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Research article

Influence of water limitation on the competitive interaction between two Cerrado species and the invasive grass *Brachiaria brizantha* cv. Piatã

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ABSTRACT

Invasive grasses inhibit the growth of other plant species, and water deficit is one of the major competition problems for native vegetation. We evaluated whether the presence of *Brachiaria brizantha* cv. Piatã has a negative influence on the competition for water and nutrients between *Anadenanthera macrocarpa* and *Anadenanthera colubrina* (Angico species). The interspecific competition was evaluated using a randomized experimental design with the following treatments: 1) free competition (FC), in which the native species were cultivated without the grass presence and 2) under competition (UC), in which the native species grew together with the invasive grass for 120 days. We analysed the water relationships in the two species, the effect of water limitation on the antioxidant stress, the nutritional content of shoots and roots, the relative competition intensity (RCI) and growth. The presence of Piatã grass reduced the soil moisture causing a decrease of 21.9% and 29.5% in the relative water content (RWC) of leaves for *A. macrocarpa* and *A. colubrina*, respectively. For the two Angico species, the quantum efficiency of Photosystem II (ΦPSII) decreased with reduction of RWC leaf, resulting in the H_2O_2 increase (57.5% at day 30 for *A. colubrina* and 38.8% at day 120 for *A. macrocarpa*). The oxidative stress was evidenced by the increase in the superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities in leaves and roots of both young native trees. In the UC treatment, reductions in water uptake also led to a decrease in root absorption of N, P, K, a Mg and low transport of these nutrients to the leaves of both Angico species. *A. macrocarpa* and *A. colubrina* showed less growth caused by limitation of water uptake, but the joint activity of the physiological and biochemical adjustments provided competitive ability.

1. Introduction

The Brazilian Cerrado is recognized as a global biodiversity hotspot (Myers et al., 2000) and its resources are highly explored for agricultural activities, such as the deforestation of native vegetation areas for the implantation of pastures (Hoffmann and Haridasan, 2008). The Cerrado fields are constantly invaded by grasses, which can lead to a reduction in their diversity since those grasses spread out through large extensions of natural ecosystems (Klink and Machado, 2005).

The competitive interactions between invasive and native species and their relationships with resource gradients have been a subject of much discussion (Leffler et al., 2014). The capacity to explore available resources depends on the functional differences among species, which is considered as proportional to their establishment abilities (van Kleunen et al., 2010). Therefore, the success of the invasive species occurs when there are opening niches after disturbances that increase the resource availability (Davis and Pelsor, 2001), or when competitors and natural enemies suppress the performance of native species (Leffler et al., 2014). Several studies analysed whether the competition for resources is a mechanism that invasive species use to cause the declining in the establishment of native trees or is a consequence of changes in the environmental conditions (Levine et al., 2003; Rossatto and Franco, 2017; Bhadouria et al., 2017). Different niche components such soil moisture, rooting depth, nutrient availability, among others, can be indicators of successful invasion when it comes to resource impacts, leading to potential coexistence (MacDougall et al., 2009; Goldstein and Suding, 2014).

In dry tropical ecosystems, grasses suppress the growth of tree seedlings primarily due to their effect on soil water and nutrient availability (Bhadouria et al., 2017). Particularly in the Cerrado, the seasonal low humidity and high daytime temperatures impose a regularly high evaporative demand during the prolonged dry season. During this period, water in the upper soil layers is severely depleted as

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evidenced by the low water potential in the upper portion of the soil profile (Goldstein et al., 2008). The grasses apparently decrease the soil moisture by high competitive ability and resistance, causing water deficit and reducing the recruitment of tree species (Hoffmann and Haridasan, 2008; Foxcroft et al., 2010). Moreover, high growth rates and large biomass production of invasive grasses promote the shading of seedlings, often releasing allelochemicals in the soil, which result in a high mortality of young trees (Hoffmann and Haridasan, 2008). Rossatto and Franco (2017) compared the efficiency for the use of resources between grasses and other plant forms and found that grasses in the Cerrado exhibited higher rates of photosynthesis by area and leaf biomass, greater specific leaf area and intrinsic efficiency in water use than young trees, shrubs, and herbs. In addition, Rossatto et al. (2012) reported that the distribution of some of the woody vegetation of Cerrado is related to the presence of species that rely on the superficial water uptake or have a great plasticity in relation to growth strategies.

African grasses are highly competitive and accumulate large amounts of biomass. *Brachiaria* species are the most common and aggressive exotic grasses found in the Cerrado (Almeida-Neto et al., 2010). *Brachiaria brizantha* cv. Piatã (Piatã grass) is a cultivar derived from an ecotype of Ethiopia, Africa, which differs from other cultivars mainly because of its fast regrowth and high leaf/stem ratio. It is a perennial grass that can reach 110 cm in height and grows in clumps composed of a considerable number of fine tillers (Quintino et al., 2013). *Anadenanthera macrocarpa* (Benth.) Brenan and *Anadenanthera colubrina* (Vell.) Brenan are popularly known as “Angico” and have a wide distribution in several Brazilian environments. Their timber is employed in construction, and commonly used as an energy source, and in folk medicine (Paula et al., 1997; Monteiro et al., 2006). Both species are subjected to competitive interactions in Cerrado areas invaded by exotic invasive grasses. Pellizzaro et al. (2017) demonstrated that *A. colubrina* seedlings submitted to competition with grasses under no-tillage showed a high emergence rate in greenhouse and field, as well as a survival rate above 60% in subsequent years.

The Brazilian Cerrado areas display rapid loss of biodiversity influenced by dominance of exotic invasive species, and tree seedlings are often used to restore these areas, due to their fast growth rates and higher seed production (Pellizzaro et al., 2017). Alongside, the seasonal contrast between the dry and rainy season of the dry forests, similar to what occurs in the Cerrado, may be altered with climate changes, resulting in changes in precipitation regimes and longer periods of drought (Allen et al., 2017), affecting especially the young plants (Vieira et al., 2017). Thus, Angico species can be used as an important model to understand how water and nutritional limitation resultant of the competitive interaction with invasive grass, may influence their establishment capabilities. We conducted this study to evaluate how the presence of Piatã grass affects the responses of the two Angico species when subjected to competitive interactions under controlled conditions, especially focused on how water limitation can modify the absorption of nutrients and activate the antioxidant defense in *A. macrocarpa* and *A. colubrina*.

