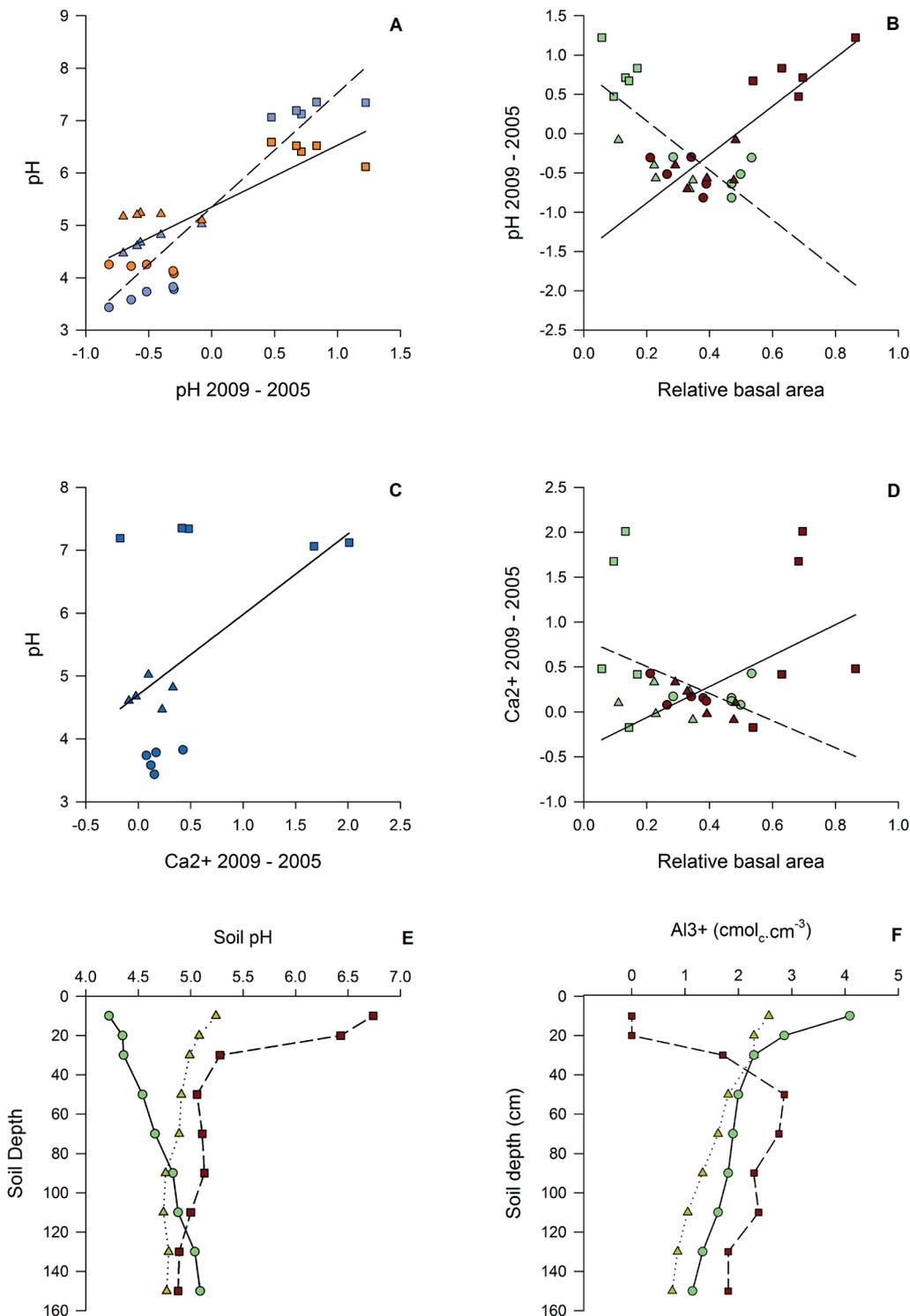
Figure 2. A – Generalized linear model (GLM) of pH variation (pH2009–2005), 2005 pH (pH, blue polygons, dashed line) and 2009 pH (pH, orange polygons, solid line). B – GLM of the LEG relative basal area (light green polygons, dashed line) and DRY relative basal area (red polygons, solid line) and soil pH variation (pH 2009–pH2005). C - GLM of Ca²⁺ variation (Ca²⁺ 2009-2005) and 2009 pH (pH). D - GLM - of LEG relative basal area (light green polygons, dashed line) and DRY relative basal area (red polygons, solid line) with Ca²⁺ variation (Ca²⁺ 2009–2005). E and F – Relationship between soil depth and soil pH and Al³⁺ of the soil profiles of the Cerrado woodlands. E and F, the solid lines represent dystrophic woodlands (light green), the dotted lines represent transitional woodlands (yellow), and the dashed lines represent mesothophic/mesotrophic woodlands (red). A-F, circles are dystrophic woodlands, triangles are transitional woodlands, and squares are mesotrophic woodlands. P values are presented in Table 1. Please see the PDF version for color reference.

