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Light and plant growth-promoting rhizobacteria effects on *Brachiaria brizantha* growth and phenotypic plasticity to shade

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Abstract

This is the first report on the effect of light intensity and plant growth-promoting rhizobacteria (PGPR) on the growth of a tropical forage grass, being a relevant study to improve pasture management in conventional farming and integrated crop-livestock-forestry systems. In this study, our aim was to evaluate the effects of light intensity and *Burkholderia pyrrocinia* and *Pseudomonas fluorescens* inoculation on *Brachiaria brizantha* cv. BRS Piatã growth, and phenotypic plasticity to shade. The experiment was conducted in a semi-controlled environment. Seedlings of *B. brizantha* were allocated to full sun and shade. *P. fluorescens* and *B. pyrrocinia* were inoculated individually or co-inoculated by soil drench, 14 days after seedling emergence. We evaluated morphogenesis, structural and growth parameters. Irrespective of the light regime, co-inoculated plants had greater leaf area and SPAD index (chlorophyll content). Increase in total biomass production in co-inoculated plants was over 100% and 300%, under full sun and shade respectively. Co-inoculated *P. fluorescens* and *B. pyrrocinia* increased shade tolerance in *B. brizantha*, improving plant performance. Co-inoculation promoted growth in *B. brizantha* under both sun and shade, indicating its potential as a bio-fertilizer in conventional and integrated systems, especially in silvopastoral systems, where light availability to pasture growth may be limited.

KEYWORDS
Burkholderia pyrrocinia, forage grass, *Pseudomonas fluorescens*, shade

1 | INTRODUCTION

Integrated crop-livestock-forestry systems are efficient land-management alternatives for restoring degraded pastures, increasing food security and promoting carbon sequestration (Dias-Filho, 2011; Moraes, Carvalho, Lustosa, Lang, & Deiss, 2014; Paciullo et al., 2017; Santos et al., 2016). However, as the forage grass species planted in integrated systems may be subjected to light restriction, imposed by agricultural crops or tree species, it is necessary to know their agronomic performance under shade, to define their potential use in these systems (Dias-Filho, 2000; Gomez, Guenni, & Bravo De Guenni, 2012; Paciullo et al., 2011; Pimentel et al., 2016).

Phenotypic plasticity relates to the species adaptability to changes in the environment, such as variations in solar radiation, temperature, soil water and nutrient availability (Gomez et al., 2012; Valladares, Laanisto, Niinemets, & Zavala, 2016). Shade tolerant plants can adjust their morphophysiology, such as biomass allocation pattern, chlorophyll content and leaf area and thickness to maximize light capture (Paciullo et al., 2017; Valladares et al., 2016).

In conventional and integrated systems, forage grass production is usually limited by low soil fertility (Paciullo et al., 2011, 2017; Pimentel et al., 2016). The use of plant growth-promoting rhizobacteria (PGPR) could be an alternative for reducing the use of chemical fertilizers, with clear environmental and economic benefits (Nadeem, Ahmad, Zahir, Javaid, & Ashraf, 2014; Paredes & Lebeis, 2016). The beneficial effect of PGPR on forage grasses has been reported for *Azospirilum brasilense* on *B. briazantha* (Hungria, Nogueira, & Araujo, 2016), and *Azotobacter*, *Azospirillum* and *Herbaspirillum* on *Axonopus affinis*, *Paspalum notatum*, *Andropogon lateralis* and *Aristida laevis* (Marques et al., 2017).

Beneficial micro-organisms are known to improve nutrient uptake, phosphorus solubilization, phytohormone production and disease resistance by elicited induced systemic resistance or systemic acquired resistance. Also, beneficial micro-organisms modify the phenotypic plasticity of plants, by mitigating the negative impact of abiotic stresses (Goh, Vallejos, Nicotra, & Mathesius, 2013; Paredes & Lebeis, 2016; Vacheron et al., 2013; Vimal, Singh, Arora, & Singh, 2017), including light limitation (Konvalinková & Jansa, 2016).

Previous studies with *Pseudomonas* sp. and *Burkolderia* sp. have attested their growth-promoting potential in rice (Nascente et al., 2016; Rêgo, Borges, Filippi, Gonçalves, & Silva, 2014) and *Brachiaria brizantha* (Lopes et al., unpublished). These rhizobacteria are known to increase the auxin synthesis, nutrient uptake (e.g., nitrogen, phosphorus and iron), chlorophyll content, photosynthetic rate and biomass production (Ahemad & Kibret, 2014; Nascente et al., 2016).

Although light is known to interfere in mycorrhiza–plant mutualism (Aguilar-Chama & Guevara, 2016; Konvalinková & Jansa, 2016; Saner et al., 2011), we could not find any published study on the effect of light intensity and use of rhizobacteria on the growth and phenotypic plasticity of a tropical forage grass species.