2. Material and methods

2.1. Plant material and experimental design

The study was conducted for 120 days in dry season (May to September), in the campus of the State University of Mato Grosso do Sul, Coxim-MS, Brazil. The experiment was set up under natural light conditions (open environment) and the soil used for plant cultivation was collected from a Cerrado area and subsequently submitted to physical and chemical analyses. The seeds of *Brachiaria brizantha* cv. Piatã were obtained from EMBRAPA Gado de Corte, Campo Grande-MS, and were planted and cultivated in plastic bags (18 × 24 cm) containing 3 kg of soil. Seedlings of *Anadenanthera macrocarpa* and *Anadenanthera colubrina*, which were obtained from seeds collected from Cerrado areas, were transferred to experimental conditions for acclimatization after reaching approximately 10 cm in height. To analyze the competitive effect of the presence of the invasive grass on the Angico species, we performed a randomized experiment with two treatments: 1) Free Competition (FC), in which 100 native plants of each species were maintained in pots without the presence of the invasive grass and were irrigated with a half-strength Hoagland solution (Hoagland and Arnon, 1950); and 2) Under Competition (UC), in which the same number of plants of each species from the previous treatment was transferred to pots with *B. brizantha* cv. Piatã, and were irrigated with a nutritive solution. A single plant of Angico per pot was transplanted when the invasive grasses reached approximately, 40 cm high. The average density of grasses was 5–6 tillers (individuals) per pot. During the transfer of the plants, a space was carefully opened in the central part of each pot, and when necessary, the grass tillers were relocated into the pots to avoid any effect in the experiment. The treatments were arranged in worktop and, because they were placed outside, plants were exposed to the light conditions and relative humidity of the environment. Although the experiment was carried out during the dry season, in which rainfall events are scarce and of short duration, all plants were protected by a plastic cover (when necessary) to avoid any influence of rainwater. The maintenance of the plants in an open area, simulated experimental conditions close to the natural competition that occurs in the environment. After 30 days of plant acclimatization and of the starting of the experiment, each plant received a half-strength Hoagland solution (0.5 ml of 1.0 M $NH_4H_2PO_4$; 3 ml of 1.0 M KNO_3; 2 ml of 1.0 M $Ca(NO_3)_2$; 1 ml of 1.0 M $MgSO_4$ + 50% of micronutrient stock solution + 0.125 ml of iron stock solution) once a month (4 times throughout the experiment). Since the first application of the solution, the plants were irrigated with water every five days, that is, 6 times in each month (considering the day when the solution was applied). Physiological and biochemical analyses were performed in leaves fully expanded placed in the second internode (apex to base) of the native plants.

2.2. Soil moisture and relative water content (RWC)

The soil moisture (%) was assessed on a daily basis during 1 h (8:00 to 9:00 a.m.) using a thermohygrometer (J. Prolab/SH122). To evaluate the RWC, fresh leaves and roots of five plants of each Angico species were weighed to obtain the fresh weight (FW). Thereafter, those tissues were hydrated with distilled water for 24 h to obtain the turgid weight (TW). Finally, those samples were dried at 65 °C for 72 h to determine the dry weight (DW). The RWC was calculated monthly as follows: $100 \times (FW - DW)/(TW - DW)$.

2.3. Quantum efficiency of photosystem II and H_2O_2 content

To evaluate the quantum efficiency of the photosystem II (ΦPSII) of each Angico species every 30 days, dark-adapted leaves for 2 h were used to measure the maximum quantum yield (Fv/Fm) by emitting a saturating light pulse of 3000 μmol photons $m^{-2} s^{-1}$ for 1 s with a portable pulse amplitude modulated fluorometer (PAM–2000; Heinz Walz GmbH, Effeltrich, Germany). The H_2O_2 content was calculated monthly from samples of 100 mg of fresh leaves and roots from five plants of each native species. Thereafter, samples were ground with 0.1% trichloroacetic acid and centrifuged at 12 000 rpm for 20 min. A 300 μL aliquot of the supernatant was mixed with 300 μL of a phosphate-buffered saline (PBS; pH 7.0, 10 mM) and 800 μL of KI (1M). The H_2O_2 content was determined according to the method described by Sergiev et al. (1997), with minor modifications, evaluating the sample absorbance at 390 nm based on a standard curve.

2.4. Antioxidant enzymes

The activity of antioxidant enzymes was evaluated using fresh leaves and roots (ca. 300 mg) from five plants of each native species. The extracts were obtained from milled samples, incubated with 50 mM of a potassium phosphate buffer (pH 7.0), 0.05% of triton, 10% of polyvinylpolypyrrolidone (PVPP) and 1 Mm of acetylsalicylic acid (AsA). The mixture obtained was centrifuged at 13 000 rpm for 30 min and the supernatant was subjected to the analysis of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities in a UV–Vis spectrophotometer (SP-22, Biospectro) at 560, 240 and 290 nm, respectively. The SOD and APX activities were analysed as described in Reddy et al. (2004), with minor modifications (in the quantity of enzyme extract), while the CAT activity was determined according to Havir and Mchale (1987).

2.5. Nutritional composition

The analysis of the nutritional content was carried out in the roots, stems, and leaves of three plants of each species, within the respective treatments, according to Jones Jr. et al. (1991). The concentrations of nitrogen (N) were determined after the digestion with sulfuric acid through the Kjeldahl method, and the content of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), copper (Cu), iron (Fe), manganese (Mn), boron (B), and zinc (Zn) was determined by a nitric-perchloric acid digestion by atomic absorption spectrophotometry using an AAS instrument (Perkin Elmer AAnalyst 700 model AAS).

2.6. Initial growth

The growth parameters evaluated for Angico species were the plant length (cm), dry mass (g), leaf area (cm^2), net assimilation rate (NAR) and relative growth rate (RGR). The growth analyses were performed every 30 days in five plants of each species per treatment until the end of the experimental period (120 days). For the length measurements, we considered that the aerial part of the plant was comprised between the insertion point of the last leaf pair and the point immediately above the first branch of secondary roots. The dry mass was obtained by drying aerial parts and roots in an oven at 65 °C for 72 h and then weighed. To quantify the leaf area, a leaf of each plant was removed from the third node, scanned in a desk scanner and the obtained image was processed in software that performed the conversion of pixels to cm^2. NAR and RGR were calculated according to equations: $NAR = \{[(P_2 - P_1)/(A_2 - A_1)] * [(\log A_2 - \log A_1)/(t_2 - t_1)]\}$ and $RGR = (\log P_2 - \log P_1)/(t_2 - t_1)$, where, P_2 and P_1 correspond to the dry weight of the plant, A_2 and A_1 to the leaf area index in the period between t_2 and t_1, respectively.