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Table 1. Generalized linear models for Al^{3+}, NO_3^-, NH_4^+, pH, pH variation (pH 2009-2005) and Ca^{2+} variation (Ca^{2+} 2009-2005) with interactions with functional groups – FG (LEG/DRY) and periods (2005/2009) related to the relative basal area - RBA. See Figures 1 and 2 for graphs and for further explanation. The corresponding AICc values are presented in Table S1 in supplementary material.

DRY RBA-LEG RBA * OTHER RBA	Estimate	Std. Error	t value	P
Intercept	0.6955	0.0356	19.515	<2E-16
LEG or OTHER	-0.8461	0.1503	-5.637	2.15E-07
RBA~NH⁴⁺* FG (LEG/DRY)	Estimate	Std. Error	t value	P
Intercept	0.7519	0.05748	13.082	<2E-16
NH⁴⁺	-0.0005709	0.0001405	-4.062	0.000107
RBA~NO³⁻* FG (LEG/DRY)	Estimate	Std. Error	t value	P
Intercept	0.6507	0.0298	21.801	<2E-16
NO³⁻	-0.0028902	0.0005510	-5.245	1.11E-06
RBA~pH* FG (LEG/DRY)	Estimate	Std. Error	t value	P
Intercept	-0.1200	0.09341	-1.285	0.202
pH	0.12489	0.01743	7.166	2.49E-10
RBA~Al* FG (DRY/LEG)	Estimate	Std. Error	t value	P
Intercept	0.6887	0.0266	25.847	<2E-16
Al³⁺	-0.11325	0.01478	-7.664	2.51E-11
Part.NRI ~ Comm.NRI*FG (LEG/DRY)	Estimate	Std. Error	t value	P
Intercept	-0.5537	0.2038	-2.717	0.00796
Comm.NRI	0.8400	0.2022	-2.717	7.69E-05
pH~(pH2005-2009)* FG (LEG/DRY)	Estimate	Std. Error	t value	P
Intercept	5.347	01493	35.823	<2E-16
(pH2009-2005)	1.176+00	2.314E-01	5.082	2.7E-05
(pH2009-2005) ~ RBA*FG (LEG/DRY)	Estimate	Std. Error	t value	P
Intercept	-1.4981	0.2896	-5.173	2.13E-05
RBA	3.0802	0.5818	5.294	1.55E-05
pH~(Ca²⁺2009-2005)* FG (LEG/DRY)	Estimate	Std. Error	t value	P
Intercept	4.697	0.4253	11.05	2.57E-11
(Ca²⁺2009-2005)	1.281	5.928-01	2.16	0.0402
(Ca²⁺ 2009-2005)~RBA*FG (LEG/DRY)	Estimate	Std. Error	t value	P
Intercept	-0.4078	0.4123	-0.989	0.3317
RBA	1.7241	0.8283	2.082	0.0474

and negatively (down to 0.7) in the TW. The acidification and alkalization are explained by the basal areas of LEG and DRY (Fig. 2). The basal areas of LEG were negatively related to pH2009-2005, while in contrast the basal areas of DRY were positively related to the pH2009-2005 values (Fig. 2). The Ca^{2+} varied (Ca^{2+} 2009-2005) positively with pH (Fig. 2C), positively with DRY relative basal area and negatively with LEG basal area (Fig. 2D).

The pH of the soil profiles varied with depth. In the dystrophic woodland, the pH varied gradually, increasing with depth. In the mesotrophic woodland, the pH decreased drastically from the surface to a depth of 50 cm where there was a contrasting pH. The transitional plots had an intermediate condition that was closer to the MW pattern than the DW pattern. The Al^{3+} contents of the soil profiles varied non-linearly as expected as a result of the variation in pH (Fig. 2).