In Brazil, *Brachiaria* is the most important grass genus for pasture formation (Reis, Bernardes, & Siqueira, 2013; Santana et al., 2017; Santos et al., 2016), within which stands out *B. brizantha* (Hochst ex. A. Rich.) cv. BRS Piatã (Reis et al., 2013). We hypothesized that *Burkhoderia pyrrocinia* and *Pseudomonas fluorescens* inoculation can increase *B. brizantha* growth under limited light conditions, by increasing the shade tolerance of this grass cultivar. In this study, our aim was to evaluate the effects of light intensity and *B. pyrrocinia* and *P. fluorescens* inoculation on the growth, phenotypic plasticity and shade tolerance of *B. brizantha* cv. BRS Piatã.

2 | MATERIALS AND METHODS

2.1 | Study site, plant material and inoculant

The experiment was conducted in a semi-controlled environment in the nursery seedling production unit of the Federal Rural University of Amazonia (UFRA) (01°27′25″S, 48°26′36″W) in Belém, Pará, Brazil. The regional climate here according to Koppen classification is Af (equatorial). During the experimental period, the mean air temperature and relative humidity were 32 ± 2.8°C and 73 ± 3% (mean ± *SD*) respectively.

Seeds of *B. brizantha* cv. BRS Piatã were sown in polyethylene pots (15 × 25 × 0.5 cm) filled with soil (Ferralsol—topsoil from a second growth forest—pH, 4.2; organic matter, 18.80 g/dm^3; P, 2 mg/dm^3; K, 4 mg/dm^3; Ca, 0.2 mmolc/dm^3; Ca + Mg, 0.3 mmolc/dm^3; Al, 1.4 mmolc/dm^3) and kept under greenhouse conditions. Plants were grown under full sun and artificial shade (0% and 47% of shade, or 2,100 μmolm^{-2} s^{-1} and 1,113 μmolm^{-2} s^{-1}, at canopy height, measured at 11 a.m., local time, on a cloudless day) obtained with black polyethylene screens (solar radiation transmissivity of ca. 50%) placed 160 cm above the canopy height.

We used *P. fluorescens* (BRM-32111) and *B. pyrrocinia* (BRM-32113), supplied by the Plant Protection Laboratory in vitro collection of the Federal University of Amazonia, and originally selected from the rhizosphere of rice plants. The rhizobacteria were cultured in Petri dishes with a solid culture medium 523 (agar, casein hydrolysate, magnesium sulphate anhydrous, potassium phosphate monobasic, sucrose and yeast extract) (Kado & Heskett, 1970) and incubated for 48 hr at 28°C. The bacterial suspension was prepared in sterile water and adjusted to 550 nm (10^8 CFU). Soil-drenched inoculation was carried out 14 days after seedling emergence; each pot received a 5 ml bacterial suspension.

2.2 | Plant growth parameters

The following morphogenetic and structural parameters were calculated according to Gomide and Gomide (2000): leaf appearance rate (ratio between the difference in the number of initial and final leaves and the number of days of the evaluation interval, LApR), leaf elongation rate (ratio between the difference of the initial and final lengths of the expanded laminae and the number of days of the evaluation interval, LER), culm elongation rate (ratio between the difference of the initial and final lengths of culm and the number of days of the evaluation interval, CER), number of leaves per plant (NL) and number of tillers per plant (NT). We also determined plant height (H) and culm length (CL). In addition, we estimated the chlorophyll content by the SPAD index (SPAD-502, Konica Minolta Sensing, INC., Japan), which was measured in the youngest fully expanded leaf blade (n = 5).

At 35 days after seedling emergence, five plants per treatment were harvested and separated into shoot (leaf blades and culms) and roots. Plant material was oven dried (60°C) until constant mass. Total dry mass (TDM) was calculated by adding shoot dry mass (SDM) and root dry mass (RDM). The biomass allocation pattern was estimated as the leaf, culm and root mass ratios (respectively, the ratio between total leaf, culm, and root dry mass per plant and total dry mass per plant).

Specific leaf area (SLA, the ratio of leaf area to leaf dry mass) was determined over leaf discs of either 0.42 cm^2 or 2.28 cm^2, dried at 60°C until constant mass. Total leaf area per plant was estimated from SLA and leaf mass results. We also calculated the root dry mass/shoot dry mass ratio (RDM/SDM) and leaf area ratio (ratio of leaf area per total dry mass per plant, LAR). Relative growth rate (change in total mass per total dry mass of plant per day, RGR) was calculated for harvests at 14 and 35 days after seedling emergence. The allometric coefficient K was calculated as the ratio of the growth rate of root and shoot. All plant growth parameters were calculated according to Hunt (1990) and Barbero et al. (2013).