2.7. Relative competition intensity (RCI)

The relative competition intensity index (RCI) which represents the decline in RGR due to the presence of other species, was calculated according to Goldberg et al. (1999), and was expressed as percentage (%):

$$\frac{RCI{=}(RGR_{mono} - RGR_{mixed})}{RGR_{mono}} \times 100$$

where RGR_{mono} represents the absence of competition and RGR_{mixed} the presence of Piatã grass × each Angico species.

2.8. Statistical analysis

The experimental design was completely randomized. Data were tested for normality and submitted to analysis of variance (ANOVA) considering the presence of grasses as a factor (independent variable) and the effects of water deficit as response variables (dependent variable). Analyses were performed using BioEstat version 5.3 and significant means ($p < 0.05$) were compared by Tukey's test. Pearson's correlation coefficients were calculated between physiological and biochemical parameters and tested for significance using Student's t-test.

Table 1
Physical and chemical analysis of Cerrado soil used for cultivation of *A. macrocarpa* and *A. colubrina* under interspecific competition with Piatã grass.

Soil composition	
Physical analysis	
Sand (g/kg)	610
Silt (g/kg)	130
Clay (g/kg)	260
Chemical analysis	
pH	5.03
Phosphorus (mg/dm^3)	40.49
Potassium (cmolc/dm^3)	1.39
Calcium (cmolc/dm^3)	6.9
Magnesium (cmolc/dm^3)	3.65
Sulfur (cmolc/dm^3)	11.94
Iron (mg/dm^3)	144.92
Manganese (mg/dm^3)	187.99
Copper (mg/dm^3)	0.41
Zinc (mg/dm^3)	8.85
Boron (mg/dm^3)	0.61
Organic matter (g/dm^3)	74.8
Cation exchange capacity (cmolc/dm^3)	17.9
Base saturation (%)	66.7

3. Results

3.1. The presence of Piatã grass affected soil moisture

The results of soil physical and chemical analyses are shown in Table 1. The soil granulometric composition indicated a high sand content, with less than 35% of clay, which can be characterized as a sandy soil. Regarding the chemical composition, high levels of manganese, iron and phosphorus were detected, as well as a deficiency of boron and copper. The pH was acidic (5.03), which is a common feature in Cerrado soils. The soil moisture was affected by the presence of the Piatã grass, with a linear decrease throughout the experiment. In the UC treatment, there were significant decreases of 66.3% at day 30 for *A. colubrina* and of 64% for *A. macrocarpa* at day 90, compared with T0 plants (Fig. 1A).

3.2. Water limitation caused a decrease in the quantum efficiency of PSII in Angico species

As shown in Fig. 1B–C, the presence of the Piatã grass caused a decrease in the RWC of both native species, which was faster in treatments under competition (23% and 37.5% in the RWC of roots and 21.9% and 29.5% in the RWC of leaves for *A. macrocarpa* and *A. colubrina*, respectively). Plants under FC presented mean Fv/Fm rates of 0.77 for *A. macrocarpa* and of 0.79 for *A. colubrina* during the experiment. However, in the UC treatment the decrease in the RWC values caused a decrease in the quantum efficiency of PSII (ΦPSII) of 15.9% (< 0.05) in *A. macrocarpa* at day 90 and of 14.2% (< 0.01) in *A. colubrina*, reaching the lowest value (0.67) at day 30 (Fig. 2A). The main correlations between the physiological and biochemical parameters in the UC treatment for both species are shown in Table 2.

3.3. Water limitation increased the oxidative stress

The water deficit caused by the presence of the Piatã grass resulted in oxidative stress with increased H_2O_2 content in the leaves of both Angico species, with increases of 38.8% ($p < 0.05$) at day 120 for *A. macrocarpa* and of 57.5% and 41.6% ($p < 0.01$) at days 30 and 90 for *A. colubrina*, respectively, as shown in Fig. 2B. The activity of antioxidant enzymes was also altered in roots and leaves of both species, mainly in the UC treatment (Fig. 3). The SOD activity of roots increased

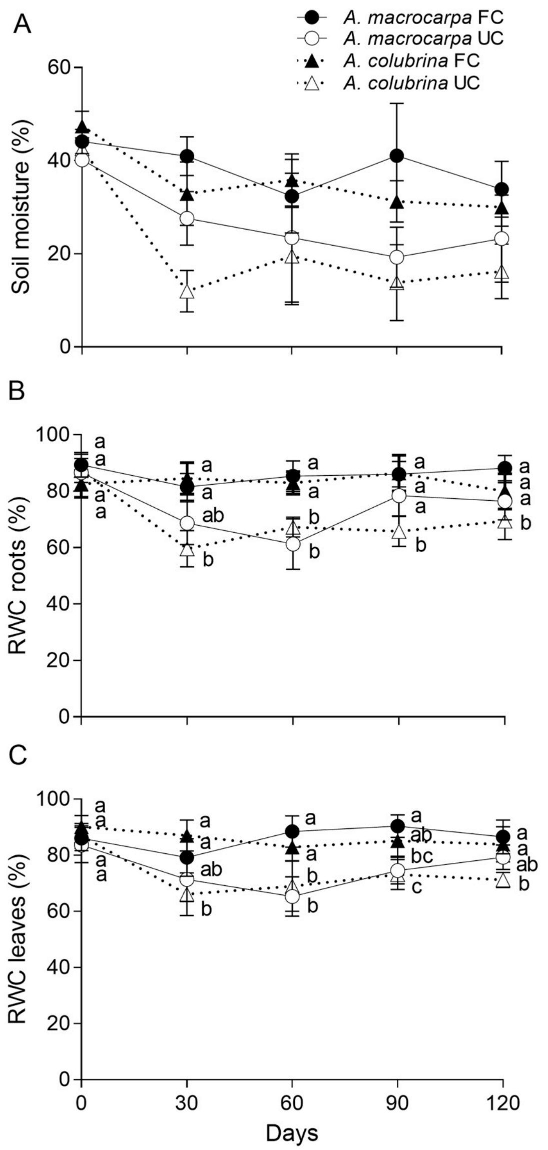

Fig. 1. Water measurement in roots and leaves of *A. macrocarpa* and *A. colubrina* subjected to interspecific competition with Piatã grass. (A) Soil moisture, (B) relative water content of roots, (C) relative water content of leaves. Different letters indicate differences of statistical significance ($p < 0.05$) by Tukey's test. The bars indicate the mean standard deviation ($n = 5$ per treatment).

