

The common mycorrhizal network originated from *Brachiaria brizantha* suppresses the growth of seedlings from the grass itself and native trees in southern Brazil

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Abstract

The common mycorrhizal network (CMN) is formed by arbuscular mycorrhizal fungi (AMF) that interconnect plant roots, and promote the exchange of nutrients, water, and signaling compounds, in addition to serving as a mechanism of competition among plants. It was evaluated the influence of CMN, originated from the adult invasive grass *Brachiaria brizantha*, on the development of seedlings of *B. brizantha* itself and of the native trees *Heliocarpus popayanensis* (early-successional) and *Cariniana estrellensis* (late-successional). The plants were grown in a substrate composed of fertile soil and sterilized sand in a 3:1 ratio, with 640 native AMF spores per gram of soil. The treatments were: 1) Seedlings of *B. brizantha*, *H. popayanensis*, and *C. estrellensis* grew under the influence of CMN originated from adult *B. brizantha* for 225 days; 2) Ninety days after the influence of CMN, two adult *B. brizantha* prunings were carried out, the first occurring 90 days after emergence and the second 180 days after the first pruning; 3) Ninety days after the influence of the CMN, the communication network was severed by the weekly rupture of the fungal hyphae; 4) The control treatment consisted of the isolated growth of five seedlings of each species without contact with the CMN. The CMN originated from *B. brizantha* suppressed the growth of seedlings of the three species. CMN severing completely eliminated the suppressive effects of adult *B. brizantha* on seedlings. The adult *B. brizantha* pruning mitigated the negative effects on seedlings, however after regrowth, suppression returned. The seedlings of *H. popayanensis* connected to the CMN originated from adult *B. brizantha* died, while the seedlings of *C. estrellensis* and *B. brizantha* itself survived, although suppressed.

Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophic organisms belonging to the phylum Glomeromycota, with 288 described species (Öpik and Davison 2016). AMF are cosmopolitan and have low specificity, characteristics that provide associations with about 80% of plant families (Berbara et al. 2006; Bertagnoli et al. 2020). Fungi absorb water and nutrients from the soil, and supply them to plants, which return carbon in the form of photoassimilates (Colozzi and Cardoso 2000; Zangaro et al. 2012). AMF are highly beneficial to plants, as they provide up to 80% of phosphorus and 25% of nitrogen needed by plants, and receive in exchange only 4 to 20% of the total carbon fixed by plants (Douds et al. 2000). The AMF which colonize a plant are able to, at the same time, colonize the roots of other plants, forming the underground communication network called the common mycorrhizal network (CMN) (Gorzela et al. 2015). CMN can also be formed by anastomoses, in which an AMF that colonizes the root of a plant fuses its hyphae with others AMF hyphae that colonize other plants (Mikkelsen et al. 2008).

Neighboring plants connected to the CMN can exchange macronutrients (Smith and Read 2008), micronutrients (Asay 2013), water (Egerton-Warburton et al. 2007), signaling compounds (Song et al. 2014; Cabral et al. 2018), allelochemicals (Nilsson 1994), and photoassimilates (Bever et al. 2010), in addition to serving as a mechanism of competition among plants (Merrild et al. 2013). Thus, CMN has the potential to influence and change the composition of a plant community (Rillig 2004; Wilson et al. 2009), especially in the seedlings establishment stage (Merrild et al. 2013).

The suppression of plants connected via CMN may occur due to the fact that different plant species contribute different amounts of carbon to the network, which may influence the amount of nutrients received in exchange (Selosse et al. 2006). Wyatt et al. (2014) reported that the amount of nutrients transferred to plants is regulated by the AMF that forms the network, which prioritizes the greatest flow of nutrients to plants that provide more carbon to the CMN. In a plant community, larger individuals obtain a disproportionately higher amount of available resources, which may limit the growth of smaller individuals, and this asymmetric effect is potentiated by CMN (Janos et al. 2013). Merrill et al. (2013) found that CMN originating from adult plants of *Cucumis sativus* strongly suppressed the growth of *Solanum lycopersicum* seedlings. However, with the pruning of *C. sativus*, the seedlings of *S. lycopersicum* connected to the CMN showed higher growth, and absorbed 6.5 times more phosphorus.

Growth rate, plant size, and root:shoot ratio are the characteristics that most influence the ability to provide carbon to the CMN. Larger plants, with high growth rates, and large leaf area provide more carbon to the network fungi, which in turn transfers more nutrients to these plants, and thus the effect of CMN will be positive for these individuals and suppressive for smaller, younger plants with a lower growth rate (Merrild et al. 2013). This way, grasses can obtain a great competitive advantage among plants belonging to the CMN, as they have a high growth rate and high photosynthetic rate, being able to dominate the CMN, and receive higher amounts of nutrients (Zangaro et al. 2018).

Several studies report seedling growth suppression via CMN (Jakobsen 2004; Janoušková et al. 2011; Janos et al. 2013; Merrild et al. 2013). However, studies with exotic plants suppressing native plants are scarce (Carey et al. 2004; Zangaro et al. 2018). Silveira et al. (2013) reported that exotic grasses are the main responsible for seedling mortality in ecological restoration areas. Carey et al. (2004) found that there was more than 15% transfer of carbon via CMN from the native species *Festuca idahoensis* to the exotic grass *Centaurea maculosa*, indicating that carbon transfer via CMN may be an important mechanism by which invasive grasses compete with native plants. Zangaro et al. (2018) verified that seedlings of native early-successional trees died when connected to the CMN originated from the grass *B. brizantha*, and that seedlings of late-successional trees survived showing limited growth and low concentrations of nitrogen and phosphorus in the shoot.

The aim of this study was to evaluate the influence of CMN originated from the adult invasive grass *Brachiaria brizantha*, on the development of seedlings of *B. brizantha* itself and the native trees *Heliocarpus popayanensis* (early-successional) and *Cariniana estrellensis* (late-successional). The hypotheses are: 1) The exchange of carbon for nutrients with the AMF which form the CMN is intensified due to the high photosynthetic rate and the high mycotrophy of *B. brizantha*, with the flow of nutrients being directed towards the adult grass, resulting in the suppression of seedlings of the three species studied; 2) The suppression in the development of the seedlings will be completely interrupted when the severing of the CMN originated from the adult grass occurs; 3) Suppression of seedling growth will be partially interrupted when the adult grass is pruned; 4) After the pruned grasses regrowth, the suppression on the seedlings will return.