2.3 | Statistical analyses

The experimental design was completely randomized in a 4 × 2 factorial arrangements (non-inoculated *B. brizantha*, inoculated with BRM-32111, inoculated with BRM-32113 and co-inoculated

(BRM-32111 + BRM-3213) × full sun and shade), with five replicates. Data were subjected to analysis of variance and, when appropriate, the means were compared by Duncan's test at 5%. The assumption of homogeneity of variances and normality was tested for each ANOVA and, when necessary, data were log-, or square-root-transformed. Transformed values were back-transformed for presentation. Statistical analyses were performed by the statistical package STATISTICA (StatSoft, Inc., Tulsa, OK, USA).

3 | RESULTS

Co-inoculation promoted growth of *B. brizantha* under both full sun and in the shade (Figures 1, 2, 4 and 5; Tables 1 and 2). On the other hand, when the bacteria were inoculated individually, plants under full sun showed the highest growth with *P. fluorescens* (BRM-32111), and with *B. pyrrocinia* (BRM-32113), when grown under shade (Figures 1, 2 and 5).

Plant height ($F_{3,32}$ = 38.05; p < .001) under co-inoculation increased by 28% and 65%, under full sun and shade, respectively, relative to non-inoculated plants (Figure 2). The highest leaf appearance ($F_{3,32}$ = 11.64; p < .001) and elongation rates ($F_{3,32}$ = 57.14; p < .001), culm elongation rate ($F_{3,32}$ = 24.97; p < .001), number of leaves ($F_{3,32}$ = 43.02; p < .001) and leaf length ($F_{3,32}$ = 5.98; p < .05) occurred in co-inoculated plants, regardless of light regime (Table 1).

Tillering was observed only in plants grown under full sun, being 50% higher in co-inoculated plants (Table 1). The increase in the culm length and in the leaf and culm elongation rates, in response to shade, was higher in co-inoculated plants (Table 1). Higher values of specific leaf area (SLA) ($F_{3,32}$ = 245.64; p < .001), and leaf area ratio (LAR) ($F_{3,32}$ = 347.52; p < .001), were obtained in non-inoculated plants, in the shade. Under full sun, the highest SLA and LAR values were found in plants inoculated with BRM-32113 (Figure 3).

Co-inoculated plants had a 130% larger leaf area ($F_{3,32}$ = 206.43; p < .05), under full sun and a 200% larger leaf area shade (Figure 4). The SPAD index ($F_{3,32}$ = 6.18; p < .05) was higher in co-inoculated plants, increasing by 13% full sun and by 17% shade.

Co-inoculation increased biomass production ($F_{3,32}$ = 616.61; p < .001), irrespective of the light intensity (Figure 5). Under full sun, co-inoculation increased leaf (139%), culm (86%) and root (88%) biomass, resulting in an increase of over 100%, in total biomass relative to non-inoculated plants (Table 2; Figure 5). Under shade, co-inoculation increased leaf (300%), culm (500%) and root (670%) biomass, resulting in an increase of about 300%, in total biomass, relative to non-inoculated plants (Table 2; Figure 5).

Co-inoculation also increased relative growth rate ($F_{3,32}$ = 119.97; p < .001) by 110%, under full sun, and 500%, in shaded plants (Figure 5). Shaded plants showed higher values of root to shoot biomass ratio ($F_{3,32}$ = 42.71; p < .05) and allometric index (K) ($F_{3,32}$ = 57.16; p < .0001), particularly when co-inoculated (Table 2). Under shade, inoculation favoured an increased biomass allocation to roots (Figure 6). Under full sun, co-inoculated plants allocated proportional amounts of biomass between leaves and roots (Figure 6).