from day 30, with the highest values observed at day 60 (147.9 units g^{-1} DW, $p < 0.01$) for *A. macrocarpa* and at day 30 for *A. colubrina* (Fig. 3A). In addition, increases of 42.6% at day 60 and of 65.5% at day 30 ($p < 0.01$) were detected in the CAT of roots of the respective species (Fig. 3B). The influence of the invasive grass also promoted changes in the APX activity of roots, with increases of 48.6% for *A. macrocarpa* at day 60 and of 52.5% for *A. colubrina* at day 30 (Fig. 3C). A significant increase was also observed for the SOD activity of leaves in *A. macrocarpa* (41.1%) and in *A. colubrina* (52.9%) at day 30 in the UC treatment, under the same comparison effects (Fig. 3D). A higher CAT activity was also observed in the UC treatment for the leaves of both species, with the highest values reaching 23 μmol $min.^{-1}$ g^{-1} DW in *A. macrocarpa* at day 60 and 31.5 μmol $min.^{-1}$ g^{-1} DW in *A. colubrina* at day 30 (Fig. 3E). Similarly, increases in the APX activity of leaves of 30.7% for *A. macrocarpa* and 51% for *A. colubrina* ($p < 0.01$) were observed at day 30 in the same treatment (Fig. 3F). The increase in H_2O_2 was positively correlated with the antioxidant enzymatic activity,

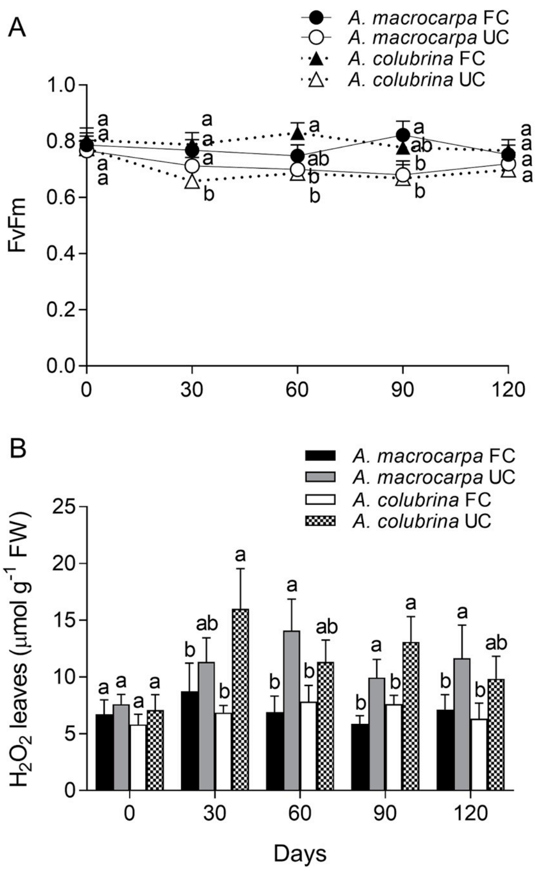

Fig. 2. Quantum efficiency of PSII (Fv/Fm) (A) and hydrogen peroxide content (H_2O_2) (B) in leaves of *A. macrocarpa* and *A. colubrina* subjected to interspecific competition with Piatã grass. Different letters indicate differences of statistical significance ($p < 0.05$) by Tukey's test. The bars indicate the mean standard deviation ($n = 5$ per treatment).

Table 2
Pearson's correlation coefficients between physiological and biochemical parameters (Student's t-test) in roots and leaves of *A. macrocarpa* and *A. colubrina* subjected to interspecific competition with Piatã grass. (UC) = under competition.

Main correlations	*r*-value	*p*-value
A. macrocarpa (UC)		
RWC roots × RWC leaves	0.94	0.0002
RWC leaves × Fv/Fm	0.70	0.0345
RWC roots × H_2O_2 content	−0.94	0.0003
RWC roots × SOD roots	−0.85	0.0041
RWC roots × CAT roots	−0.79	0.0116
RWC roots × APX roots	−0.96	0.00009
H_2O_2 leaves × SOD leaves	0.89	0.0016
H_2O_2 leaves × CAT leaves	0.84	0.0046
SOD roots × CAT roots	0.74	0.0209
SOD roots × SOD leaves	0.96	0.0001
SOD leaves × CAT leaves	0.98	0.000002
A. colubrina (UC)		
RWC roots × RWC leaves	0.96	0.00008
RWC leaves × Fv/Fm	0.93	0.0005
Fv/Fm × SOD roots	−0.89	0.0017
Fv/Fm × APX leaves	−0.75	0.0181
H_2O_2 leaves × SOD roots	0.93	0.0005
H_2O_2 leaves × APX leaves	0.94	0.0003
SOD roots × APX roots	0.94	0.0002
CAT leaves × APX leaves	0.74	0.0221
SOD leaves x CAT leaves	0.90	0.0014