Material And Methods

Study place, experimental design, and description of treatments

The experiment was carried out in a greenhouse, without temperature and relative humidity control, belonging to the Department of Animal and Plant Biology of the State University of Londrina, in the state of Paraná, Brazil, 23° 19' 29.2" S, 51° 11' 52.8" W, with an altitude of 602 m. The climate of the region is humid subtropical (Cfa) with hot summers, with an average annual temperature of 20.9° C and an average annual rainfall of 1429 mm (Köppen 1948).

The substrate used for plant growth was a mixture of high fertility soil and sterilized sand in a 3:1 ratio. Soil was collected at a depth of 0–10 cm, from an area where there was an old vegetable garden that received constant organic fertilizer, which had been abandoned for more than 10 years and is currently colonized by the grass *Paspalum notatum* Flügge. This soil contained approximately 640 AMF spores per gram, as well as other infective propagules such as colonized roots and hyphae. The most abundant AMF species in the soil were: *Rhizophagus clarus* (Nicolson & Schenck) Walker & Schüßler, *Glomus macrocarpum* Tulasne & Tulasne, *Claroideoglomus etunicatum* (Becker & Gerdemann) Walker & Schüßler, *Paraglomus occultum* (Walker) Morton & Redecker, *Acaulospora mellea* Spain & Schenck, *Acaulospora morrowiae* Spain & Schenck, and *Glomus hoi* Berch & Trappe. The soil used was classified as a clayey Rhodic Ferralsol (FAO 1994) and high fertility, with levels of 166.50 mg dm⁻³ of phosphorus, 20.42 g dm⁻³ of carbon, 5.40 of pH, 4.60 cmol_c dm⁻³ of potential acidity (H + Al), 5.80 cmol_c dm⁻³ of calcium, 2.06 cmol_c dm⁻³ of magnesium, 0.97 cmol_c dm⁻³ of potassium, 8.83 cmol_c dm⁻³ of sum of bases (SB), 13.43 cmol_c dm⁻³ cation exchange capacity (CEC), and 65.7% percent base saturation (BS). The main soil characteristics include an advanced degree of weathering, low activity (low

CEC), predominance of clay (65% clay, 15% silt, and 20% sand), and a well-developed microgranular structure (Santos et al., 2006). The plants were grown in the substrate, using plastic pots with a capacity of 5 L, which were randomly placed on the tables of the greenhouse.

The plant species used were: the grass *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf (Poaceae), the early-successional tree *Heliocarpus popayanensis* (Kunth) K. Schum. (Malvaceae), and the late-successional tree *Cariniana estrellensis* (Raddi) Kuntze (Lecythidaceae). To evaluate the effects caused by CMN originated from adult *B. brizantha* on the seedlings of *B. brizantha*, *H. popayanensis*, and *C. estrellensis*, two *B. brizantha* plants, which were the originators of CMN, were grown on the opposite sides of the plastic pots. In the central region were grown the seedlings of the species that suffered the effects of CMN. To eliminate the competitive effect of the roots and maintain only the effects caused by the CMN, the central seedlings were grown inside a polyethylene mesh cylinder with 30 µm pores and with a capacity of 1 L of soil, located in the center of the plastic pots. The small pores of the polypropylene mesh that involved the soil cylinder prevented the *B. brizantha* roots from the sides of the pot to compete with the seedling roots inside the cylinder, allowing only the passage of hyphae and the formation of CMN.

The two *B. brizantha* plants on the sides of the pots grew for 70 days, a period sufficient for the growth of the root system and the hyphae of the AMF, which crossed the pores of the cylinder to explore the soil, forming the CMN between the roots of the plants. Five seeds of each plant species were sown in the center of the cylinder, being thinned to just one seedling soon after emergence.

The experiment consisted of three treatments and a control, in five replications: 1) The seedlings of *B. brizantha*, *H. popayanensis*, and *C. estrellensis* suffered the direct effects of CMN originated from adult *B. brizantha*, which remained under the effect of CMN for 225 days; 2) Ninety days after the CMN influence, the pruning of the adult grasses that gave rise to the CMN was carried out. Pruning was performed twice, the first 90 days after emergence and the second 180 days after the first pruning; 3) After 90 days of CMN influence, the network was severed weekly, preventing communication via CMN between adult *B. brizantha* and seedlings. The rupture of the CMN occurred through 360° rotation of the polyethylene meshes, causing the rupture of the fungal hyphae. Seeking to avoid a new formation of the CMN by anastomosis, this process was repeated weekly; 4) The control consisted of growing seedlings of each of the three species in isolation, without contact with CMN, in cultivation bags with a capacity of 1 L.

Data collect

Every 45 days, data on height, number of leaves, and leaf area of seedlings were collected. After 225 days, the plants were carefully removed from the substrate and the roots were washed in running water. The roots and shoots were separated, and subsequently dried in an oven at 65 °C until reaching constant weight to determine the dry mass. The ratio between root and shoot was calculated to verify in which system the plant is investing greater amounts of resources.

The net CO₂ assimilation rate of adult plants of the three plant species used in the experiment was determined, without any contact with CMN. Data were obtained using an infrared gas analyzer (IRGA) model LI-6400 (LI-COR, Inc., Lincoln, NE, USA), which includes a blue-red light source LI-6400-02B (LI -COR) and CO₂ concentration and temperature controller. Measurements were performed only when the coefficient combining the variations of water, CO₂, and air flow was below 1%.

The competitive response of seedlings of the three species that grew under the influence of CMN originated from adult *B. brizantha* was calculated by the equation: $CR = \ln \frac{DMC}{DMW}$, in which CR is the competitive response, DMC is shoot dry mass of the seedling grown under the influence of CMN originated from adult *B. brizantha*, and DMW is shoot dry mass of the seedling grown isolated, without any contact with CMN (Cahill 2002). The relative growth rate (RGR) of the seedlings was determined according to Hunt (1982), using the equation: $RGR = \frac{(\ln W_{t2} - \ln W_{t1})}{t2 - t1}$, in which W is the

average of the total seedling dry mass and t is the time measured in days (t_1 = seedling emergence; t_2 = days elapsed after seedling emergence until the end of experiment).

To estimate the mycorrhizal colonization of fine roots, a portion of approximately 1 g of fresh roots from each sample was clarified in 10% KOH, acidified in 1% HCl, washed in running water and stained with 0.05% trypan blue (Brundrett et al. 1996). Then, the intersection method on gridded plates, described by McGonigle et al. (1990), was employed, considering the presence of fungal structures (coenocytic hyphae, arbuscules and vesicles) at $\times 100$ magnification.

After obtaining the dry mass data, the seedlings shoot of the three species studied was ground and passed through a 1 mm diameter sieve to determine the chemical attributes, and the values were corrected by the dry mass to obtain the total nutrient content in the seedling. The elements determined were: nitrogen, phosphorus, potassium, calcium, magnesium, copper, zinc, boron, and manganese in atomic emission spectrometer - ICP, Thermo Jarrel Ash ICAP 61E (Miyazawa et al. 1984).