Discussion

All study plots in the Cerrado woodlands encompassed LEG and DRY trees. The negative relationship between the basal areas (proxy of biomass) of LEG and DRY suggests a functional antagonism between them.

We found evidence of antagonism between LEG and DRY in the relationship between their relative basal areas and soil biogeochemical characteristics. Over four years, the uppermost layer of the soil of LEG-dominated plots experienced acidification, and the uppermost layer of soil of DRY-dominated plots experienced alkalization. Congruently, in LEG-dominated plots, the Ca^{2+} content decreased whereas in DRY-dominated plots the Ca^{2+} content increased. The relationship between the alkalization in the uppermost 20 cm of the soil in DRY-dominated plots and the acidification in the uppermost 20 cm of the soil in LEG-dominated plots over 4 years (Fig. 2) suggests a causal relationship between the type of plant community and soil biogeochemistry. The uppermost 20 cm of the soil in the studied woodlands in the Paraopeba Reserve has the highest content of organic matter, according to Neri *et al.* (2013). This indicates the strong biotic influence in this upper soil horizon and supports the hypothesized causality between plant composition and soil functioning.

The acidic soils of LEG-dominated plots in the dystrophic woodland were generally associated with very high levels of Al^{3+}, which are stressors for DRY species (Ratter *et al.* 1977; Crawley 1996; Haridasan & Araújo 2005). Additionally, if LEG avoid Al^{3+} uptake, they contribute to the accumulation of Al^{3+} in the soil. The pH dynamics indicate that LEG-dominated soils had become more acidic and that LEG were related to this acidification. These findings are consistent with the negative relationship between LEG and pH, and the positive relationships between LEG and Al^{3+}, NH^{4+} and even NO^{3-}, as the nitrification of NH^{4+} occurs continuously in the N cycle (e.g., Booth *et al.* 2005).

The processes that caused these relationships are not completely understood, indeed. The cause of the relationships between LEG basal area and soil properties is likely the N-fixing process that fixes NH^{4+}, as well as the preference to take up NH^{4+}, which causes acidification of the rhizosphere (Riley & Barber 1971; Haynes 1990; Bolan *et al.* 1991; Zahran 1999; Kanu *et al.* 2014). Although we did not test nitrogen fixation, we tested the potentiality for fixation expressed by the basal area of LEG.

Inversely, DRY-dominated communities of the MW are less affected by Al^{3+} toxicity because of the alkalization of the uppermost soil layer. The cause might be the preference of calcicole-DRY for NO^{3-}, alkalizing the uppermost soil layer; however, the results are not conclusive. Another explanation for the soil alkalization is that DRY are calcicoles and have high exudation rates for organic acids for P uptake (Ratter *et al.* 1977; Ström *et al.* 2005). Congruently, the N/P ratio of the leaves of 23 non-leguminous species from the Paraopeba Reserve ranged from 13.3 to 57.9, with an average value of 21.5 (Nascimento 2011), which is higher than the average value of 18 reported by Bustamante *et al.* (2006). Among the 23 species sampled by Nascimento (2011), eight species were DRY and had N/P ratios lower than the average; of these, seven had N/P ratios between 13.3 and 16.9, indicating they were not P-limited or that they were not only P-limited (Koerselman & Meuleman 1996).

Results suggest that LEG and DRY are antagonists because of the negative relationship of basal area to each other and because they likely change pH, Ca^{2+} and, consequently, Al^{3+} in opposite ways. Thus, LEG and DRY do not only have different preferences for contents or levels of soil factors along a gradient; LEG are also positively related to contents of NH^{4+} and NO^{3-} in the upper layers of soil, whereas DRY are negatively related.

The confirmation of our results may allow generalizations to be made for the Cerrado domain and for other tropical vegetation occurring on acidic soils. If the results are confirmed, at least for the Cerrados, the understanding of the Cerrado MW as a transitional ecosystem between Cerrado and seasonal forests (e.g., Durigan & Ratter 2006) shifts toward another perspective. Our results suggest that woodland composition with dominance of LEG or DRY influence the way the Cerrado woodlands change: toward dystrophic woodlands when the LEG are dominant or toward mesotrophic woodlands when the DRY are dominant.

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