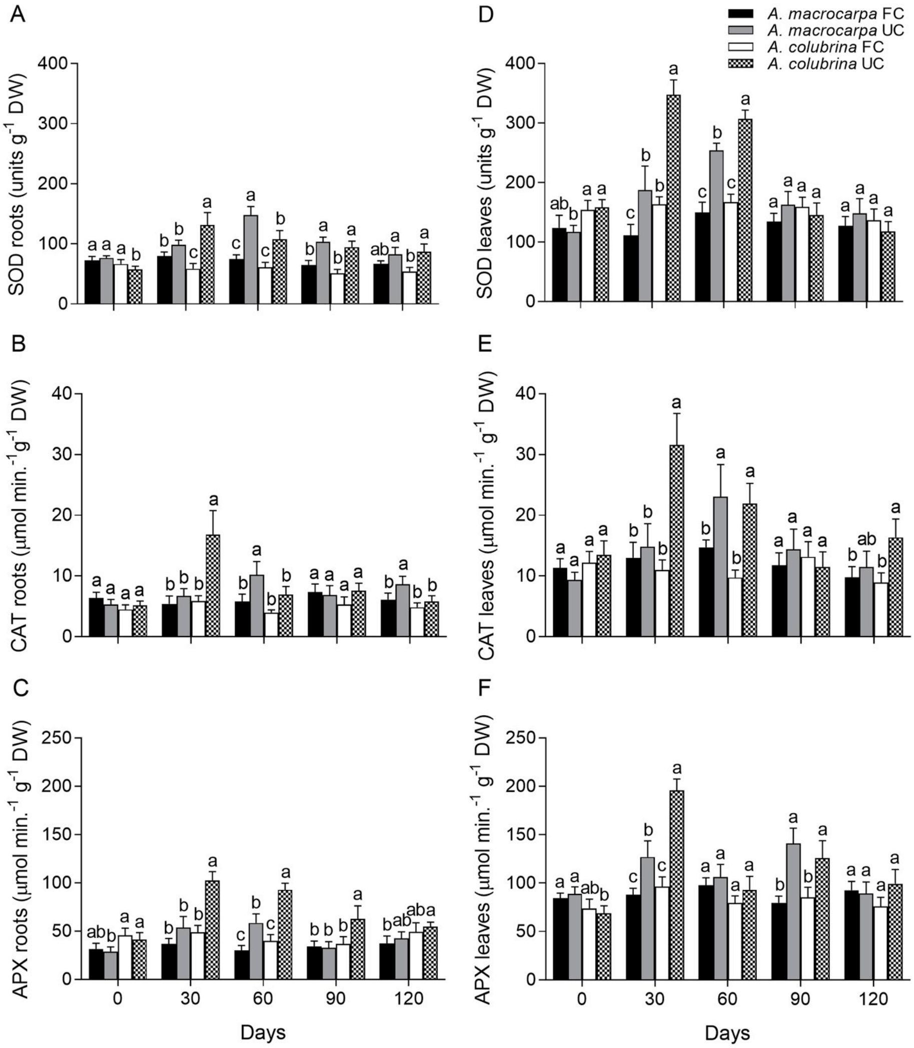

Fig. 3. Antioxidant activity of SOD (A, D), CAT (B, E) and APX (C, F) in roots and leaves of *A. macrocarpa* and *A. colubrina* subjected to interspecific competition with Piatã grass. Different letters indicate differences of statistical significance ($p < 0.05$) by Tukey's test. The bars indicate the mean standard deviation (n = 5 per treatment).

which was in turn integrated, in the UC treatment, for both species (Table 2).

3.4. Interspecific competition reduced the absorption of nutrients

The macronutrient content of the young Angico species is shown in Table 3. The concentrations of macronutrients were higher in the FC treatments, especially in the leaves. The young plants of *A. colubrina* showed a higher concentration of nutrients than *A. macrocarpa*. Regarding the relative order of macronutrient concentrations, the overall sequence was N > Ca > K > Mg > S > P. In this series, the N and Mg values were higher in the leaves of *A. colubrina* subjected to FC, while Ca was present in the leaves of the same species, although in the UC treatment, and S accumulated in higher amounts in the roots of *A. colubrina* subjected to FC. Significant differences were observed in the N content, with a decrease of 33.4%, 50% and 60.8% in the leaves, stems, and roots of *A. colubrina*, respectively and of 50%, 68% and 65% in the respective organs of *A. macrocarpa* in the UC treatment. Similar results were observed for the K values, with a decrease of 84.8% and 47.2% in the leaves. The Mg content showed a decrease in all organs of *A. macrocarpa* subjected to UC (Table 3).

The two species presented differences in the uptake of micronutrients according to the treatments (Table 4). The micronutrient concentrations in the different organs analysed followed the decreasing order: Fe > Zn > Mn > B > Cu. The Fe concentrations, although higher in the leaves and roots of plants subjected to FC, showed a decrease of 40.5% in the leaves and of 46% in the roots of *A. colubrina* subjected to the UC treatment. On the other hand, the Zn concentration increased by about 25%, 32.8% and 56.6% in leaves, stems, and roots of *A. macrocarpa* in the same treatment, respectively. The values of Mn decreased in approximately 64.6% and 41.7% in the roots of both species subjected to the UC treatment and expressive reductions were also observed in the B content in the stems and roots of *A. colubrina* in the presence of the Piatã grass (Table 4).

3.5. The Piatã grass affected the growth of the Angico species

The two Angico species showed a decrease in the growth rates due to the presence of the grass (Fig. 4). In the UC treatments, both species presented lower root length than in the FC treatments (Fig. 4A). Although a decrease in root growth occurred in the UC treatment, a large number of thin lateral roots were observed in both species. Reductions in the length of the aerial part were also observed, especially for *A. colubrina* (36.9%) in the UC treatment at day 120 (Fig. 4B). Plants of *A. macrocarpa* showed a more expressive growth in all treatments. The influence of competition also caused a deficit in the biomass allocation in the two species, although *A. colubrina* was more affected than *A. macrocarpa*. The dry mass of roots and shoots and the leaf area of both species presented a lower allocation in plants subjected to competition

Table 3
Macronutrients content in leaves, stems and roots of *A. macrocarpa* and *A. colubrina* plants subjected to interspecific competition with Piatã grass. (FC) = free competition and (UC) = under competition (n = 3 per treatment).