For soil chemical analyses, the samples were air-dried and sieved through a 2 mm mesh. The organic carbon content was determined via the humid oxidation method of Walkley and Black (1934). pH was determined with CaCl_2 0.01 mol L⁻¹ at a proportion of 1:2.5 (mass:volume). Calcium and magnesium were extracted with KCl 1 mol L⁻¹ at a 1:10 ratio (mass:volume) and determined by atomic absorption spectrophotometry. Phosphorus and potassium were extracted by Mehlich-1 (HCl 0.05 mol L⁻¹ + H₂SO₄ 0.025 mol L⁻¹) and determined by spectrophotometry and flame photometry, respectively (Pavan et al. 1992). Potential acidity (H + Al) was determined by a buffer solution called Shoemaker-McLean-Pratt (SMP), reported as pH_{SMP} (Quaggio et al. 1985), from the calibration curve of pH_{SMP} versus H + Al for Paraná soils. Cation exchange capacity (CEC) was calculated from the sum of calcium, magnesium, potassium, and H + Al. Sum of bases (SB) from the sum of calcium, magnesium, and potassium, and percent base saturation (BS) from the SB:CEC ratio multiplied by 100.

Statistical analysis

The data obtained were analyzed for normality using the Shapiro-Wilk test and for homoscedasticity using the Levene test. Data that did not meet the assumptions were transformed by the Box-Cox method and data expressed as percentages were previously transformed into arc sine $\sqrt{\left(\frac{x}{100}\right)}$.

For the analyzes that compared only the seedlings with the presence or absence of CMN, the Student's t test was used; to compare the means of seedlings grown in the absence of CMN, with the shoots of adult *B. brizantha* pruned, and with the CMN severed, an ANOVA followed by the Tukey's test was used. In both tests, values of 95% confidence were adopted ($p < 0.05$). All statistical analyzes were performed using the R v 4.0.2 program (R Core Team 2020).

Results

Influence of CMN on height, number of leaves, and leaf area of seedlings

The effects of CMN originated from adult *B. brizantha* on the height, number of leaves, and leaf area of seedlings of *B. brizantha*, *H. popayanensis*, and *C. estrellensis*, during 225 days, are shown in Fig. 1. *B. brizantha* seedlings were negatively affected by CMN in all evaluated periods, showing shorter height (Fig. 1a), smaller number of leaves (Fig. 1b) and smaller leaf area (Fig. 1c) when compared to *B. brizantha* seedlings grown isolated, without contact with CMN. Differences in height, number of leaves, and leaf area were identified in the first evaluation (45 days after seedling emergence) and increased throughout the period. After 225 days, the mean height of seedlings of *B. brizantha* isolated,

without CMN, was 40.1 cm and of seedlings that grew connected to the CMN was 6.2 cm, representing a 6.4 times difference in seedling height. In addition, seedlings without CMN had 17 leaves per plant and 267 cm² of leaf area, while seedlings with CMN had 3.4 leaves per plant and 12.4 cm² of leaf area, differing by 5 times in the number of leaves and in 21.5 times the leaf area.

The early-successional species *H. popayanensis* was also negatively affected by CMN, presenting shorter height (Fig. 1d), smaller number of leaves (Fig. 1e), and smaller leaf area (Fig. 1f) than the seedlings grown without CMN. A hundred eighty days after emergence, all seedlings connected to the CMN did not resist the suppressive effects and died, while seedlings without CMN remained growing.

The late-successional species *C. estrellensis* was also suppressed by CMN originated from adult *B. brizantha*, showing shorter height (Fig. 1g), smaller number of leaves (Fig. 1h), and smaller leaf area (Fig. 1i) than the seedlings without CMN. Since the first evaluation, 45 days after emergence, the suppression of *C. estrellensis* seedlings grown with CMN was verified, and this effect remained throughout the 225 days. After this period, the seedlings without CMN had a height of 29.6 cm, while the seedlings with CMN had 7.1 cm, showing a growth limitation of 4.2 times. Regarding the number of leaves and leaf area, the seedlings without CMN had 22.6 leaves per plant and 368.8 cm², respectively, while the seedlings with CMN had 6.4 leaves and 20.9 cm² of leaf area, showing a difference of 3.5 and 17.6 times, respectively.

The interferences, represented by the pruning of the adult *B. brizantha* grass and the severing of the CMN, positively influenced the height, number of leaves, and leaf area of native tree seedlings of *H. popayanensis* and *C. estrellensis* (Fig. 2). The seedlings of *H. popayanensis* presented taller height (Fig. 2a), larger number of leaves (Fig. 2b), and larger leaf area (Fig. 2c) with the pruning of adult *B. brizantha* and with the severing of the CMN, while the seedlings that remained connected to the CMN of adult *B. brizantha* died within 90 days of the beginning of the interference. *H. popayanensis* seedlings grown connected to the CMN originated from pruned *B. brizantha* showed taller height, larger number of leaves, and larger leaf area than seedlings grown with the CMN severed. After the period of regrowth of the shoot of adult *B. brizantha*, the negative influence on the growth of the seedlings occurred again, with no differences in the growth between these and the seedlings with the CMN severed. Differences in seedling growth were verified after the two pruning of *B. brizantha* (0 and 180 days), while the disappearance of these differences occurred after two grass regrowth. Two hundred forty days later, the height, number of leaves, and leaf area were 20 cm, 7.8, and 102.8 cm², respectively, for seedlings grown with pruned *B. brizantha*, and 15 cm, 5, and 44.3 cm², respectively, for seedlings grown with the CMN severed.

Interferences were also beneficial for the late-successional native tree species *C. estrellensis*, which presented taller height (Fig. 2d), larger number of leaves (Fig. 2e), and larger leaf area (Fig. 2f) when compared to the seedlings that remained connected to the CMN without interference from *B. brizantha*. Two hundred forty days later, the seedlings that were connected to the adult *B. brizantha* CMN showed 8.6 cm in height, 9.1 leaves per plant, and 25.5 cm² of leaf area, while those connected to the pruned adult *B. brizantha* CMN presented height of 17.5 cm, number of leaves of 16.8 per plant, and leaf area of 240 cm². The CMN severed seedlings presented 16.7 cm in height, 17 leaves per plant, and 181.3 cm² of leaf area, representing a suppression of 2 and 1.9 times in height, 1.8 and 1.8 times in the number of leaves, and 9.4 and 7.1 times in the leaf area, respectively.