Treatments	Macronutrients (g/Kg)					
	N	P	K	Ca	Mg	S
A. macrocarpa						
FC	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Leaves	30.80 ± 2.16 a*	1.92 ± 0.12 a	10.61 ± 1.69 b	11.03 ± 1.11 b	3.04 ± 0.24 b	2.81 ± 0.32 a
Stems	22.40 ± 1.12 a	1.86 ± 0.17 a	9.43 ± 0.72 b	9.90 ± 1.02 b	2.31 ± 0.19 b	2.19 ± 0.22 a
Roots	16.81 ± 1.07 a	1.61 ± 0.09 a	6.05 ± 0.94 b	6.33 ± 0.86 ab	2.00 ± 0.10 b	2.73 ± 0.46 b
UC						
Leaves	15.42 ± 1.16 b	1.90 ± 0.23 a	5.57 ± 0.56 c	12.20 ± 1.62 b	2.43 ± 0.80 b	3.02 ± 0.41 a
Stems	7.04 ± 0.47 c	1.75 ± 0.13 a	2.65 ± 0.21 c	9.37 ± 1.24 b	1.15 ± 0.28 c	2.45 ± 0.36 a
Roots	5.65 ± 0.63 c	1.48 ± 0.15 a	3.59 ± 0.34 b	5.92 ± 0.75 b	1.18 ± 0.19 b	2.49 ± 0.15 b
A. colubrina						
FC						
Leaves	33.63 ± 2.48 a	2.22 ± 0.21 a	15.80 ± 1.87 a	14.71 ± 1.49 b	4.94 ± 1.05 a	2.62 ± 0.18 ab
Stems	28.03 ± 1.79 a	1.95 ± 0.14 a	19.06 ± 2.12 a	13.12 ± 1.70 a	4.20 ± 0.34 a	1.90 ± 0.13 a
Roots	25.22 ± 1.53 a	1.84 ± 0.10 a	17.32 ± 1.73 a	8.32 ± 1.12 a	3.89 ± 0.52 a	4.27 ± 0.54 a
UC						
Leaves	22.47 ± 1.89 b	1.52 ± 0.08 a	2.46 ± 0.21 c	21.3 ± 2.07 a	2.37 ± 0.66 b	2.07 ± 0.29 b
Stems	14.05 ± 1.26 b	1.34 ± 0.14 b	1.22 ± 0.54 c	6.85 ± 1.39 b	1.82 ± 0.14 b	0.83 ± 0.03 b
Roots	9.82 ± 1.18 b	1.20 ± 0.11 b	3.69 ± 0.78 b	7.15 ± 0.90 ab	1.56 ± 0.23 b	1.42 ± 0.10 c

*Means followed by different letters in the columns differ by Tukey test at $P < 0.05$ and indicate differences of the same organ between treatments.

than in the FC treatment (Fig. 4C–E). In the FC treatment, there was a substantial increase in the allocation of dry mass to shoots, with an increase of 14.6% in *A. macrocarpa* compared with *A. colubrina*, influenced by an increase in the leaf area. The values of LAR and RGR throughout the experiment evidenced different strategies for resource assimilation and growth among the studied species (Fig. 4F and G). The LAR values for the two species were significantly different, with a linear increase for *A. macrocarpa* in the FC treatment from day 60 and variable values for the two species under competition (Fig. 4F). The decrease in LAR influenced the RGR, causing a reduction in growth in both species subjected to the UC treatment throughout the 120 days (Fig. 4G). The RCI was negative for both species, evidencing that *A. macrocarpa* was less affected by competition than *A. colubrina* during the 120 days. The most negative RCI value was observed between days 30–60 for *A. macrocarpa,* indicating the greatest growth difference between the two species during this period, when subjected to competition with the Piatã grass (Fig. 4H).

4. Discussion

The presence of the Piatã grass negatively influenced the development of Angico species, with potentially important implications for the understanding of the establishment dynamics of young native trees and of grass coexistence, particularly under water limiting conditions. *Brachiaria brizantha* cv. Piatã proved to be very competitive and to have a fast growth, with a high shoot rate and biomass production as reported by Morais et al. (2016), which contributed to the rapid decrease in soil moisture in the UC treatment. The reduction of soil moisture affected root water absorption resulting in the RWC decrease in roots and leaves, which negatively influenced the ΦPSII (Fv/Fm) of both Angico species.

Soil water deficit appeared to be a determining factor for the low competitiveness of the two Angico species under the influence of the Piatã grass. According to Tron et al. (2015), the root density, especially on the soil surface, becomes the most relevant feature to exploit soil

Table 4
Micronutrients content in roots, stems and leaves of *A. macrocarpa* and *A. colubrina* plants subjected to interspecific competition with Piatã grass. (FC) = free competition and (UC) = under competition (n = 3 per treatment).

Treatments	Micronutrients (mg/Kg)				
	Cu	Fe	Zn	Mn	B
A. macrocarpa					
FC	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Leaves	6.32 ± 1.13 a*	421 ± 15.26 ab	39.22 ± 3.54 a	65.31 ± 4.67 a	50.71 ± 3.83 b
Stems	6.04 ± 0.84 a	177 ± 10.91 b	41.30 ± 3.94 c	21.60 ± 1.94 a	26.40 ± 2.14 b
Roots	3.71 ± 0.29 b	1136 ± 38.57 a	30.43 ± 2.18 b	24.13 ± 2.86 ab	24.79 ± 2.94 b
UC					
Leaves	5.95 ± 1.57 a	411 ± 21.42 ab	52.74 ± 4.73 a	60.20 ± 5.10 a	46.36 ± 2.40 b
Stems	3.67 ± 0.38 b	348 ± 14.60 a	61.23 ± 6.24 b	23.51 ± 1.87 a	27.85 ± 1.77 b
Roots	3.24 ± 0.46 b	633 ± 32.27 b	69.81 ± 9.08 a	13.98 ± 1.18 c	24.61 ± 1.38 b
A. colubrina					
FC					
Leaves	6.14 ± 1.42 a	522 ± 23.79 a	42.27 ± 7.91 a	49.54 ± 3.11 b	69.82 ± 5.26 a
Stems	5.10 ± 1.30 ab	151 ± 8.03 b	58.14 ± 10.48 bc	18.27 ± 1.19 ab	51.63 ± 3.09 a
Roots	5.35 ± 0.96 a	593 ± 32.41 b	79.95 ± 18.56 a	31.35 ± 2.06 a	37.92 ± 2.27 a
UC					
Leaves	7.23 ± 1.28 a	311 ± 15.07 b	50.32 ± 2.82 a	47.03 ± 3.32 b	62.48 ± 3.11 a
Stems	4.76 ± 0.41 ab	127 ± 11.59 b	88.09 ± 11.70 a	14.89 ± 1.68 b	29.08 ± 4.53 b
Roots	3.30 ± 0.67 b	321 ± 29.50 c	76.35 ± 5.13 a	11.43 ± 2.14 c	23.59 ± 2.19 b

*Means followed by different letters in the columns differ by Tukey test at $P < 0.05$ and indicate differences of the same organ between treatments.