Influence of CMN on the competitive response, nutrient content, and dry mass of seedlings

The competitive response (CR) of the seedlings is represented in Fig. 3. When connected to the CMN of adult *B. brizantha*, the seedlings of *B. brizantha* had a lower CR (-2.07) when compared with those of *C. estrellensis* (-0.60), not being possible to calculate for *H. popayanensis* due to seedling death (Fig. 3a). Regarding seedlings grown under the influence of pruned adult *B. brizantha* CMN, the seedlings of *H. popayanensis* showed lower CR (0.66) than those of *C. estrellensis*

(1.22) (Fig. 3b). With the CMN severed, the seedlings of *H. popayanensis* showed a lower CR again, with 0.93 compared to those of *C. estrellensis* with 1.15 (Fig. 3c).

The nutrient contents in the shoot of seedlings of *B. brizantha*, *H. popayanensis*, and *C. estrellensis*, grown in the presence and absence of CMN are shown in Table 1. The seedlings of *B. brizantha* without CMN had higher nitrogen content than the *B. brizantha* that grew connected to the CMN. It was not possible to analyze other nutrients content due to the low biomass production by the seedlings of *B. brizantha* connected to the CMN, not providing enough material for analysis. The seedlings of *H. popayanensis* connected to the CMN originated from *B. brizantha* died, and it was not possible to verify the nutrient content, while the seedlings of *C. estrellensis* grown without CMN had a higher content of all nutrients than those grown connected to the CMN.

Table 1

Nutrient content in the shoot of seedlings of *B. brizantha*, *H. popayanensis*, and *C. estrellensis* grown for 225 days under the influence of the common mycorrhizal network (CMN) originated from adult *B. brizantha* (+ CMN) or without CMN (- CMN). Means followed by the same letter in the column, for the same species, did not differ from each other by Student's *t* test at 95% ($p < 0.05$). The standard error is in parentheses, IM indicates insufficient material for analysis, and † indicates seedling death.

		N	P	K	Ca	Mg	Cu	Zn	B	Mn
Plant	Treatment	mg seedling ⁻¹					µg seedling ⁻¹			
<i>B. brizantha</i>	- CMN	12,42 a (0,55)	11,35 (0,23)	66,17 (1,81)	13,47 (0,97)	11,32 (0,29)	0,77 (0,01)	110,03 (1,84)	54,35 (2,63)	194,74 (4,57)
	+ CMN	0,41 b (0,01)	IM	IM	IM	IM	IM	IM	IM	IM
<i>H. popayanensis</i>	- CMN	14,20 (0,58)	9,67 (0,24)	44,57 (1,00)	42,18 (1,65)	10,57 (0,32)	9,31 (0,62)	90,94 (3,80)	122,02 (5,53)	305,64 (16,99)
	+ CMN	†	†	†	†	†	†	†	†	†
<i>C. estrellensis</i>	- CMN	14,75 a (1,31)	4,72 a (0,15)	43,06 a (1,27)	47,94 a (0,73)	12,95 a (0,41)	32,63 a (1,25)	44,63 a (2,37)	256,59 a (4,49)	287,80 a (1,39)
	+ CMN	0,95 b (0,09)	0,71 b (0,04)	3,48 b (0,11)	2,73 b (0,10)	1,49 b (0,09)	1,99 b (0,15)	5,97 b (0,05)	21,40 b (0,04)	13,14 b (0,10)

The nutrient content in the seedlings of *H. popayanensis* and *C. estrellensis*, grown with pruned *B. brizantha* and severed CMN, is shown in Table 2. The *H. popayanensis* seedlings grown when *B. brizantha* was pruned had higher content compared to seedlings that had the CMN severed, except for zinc, which showed less accumulation and manganese, which did not differ between treatments. *H. popayanensis* seedlings grown with pruned *B. brizantha* showed higher nutrient content compared to seedlings that had CMN severed, except for zinc, which showed less accumulation and manganese, which did not differ between treatments. *C. estrellensis* seedlings showed higher content for all nutrients when there was interference (*B. brizantha* pruning or severing CMN) when compared to seedlings connected to the CMN originated from adult *B. brizantha*.

Table 2

Nutrient content in the shoots of seedlings of *H. popayanensis* and *C. estrellensis* grown under the influence of the common mycorrhizal network (CMN) originated from adult *B. brizantha* (+ CMN), *B. brizantha* pruned (Bb pruned), and with the CMN severed (CMN severed). Means followed by the same letter in the column did not differ from each other by Student's *t* test for *H. popayanensis* or by Tukey's test for *C. estrellensis* at 95% ($p < 0.05$). The standard error is in parentheses, CV indicates coefficient of variation, and † indicates seedling death.

		N	P	K	Ca	Mg	Cu	Zn	B	Mn
Plant	Treatment	mg seedling ⁻¹					µg seedling ⁻¹			
<i>H. popayanensis</i>	+ CMN	†	†	†	†	†	†	†	†	†
	CMN severed	5,80 b (0,33)	2,81 b (0,10)	10,05 b (0,24)	10,17 b (0,37)	3,63 b (0,07)	1,16 a (0,04)	22,49 b (1,12)	22,13 b (0,67)	96,87 a (4,85)
	Bb pruned	9,68 a (0,13)	4,08 a (0,06)	16,54 a (0,05)	18,96 a (0,19)	6,06 a (0,06)	0,97 b (0,01)	36,78 a (0,05)	35,90 a (0,45)	93,89 a (2,37)
<i>C. estrellensis</i>	+ CMN	0,95 c (0,09)	0,71 c (0,04)	3,48 b (0,11)	2,73 c (0,10)	1,49 c (0,09)	1,99 b (0,15)	5,97 c (0,05)	21,40 c (0,04)	13,14 c (0,10)
	CMN severed	9,21 b (0,25)	3,09 a (0,04)	15,23 a (0,13)	15,93 b (0,17)	6,48 b (0,02)	23,67 a (0,63)	26,43 b (0,93)	69,08 b (1,04)	121,76 a (0,90)
	Bb pruned	11,36 a (0,23)	2,88 b (0,04)	15,73 a (0,12)	18,12 a (0,30)	8,07 a (0,12)	22,25 a (0,12)	34,25 a (0,26)	102,67 a (1,72)	113,10 b (0,72)
	CV (%)	4,86	2,94	1,64	2,88	2,56	3,99	4,40	3,16	1,38

The net assimilation of CO₂ by plants decreased as the successional group progressed, with *B. brizantha* presenting an average of 11.8 µmol CO₂ m⁻² s⁻¹, the early-successional *H. popayanensis* with 8.2 µmol CO₂ m⁻² s⁻¹, and the late-successional *C. estrellensis* with 3.6 µmol CO₂ m⁻² s⁻¹ (Table 3).

Table 3

Net CO₂ assimilation of adult *B. brizantha*, *H. popayanensis*, and *C. estrellensis* grown without CMN influence, predominant morphotype of arbuscules and percentage of mycorrhizal colonization. Means followed by the same letter in the lines do not differ from each other by the Tukey test at 95% ($p < 0.05$). The standard error is inside the parentheses and CV indicates coefficient of variation.

	<i>B. brizantha</i>	<i>H. popayanensis</i>	<i>C. estrellensis</i>	CV (%)
Net CO ₂ assimilation (µmol CO ₂ m ⁻² s ⁻¹)	11,81 a (1,87)	8,18 b (0,59)	3,58 c (0,46)	20,87
Arbuscule morphotype	<i>Arum</i>	<i>Arum-Paris</i>	<i>Paris</i>	-
Mycorrhizal colonization (%)	91,67 a	72,67 b	41,33 c	6,98

Root dry mass (RDM), shoot dry mass (SDM), root:shoot ratio (R:S), and relative growth rate (RGR) of seedlings of *B. brizantha*, *H. popayanensis*, and *C. estrellensis* grown under the influence of CMN originated from adult *B. brizantha* are

shown in Table 4. Seedlings of *B. brizantha* and *C. estrellensis* without CMN showed higher RDM, SDM, R:S, and RGR than seedlings that grew connected to the CMN. CMN suppressed *B. brizantha* seedlings by 92.6 times for RDM and 41 times for SDM. For *C. estrellensis* seedlings the suppression was 10.5 times for RDM and 10.7 times for SDM. Regarding interferences (pruning *B. brizantha* and severing CMN) the values are shown in Table 5. The seedlings of *C. estrellensis* that grew connected to the CMN of pruned *B. brizantha* and to the CMN severed showed higher RDM and SDM than the seedlings connected to the CMN originated from adult *B. brizantha*, except for the R:S in which only the seedlings that grew with the CMN severed showed higher values and for the RGR that did not differ among treatments.

Table 4

Root dry mass (RDM), shoot dry mass (SDM), root:shoot ratio (R:S) and relative growth rate (RGR) of seedlings of *B. brizantha*, *H. popayanensis*, and *C. estrellensis* grown under the influence of the common mycorrhizal network (CMN) originated from adult *B. brizantha* (+ CMN) or without CMN (- CMN). Means followed by the same letter on the line do not differ from each other by Student's *t* test at 95% ($p < 0.05$). The standard error is in parentheses and † indicates the death of the seedling.

	<i>B. brizantha</i>		<i>H. popayanensis</i>		<i>C. estrellensis</i>	
	- CMN	+ CMN	- CMN	+ CMN	- CMN	+ CMN
RMD (g)	4,63 a (0,46)	0,05 b (0,01)	2,26 (0,07)	†	3,26 a (0,26)	0,31 b (0,26)
SDM (g)	3,28 a (0,34)	0,08 b (0,01)	2,39 (0,08)	†	2,89 a (0,07)	0,27 b (0,04)
R:S	1,44 a (0,25)	0,63 b (0,08)	0,97 (0,06)	†	1,13 a (0,10)	1,10 a (0,09)
RGR (mg g ⁻¹ d ⁻¹)	32,96 a (0,00)	14,32 b (0,00)	0,04 (0,00)	†	0,02 a (0,00)	0,01 b (0,00)

Table 5

Root dry mass (RDM), shoot dry mass (SDM), root:shoot ratio (R:S) and relative growth rate (RGR) of *H. popayanensis* and *C. estrellensis* grown under influence of the common mycorrhizal network (CMN) originated from adult *B. brizantha* (+ CMN), *B. brizantha* pruned (*Bb* pruned), and with the CMN severed (CMN severed). Means followed by the same letter on the line do not differ from each other by Student's *t* test for *H. popayanensis* or by Tukey's test for *C. estrellensis* at 95% ($p < 0.05$). The standard error is in parentheses, CV indicates coefficient of variation, and † indicates seedling death.

	<i>H. popayanensis</i>			<i>C. estrellensis</i>			
	<i>Bb</i> pruned	CMN severed	+ CMN	<i>Bb</i> pruned	CMN severed	+ CMN	CV (%)
RDM (g)	1,13 a (0,10)	0,90 a (0,04)	†	1,78 a (0,33)	2,12 a (0,18)	0,31 b (0,26)	34,91
SDM (g)	1,46 a (0,19)	1,04 a (0,05)	†	1,53 a (0,22)	1,32 a (0,10)	0,27 b (0,04)	30,24
R:S	0,80 a (0,07)	0,87 a (0,08)	†	1,16 b (0,13)	1,61 a (0,08)	1,10 b (0,09)	17,02
RGR (mg g ⁻¹ d ⁻¹)	0,02 a (0,00)	0,02 a (0,00)	†	0,01 a (0,00)	0,01 a (0,00)	0,01 a (0,00)	15,75

Discussion

The four hypotheses tested in this study were accepted, corroborating the reciprocal reward mechanism acting in the CMN. The suppression exerted via CMN originated from adult *B. brizantha* on the seedlings of the three studied species can be explained according to the reciprocal reward mechanism. This mechanism occurs between interconnected plants and AMF, whereas the plant that supplies the greatest amount of carbon to the CMN receives in exchange greater amounts of nutrients (Lekberg et al. 2010; Kiers et al. 2011; Merrild et al. 2013; Wyatt et al. 2014). Thus, a preference in the flow of nutrients was established for plants that have higher photosynthetic rates, to the detriment of those with lower rates.

In this study, the adult *B. brizantha* grass showed the highest rate of net CO₂ assimilation and, therefore, could produce and supply more carbohydrates to the CMN than the seedlings of the *B. brizantha* species itself. These seedlings were limited in receiving nutrients while connected to the CMN originated from adult *B. brizantha*. As a result, they presented smaller shoots, and thus produced and supplied smaller amounts of carbohydrates for CMN. The suppression action was also intense on the seedlings of the early-successional tree *H. popayanensis* and the late-successional tree *C. estrellensis*. These plants also present lower net CO₂ assimilation than adult *B. brizantha* and, thus produced less carbohydrates, contributing to a lower carbon supply to the CMN. The greater flow of nutrients via CMN towards the adult *B. brizantha* and not to the seedlings may have been the main reason for the extremely low values of root and shoot dry mass in *B. brizantha* and *C. estrellensis* seedlings, as well as the death of *H. popayanensis* seedlings. The limitation of nutrient transfer to these seedlings causes lower metabolic activity, lower photosynthetic rate and lower formation of plant tissue (Fernandes et al. 2019). Therefore, the establishment of the reciprocal reward mechanism limited the seedlings to receive nutrients by the CMN, which was evidenced by the suppression in the growth and development of the seedlings. The preference of AMF forming the CMN to direct the flow of nutrients to plants that provide more carbon has been reported in several studies (Janoušková et al. 2011; Hammer et al. 2011; Merrild et al. 2013; Zangaro et al. 2013; Zangaro et al. 2018).