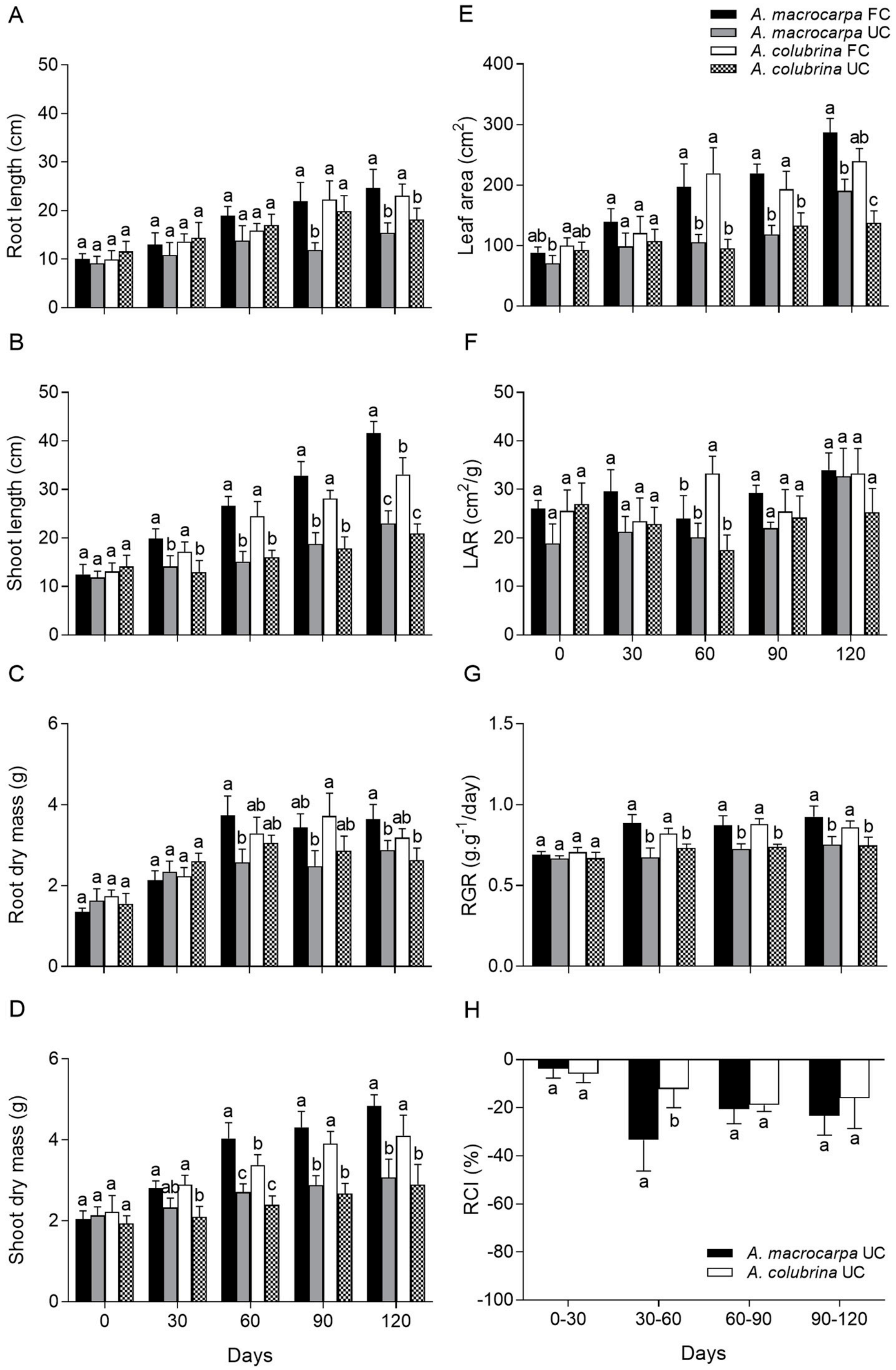

Fig. 4. Growth parameters (A–G) and Relative Competition Intensity index (RCI) (H) in *A. macrocarpa* and *A. colubrina* subjected to interspecific competition with Piatã grass. Different letters indicate differences of statistical significance ($p < 0.05$) by Tukey's test. The bars indicate the mean standard deviation (n = 5 per treatment).

moisture during the development of the root system. Thus, the grasses have the initial capacity of water absorption increased and the access to water sources and nutrients in the soil deepest layers before the roots are developed in larger plants (Eggemeyer et al., 2009). This process, together with the low water retention by the sandy soil and other climatic factors such as irradiance and temperature, can explain the lower RWC values in the roots and leaves of *A. macrocarpa* and *A. colubrina*. Similar results were observed for the establishment of *Quercus faginea* seedlings in a controlled experiment in which the water availability of the soil was compared; the authors showed that the reduction of water availability in the presence of the grass was highly related to the poor development of *Quercus faginea* (Rey Benayas et al., 2003). Donzelli et al. (2013) suggest that in dry savannas the coexistence between tree and grasses is permitted by differential resource access strategies, where trees are superior competitors for soil nitrogen, while grasses are superior competitors for water. Thus, the high correlation observed between the decrease of RWC in roots and leaves and the reduction of Fv/Fm in our study (Table 2), reinforces the efficiency of Piatã grass in removing water from the soil.

The significant reduction of Fv/Fm suggests that underground competition affected the carbon metabolism in both Angico species. It is possible that changes in the ΦPSII reflected reductions in the electron transport due to the decrease in the RWC, indicating photoinhibition, in which the excess of energy absorbed by the leaves exceeded that consumed by photosynthesis. According to Bhadouria et al. (2017), one of the indirect effects of interspecific competition is water limitation, which makes the leaf water content to decline quickly in most native plants and reduces the electron transport, leading to oxidative stress (Wang et al., 2013). Similar reductions in the ΦPSII were observed in seedlings of *Cedrela tonduzii*, *Inga punctata*, *Ocotea whitei* and *Tapirira mexicana* when subjected to competition with African grasses in Southern Costa Rica (Loik and Holl, 2001). In fact, the modified ΦPSII performance in *A. macrocarpa* and *A. colubrina* under competition resulted in consequences to the redox state of the cell mainly in the leaves. Therefore, its feedback was reflected in the H_2O_2 content and the level of oxidative-stress enzymes. The high correlation observed between these data reinforces that the oxidative stress was caused by water limitation (Table 2). The major scavenging mechanisms of Reactive Oxygen Intermediaries (ROI-scavenging) in plants include SOD, found in almost all cell compartments, the water-water cycle in chloroplasts, the ascorbate-glutathione cycle in chloroplasts, mitochondria, cytosol, apoplast and peroxisomes, and the CAT in peroxisomes (Mittler et al., 2004; Januškaitienė et al., 2018). In our study, the high SOD and CAT activities of Angico species in UC indicate that the water-water and catalase cycles were involved, as evidenced by the high correlations between these enzymes. The SOD activity removes O_2^- by catalyzing its dismutation and decreases the risk of $OH^\bullet$ formation (Gai et al., 2017; Gill and Tuteja, 2010), whereas CAT is important in the removal of the H_2O_2 generated in the peroxisomes by oxidases involved in β-oxidation of fatty acids (Gill and Tuteja, 2010). In addition, the antioxidant defense system of the ASC/GSH cycle involving reduced ascorbate (ASC) and glutathione (GSH) was also efficiently activated, as observed by the increased APX activity in roots and leaves, especially in *A. colubrina*.