The preference in the allocation of resources among individuals connected to the CMN may also be related to the amount and morphotype of arbuscules (nutrient exchange structure) formed by the AMF in the cortical cells of the roots, whereas the greater amount of carbohydrates in the roots of plants induces the formation of arbuscules by AMF (Kiers et al. 2011). Lekberg et al. (2010) found twice as many arbuscules in the roots of plants that provided more carbon to the CMN while receiving up to ten times more phosphorus than plants that provided less carbon to the network. In the present study, it was observed that adult *B. brizantha* showed root colonization with a high presence of arbuscules (not quantified) in the cortex of the fine roots, which may have allowed greater transfer of carbon to the fungi in the CMN to receive in exchange greater amounts of nutrients. On the other hand, a lower presence of arbuscules was observed in the roots of seedlings of the three species studied, suggesting that plants with lower carbohydrate supply capacity may also have fewer arbuscules in their cortical cells, resulting in a less efficient exchange of carbon for nutrients (Wyatt et al. 2014).

The arbuscle morphotype formed in the cells of the root cortex also influences the exchange of substances between the host plants and the mycorrhizal fungi that form the CMN (Lekberg et al. 2010). The formations of the *Arum*-type are composed of finer structures and with more branches, being more efficient in the exchange of substances between the AMF and the host plant. On the other hand, formations of the *Paris*-type are composed of thicker structures with fewer branches, being less efficient in the exchange of substances between the AMF and the host plant (Ahlu et al. 2005). In this study, the fine roots microscopic observations revealed that the control seedlings (without CMN) of *B. brizantha* presented the predominance of the formation of structures of the *Arum*-type, the early-successional tree seedlings *H. popayanensis* presented both morphotypes in similar amounts, and in the late-successional tree *C. estrellensis* predominated the formation of the *Paris*-type. In this sense, the arbuscle morphotype formed by the AMF inside the

cortical cells of the seedlings can also influence the suppression capacity and the survival of seedlings of *B. brizantha*, *H. popayanensis*, and *C. estrellensis*, when connected to the CMN originated from adult *B. brizantha*.

Regarding the tree species, only the seedlings of the early-successional *H. popayanensis* died when grown connected to the CMN originated from adult *B. brizantha*, while the seedlings of the late-successional *C. estrellensis* survived. In addition to the arbuscule morphotype produced, the difference in the survival of the two tree species may also be related to differences in colonization intensity and mycorrhizal responsiveness, whereas the early-successional species has greater colonization intensity and greater responsiveness to AMF (Zangaro et al. 2003). Furthermore, Zangaro et al. (2018) also found that tree species from the late-successional phase survived the competitive effects of CMN originated from adult grass, which they attributed to the typically longer life span of late-successional species, to the morphological characteristics of fine roots (larger diameter and few branches) and the low intensity of mycorrhizal colonization, which allows less interference by AMF or CMN. In turn, the death of individuals of the early-successional tree species *H. popayanensis* may have been due to their typically shorter life span and high mycorrhizal colonization, which increased the amount of AMF hyphae that could connect to the CMN controlled by adult *B. brizantha*. Furthermore, when the effect of competition via CMN was excluded (control treatment), *H. popayanensis* seedlings showed higher growth rates and mycorrhizal colonization.

The higher competitive response with *B. brizantha* connected to the CMN was more evident in the seedlings of the late-successional tree *C. estrellensis* than in the seedlings of the other two species. This may be due to the morphological characteristics of the radicle cells of *C. estrellensis*, which are thick, little branched and have low mycorrhizal colonization, which implies a smaller number of hyphae that could connect to the CMN originated from adult *B. brizantha*. In addition, the late-successional species *C. estrellensis* has lower mycorrhizal responsiveness when compared to the grass *B. brizantha* and the early-successional *H. popayanensis* (Zangaro et al. 2013). Seedlings of *B. brizantha* and *H. popayanensis* have fine roots with morphological characteristics that provide greater colonization and greater mycorrhizal responsiveness and, therefore, may be more affected by CMN originated from adult *B. brizantha* (Zangaro et al. 2018; Rondina et al. 2019).

The greater amount of nutrients that adult *B. brizantha* received from the CMN may also be related to the greater efficiency in the absorption of nutrients from the soil that these plants have compared to the seedlings connected to the CMN. Weremijewicz et al. (2016) found that roots of slow-growing plants have low absorption efficiency, while in adult plants, under favorable light conditions and with a high metabolic rate, they have high absorption efficiency and can influence the direction of nutrient flow in the CMN. In addition, the CMN preference for certain plant species may be related to the supply of manganese by AMF to plants that have high rates of carbon fixation, whereas manganese is essential for the manganic complex of photosystem II, therefore crucial for photosynthesis (Yachandra et al. 1996). This way, carbohydrate production is increased and greater amounts of carbon can be made available to CMN fungi, which reciprocate with greater amounts of manganese and other soil nutrients (Kiers et al. 2011; Weremijewicz and Janos 2013).

Interferences (pruning *B. brizantha* and severing CMN) increased the survival and growth of individuals of the early-successional tree species *H. popayanensis*, whereas, 225 after growth, the seedlings that were connected to the CMN originated from adult *B. brizantha* died. This early-successional tree showed greater growth, greater number of leaves, greater leaf area, and greater nutrient content when the *B. brizantha* was pruned than when the seedlings had their CMN severed. The pruning of adult *B. brizantha* may have caused a decrease in the contribution of carbon to the CMN and, thus, the seedlings of *H. popayanensis* became the main carbon suppliers for the fungi in the network. Thereby, the seedling of *H. popayanensis* acquired a larger area of soil exploration and obtained more nutrients because it may have temporarily used all the CMN originated from the grass in its favor, until the regrowth of the adult *B. brizantha*. In contrast, the *H. popayanensis* seedlings that had the CMN severed showed lower growth, probably because they were restricted to exploring only the soil contained within the polyethylene cylinder. After regrowth of the adult *B. brizantha* shoot, the

growth of *H. popayanensis* seedlings connected to the CMN was suppressed again. However, the suppression was interrupted one more time and the seedlings started to grow once again when the second pruning of the adult *B. brizantha* took place. After the second regrowth of the grass, the seedlings were suppressed again. Similar results occurred with individuals of the late-successional species *C. estrellensis* after the two pruning of the adult *B. brizantha*. These results are in line with the reciprocal reward mechanism (Lekberg et al. 2010; Kiers et al. 2011) and demonstrate that CMN increases intra and interspecific competition and behaves asymmetrically in the distribution of nutrients between symbiosis partners (Janos et al. 2013).