Changes in the nutrient transportation and accumulation in the organs of Angico species may have been influenced by the decrease in water absorption due to the water limitation in UC. The slow diffusion of mineral nutrients from the soil to the root surface under water restriction conditions reduces the translocation speed to the leaves. Thus, the N mobility is disrupted and plants exposed to water limitation face N deficiency and have reduced growth. The decrease in leaf area and photosynthetic rates are immediate effects of the altered N uptake, as well as of the decreased enzymatic activity (Ahanger et al., 2016). Similarly, plants under water restriction may also have P deficiency resulting in decreased plant height, leaf area and leaf water content (Singh et al., 2006), as well as a deficiency in K, which decreases aquaporin activity, root hydraulic conductivity and water supply as a whole (Kanai et al., 2011). We should consider that the application of the solution might have increased the macronutrients consumption by the Piatã grass, decreasing the availability of N, P and K for Angico species. Fast-growing plants that have a high rate of biomass production, such as invasive grasses, can maximize the consumption of some nutrients when there is a high availability (Eller and Oliveira, 2016). In addition, even if plants grow in nutrient-rich soils, water limitation can cause a nutrient deficiency in tissues due to the direct effect on physicochemical properties, reducing their mobility and absorption by plants. Thus, coupled with the water limitation, it is possible that the Piatã grass has used this strategy of high nutrient consumption and affected nutrient availability for Angico species. Morais et al. (2016) showed that fertilization with N and P increased shoot dry mass production of Piatã grass plants cultivated in Cerrado soil. Manea and Leishman (2015) also demonstrated the competitive effect of some native grasses on the development of seedlings of Australian tree species, emphasizing that the high total biomass of grasses may have increased the competition for water and soil nutrients, reducing the growth of saplings of woody plants.

With regard to micronutrients, the decrease in Fe content, mainly in the roots and leaves of *A. colubrina*, was also associated with a lower absorption under competitive conditions. A low soil humidity and Fe^{2+}/Fe^{3+} ratio are possibly due to the increase in the soil O_2 levels. A high amount of O_2 reduces the iron availability for plant absorption because Fe^{3+} is less soluble (Sardans et al., 2008). Reductions in the amount of Fe and Mn in both Angico species under competition may have promoted imbalances in the photosynthetic apparatus, as evidenced by the decrease in Fv/Fm values. While Fe is involved in the production of chlorophyll, energy transfer and nitrogen reduction (Ahanger et al., 2016), Mn is linked to the functioning of the photosynthetic system, maintaining the chlorophyll concentration, superoxide dismutase activity, ATP synthesis and RuBP carboxylase reactions (Upadhyaya et al., 2012). On the other hand, the increase in Zn content observed in the Angico species under UC treatment can be related to the maintenance of a greater enzymatic activity due to the soil water deficit. Although the absorption of Zn by plant roots is reduced by the low water availability in the soil, the activity of antioxidant enzymes in *A. macrocarpa* and *A. colubrina* was certainly improved by the Zn increase in the tissues. The Zn increase can reduce the effects of water limitation on plant growth, reducing membrane-bound NADPH oxidase activity, preventing photo-oxidative damage, reducing the generation of ROS and increasing the SOD and CAT activities involved in ROS detoxifying (Hajiboland, 2012; Ahanger et al., 2016). The higher correlations in SOD, CAT and APX activities in leaves and roots in both Angico species under competition confirmed this relationship.

The competitive efficiency below ground of the Piatã grass is evidenced by the RCI values and the lowest absorption of water and nutrients that resulted in a growth deficit and a lower final development of Angico species. According to Galon et al. (2013), the best competitive adaptation of grasses occurs because they have a more efficient enzymatic system for the capture and use of CO_2 under optimal conditions of moisture, temperature, and luminosity, in which species such as *Brachiaria* have a greater ability to produce dry mass. In addition, plant height is an important attribute that can strongly influence the competition depending on the species and on the period of interaction. This characteristic, together with a rapid development of the leaf area and a high density of individuals, can increase the competitive ability of the invasive species (Galon et al., 2013). Since in this study the length of the Piatã grass was not affected by competition, it is possible that such effect influenced light penetration, reducing the leaf area and the LAR of *A. macrocarpa* and *A. colubrina*. Consequently, the decrease in LAR influenced the RGR in both species evaluated. Sun and Dickinson (1996) observed that young plants of *Alphitonia petriei* cultivated in the field and subjected to competition with *Brachiaria* in northeastern Australia had low values of biomass allocation and reduced growth rates. The negative RCI indicated that when the water supply was limited, the interspecific competition affected the growth of *A. macrocarpa* more than the competition with *A. colubrina*, only between the 30–60 days. *Anadenanthera macocarpa* showed higher RGR rates over 120 days, although the RCI results evidenced large differences between each Angico species and its respective FC and UC treatments. As RGR is an estimate of the plant efficiency in accumulating dry matter, we might infer that the competition with the Piatã grass was responsible for such variations in the parameters herein evaluated, since the display of morphological plasticity over time can be considered as adjustments to a particular interaction.

5. Conclusion

Plants of *A. macrocarpa* and *A. colubrina* showed reduced development during the initial growth stages, imposed by the presence of the Piatã grass. The rapid growth and the high competitive capacity of the grass negatively influenced the absorption of water and nutrients by the native plants, due to the reduction in soil moisture. The negative effects of water limitation with the consequent decrease in RWC of the tissues and in the leaf Fv/Fm was minimized by the antioxidant activity. Thus, a better understanding of tree seedling establishment is important for the development of management plans that can improve the diversity of tree species in Cerrado areas under the changing climate scenario.

CRediT authorship contribution statement

Evandro Alves Vieira: Formal analysis, Writing - original draft. **Fernanda Cristina Andrade Galvão:** Formal analysis, Writing - original draft. **Ana Lúcia Barros:** Formal analysis, Writing - original draft.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2018.12.002.

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