Malcová et al. (2001) also found beneficial effects promoted by CMN originated from the adult grass *Calamagrostis epigejos* with the shoot pruned in the establishment and growth of seedlings of the same species. Likewise, Pietikäinen and Kytöviita (2007) studied different levels of shoot limitation of adult plants and found that the greater the loss of shoot, the greater the growth of seedlings of the same species connected to the CMN, showing increased biomass and greater nitrogen uptake.

Invasive grasses are the main responsible for the mortality of seedlings of tree species in areas of ecological restoration, which often invade and compete with native species (Silveira et al. 2013). Based on the results of this study, the suppression and death of seedlings of native tree species that grow in restoration areas in the presence of *B. brizantha* can be mitigated by constant pruning of this grass. Furthermore, the constant pruning of the suppressor grass can become a very important strategy to accelerate the growth of tree species seedlings in restoration areas. It is expected that after the pruning of the adult *B. brizantha*, the seedlings of the tree species will temporarily become the main carbon suppliers for the CMN. During this period, the CMN can be fully available for use by seedlings of tree species to expand their area of absorption of water and nutrients from the soil.

Conclusion

The CMN originated from adult exotic grass *B. brizantha* negatively affected the growth and development of the seedlings of *B. brizantha* and the tree species *H. popayanensis* and *C. estrellensis*, which is in agreement with the first hypothesis of this study. Physiological characteristics and favorable environmental conditions give *B. brizantha* high carbohydrate production, which contributes large amounts of carbon to CMN fungi. In this way, the flow of nutrients from the network is directed to the grass and, at the same time, makes the seedlings connected to the CMN limited in terms of the supply of nutrients, reducing their growth.

The CMN originated from adult *B. brizantha* caused the death of seedlings of the early-successional tree species *H. popayanensis*, while the seedlings of the late-successional tree species *C. estrellensis* and *B. brizantha* itself survived, but with reduced growth rates.

The CMN severed originated from the adult *B. brizantha* totally eliminated the suppressive effects on the seedlings connected to the network, validating the second hypothesis.

The pruning of the adult *B. brizantha* mitigated the suppressive effects on the seedlings of the tree species, however, with the regrowth and the reestablishment of the shoot, the adult plants of *B. brizantha* were back to receiving the flow of nutrients from the CMN and suppressing the seedlings connected to the network, which validates the third and fourth hypotheses.

Declarations

Ethical Approval

This declaration is “not applicable”.

Competing interests

This manuscript has no conflict of interest.

Authors' contributions

BGPB and WZ conducted the experiments, performed the laboratory analyzes, and wrote the manuscript. ACF and JAP corrected and assisted in writing the manuscript

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Availability of data and materials

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Figures

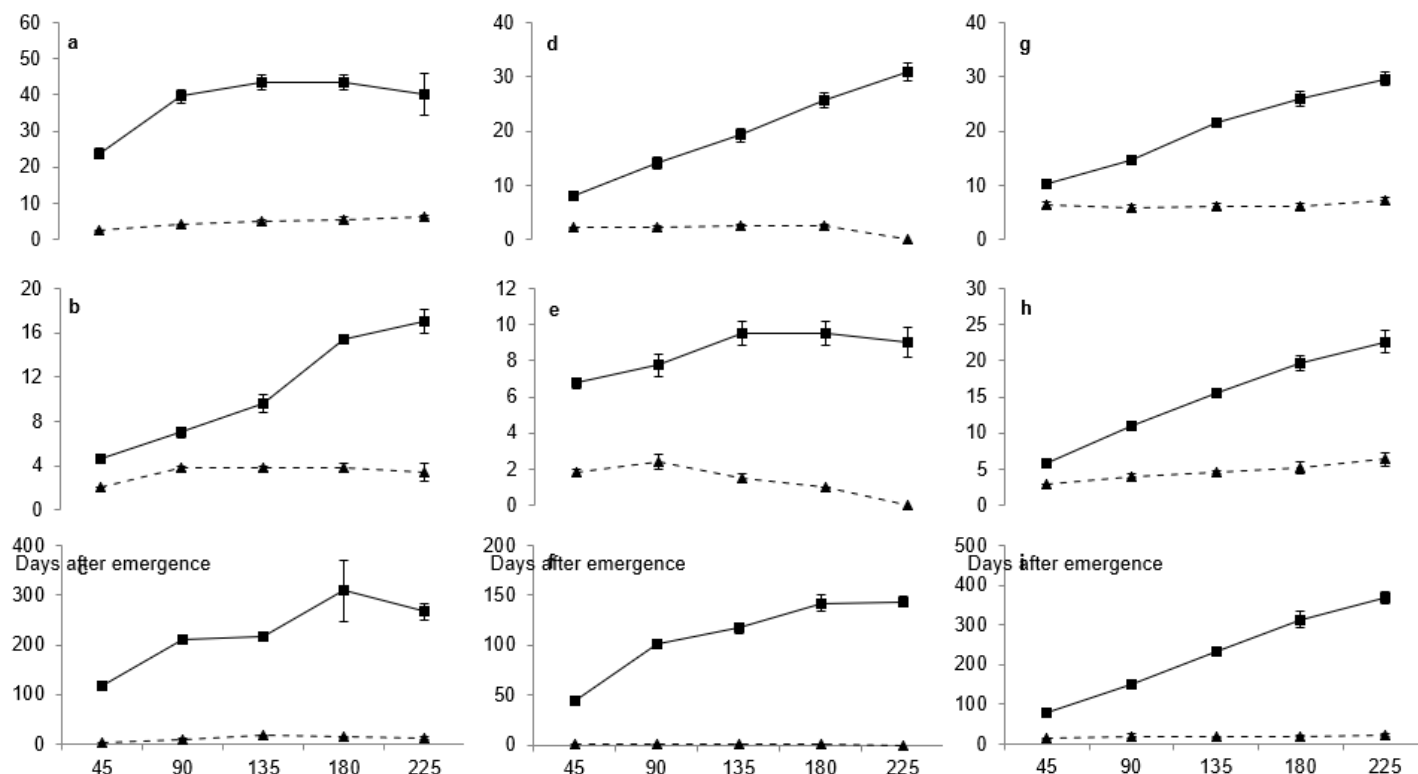


Fig 1. Height, number of leaves, and leaf area of *B. brizantha*, *H. popayanensis*, and *C. estrellensis* grown for 225 days under the influence of the common mycorrhizal network (CMN) originated from adult *B. brizantha* (---▲---) or without CMN (—■—). Means followed by the same letter, on the same day after emergence, did not differ from each other by Student's *t* test at 95% ($p < 0.05$). Bars indicate standard error and † indicates seedling death.

Figure 1

See image above for figure legend

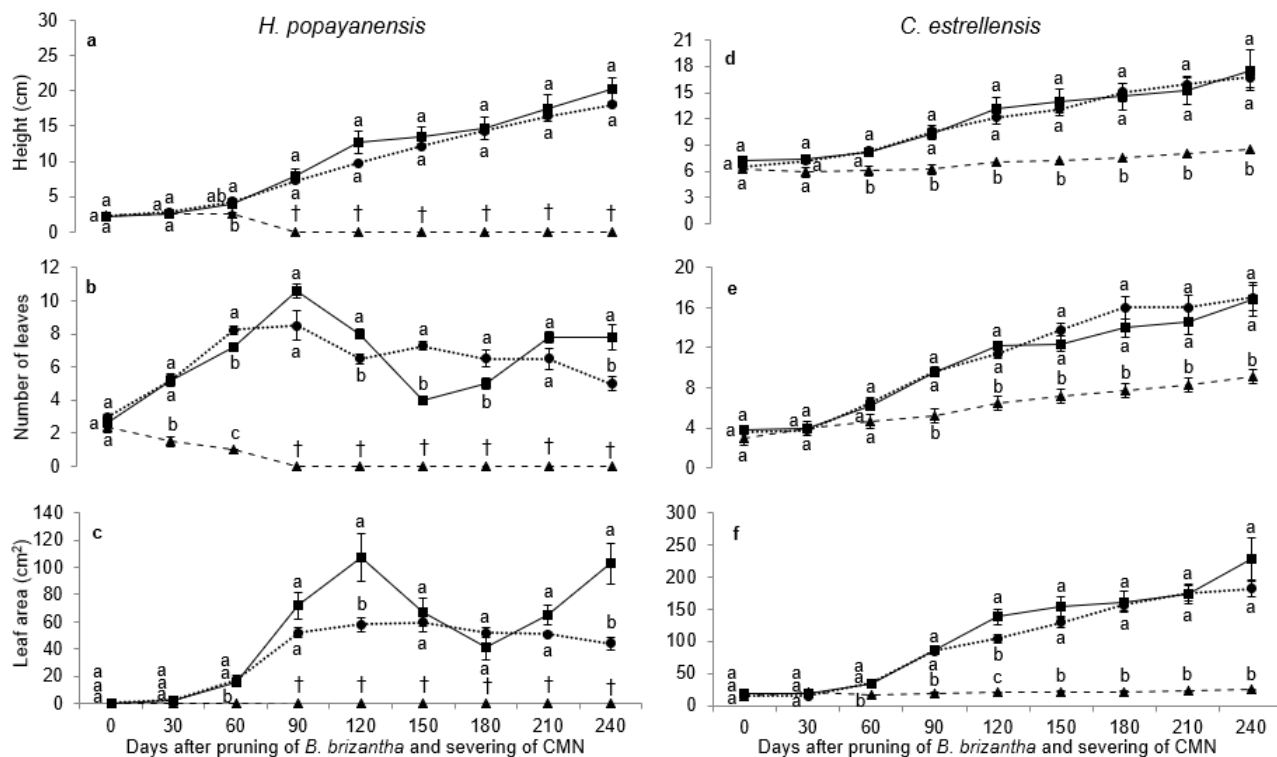


Fig 2. Height, number of leaves, and leaf area of *H. popayanensis* and *C. estrellensis* grown under the influence of the common mycorrhizal network (CMN) originated from adult *B. brizantha* (---▲---), from *B. brizantha* pruned (—■—), and with the CMN severed (····●····). Means followed by the same letter, on the same day after pruning of *B. brizantha* and severing of CMN, did not differ from each other by Tukey's test at 95% ($p < 0.05$). Bars indicate standard error and † indicates seedling death. The severing of CMN occurred weekly 90 days after the seedling emergence and pruning of *B. brizantha* occurred at 90 and 270 days (days 0 and 180 of interference) after seedling emergence.

Figure 2

See image above for figure legend

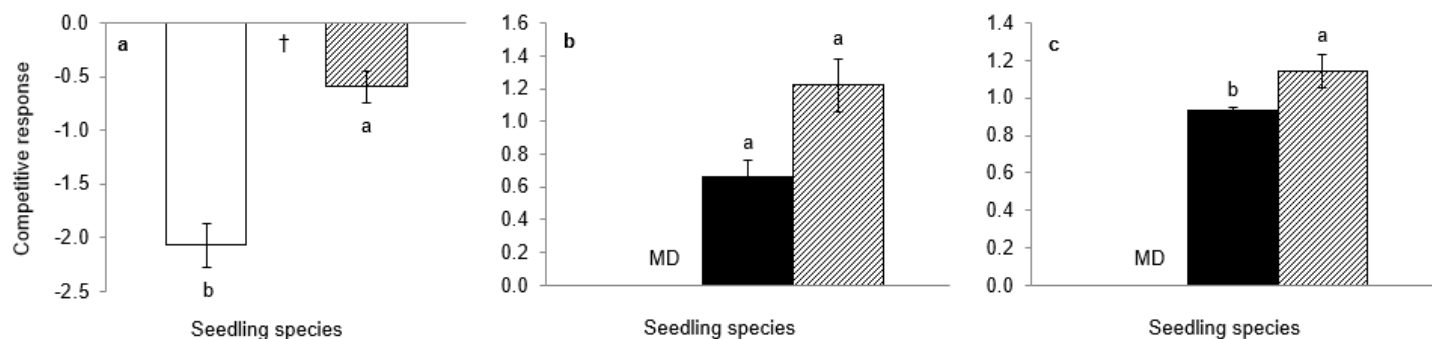


Fig 3. Competitive response of *B. brizantha* (□), *H. popayanenses* (■), and *C. estrellensis* (▨) grown under the influence of the common mycorrhizal network (CMN) originated from adult *B. brizantha* (A), *B. brizantha* pruned (B), and with the CMN severed (C). Means followed by the same letter did not differ from each other by Student's *t* test at 95% ($p < 0.05$). Bars indicate standard error, MD indicates missing data, and † indicates seedling death.

Figure 3

See image above for figure legend