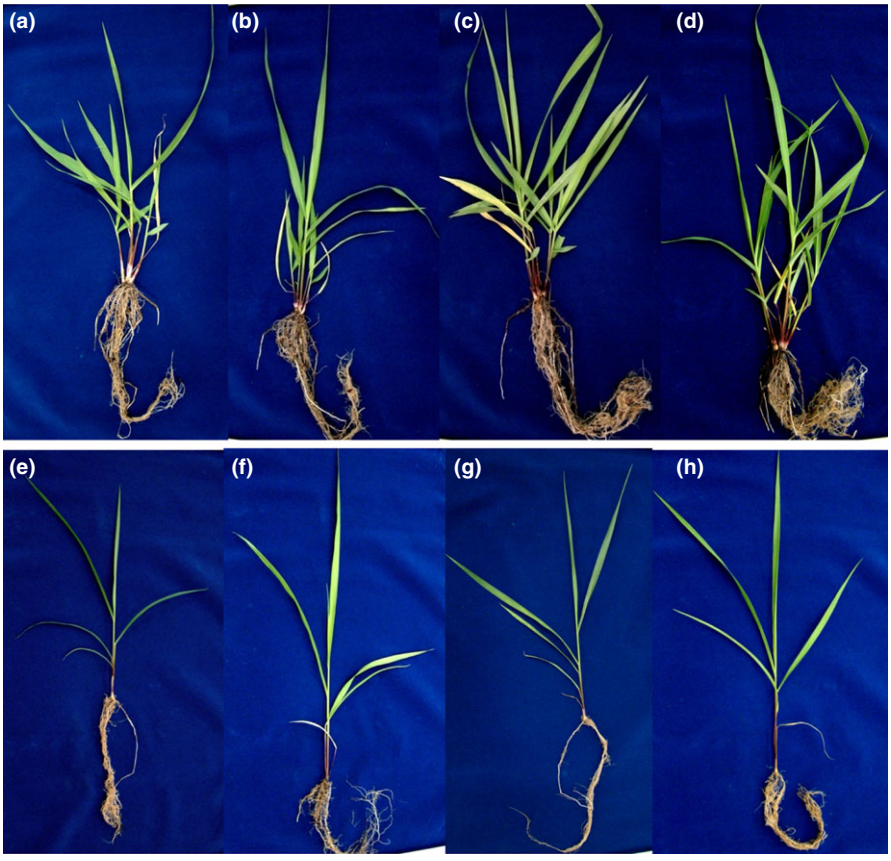

FIGURE 1 Plants of *Brachiaria brizantha* cv. Piatã under full sun (a–d) and shade (e–h). 21 days after inoculation (35 days after seedling emergence). Non-inoculated plant (a, e), inoculated with *Pseudomonas fluorescens* (BRM-32111) (b, f), inoculated with *Burkholderia pyrrocinia* (BRM-32113) (c, g) and co-inoculated with BRM-32111 + BRM-32113 (d, h) [Colour figure can be viewed at wileyonlinelibrary.com]

Treatments	NT	NL	EF	C	LApR	LER	CER
Full sun							
Non-inoculated	2 c	11 d	24.83 d	10.80 c	0.37 c	0.88 c	0.43 b
BRM-32111	2 c	13 c	27.88 c	11.75 b	0.45 b	0.84 d	0.42 b
BRM-32113	2 b	16 b	33.6 b	12.05 a	0.45 b	0.96 b	0.44 b
MIX	3 a	17 a	36.5 a	12.01 a	0.51 a	1.32 a	0.60 a
Shade							
Non-inoculated	0	5 d	21.91 c	10.35 d	0.10 c	0.61 c	0.26 d
BRM-32111	0	6 b	27.77 b	13.83 b	0.20 b	1.45 b	0.63 b
BRM-32113	0	5 c	28.2 b	11.66 c	0.18 b	1.41 b	0.56 c
MIX	0	6 a	34.07 a	15.60 a	0.3 a	2.05 a	0.88 a

Means followed by different letters in each column and within each light intensity are significantly different ($p < .05$, Duncan's test). BRM-32111 = *Pseudomonas fluorescens*; BRM-32113 = *Burkholderia pyrrocinia*; MIX = BRM-32111 + BRM-32113.

TABLE 1 Effect of light intensity and plant growth-promoting rhizobacteria (PGPR) on number of tillers (NT), number of leaves per plant (NL), expanded leaf length (EF—cm), culm length (*C*—cm), leaf appearance rate (LApR—$L^{-}.day^{-1}$), leaf elongation rate (LER—$L^{-}.day^{-1}$) and culm elongation rate (CER—$L^{-}.day^{-1}$) of *Brachiaria brizantha*

TABLE 2 Effect of light intensity and plant growth-promoting rhizobacteria (PGPR) on the leaf (LDM—g), culm (CDM—g), root (RDM—g) and root/shoot dry mass production, and allometric coefficient *K* (ratio of the growth rate of root and shoot) of *Brachiaria brizantha*

Treatments	LDM	CDM	RDM	Root/Shoot	K
Full sun					
Non-inoculated	0.80 c	0.35 c	1.01 c	0.88 a	0.91 a
BRM-32111	0.92 c	0.28 d	1.01 c	0.85 a	0.87 a
BRM - 32113	1.32 b	0.42 b	1.08 b	0.63 c	0.64 c
MIX	1.91 a	0.65 a	1.90 a	0.75 b	0.76 b
Shade					
Non-inoculated	0.11 d	0.04 c	0.10 d	0.43 c	0.48 c
BRM-32111	0.29 b	0.14 b	0.37 b	0.90 c	0.96 b
BRM-32113	0.20 c	0.10 c	0.23 c	0.83 c	0.91 b
MIX	0.44 a	0.24 a	0.77 a	1.16 a	1.21 a

Means followed by different letters in each column and within each light intensity are significantly different ($p < .05$, Duncan's test). BRM-3211 = *Pseudomonas fluorescens*; BRM-3213 = *Burkholderia pyrrocinia*; MIX = BRM-3211 + BRM-3213.

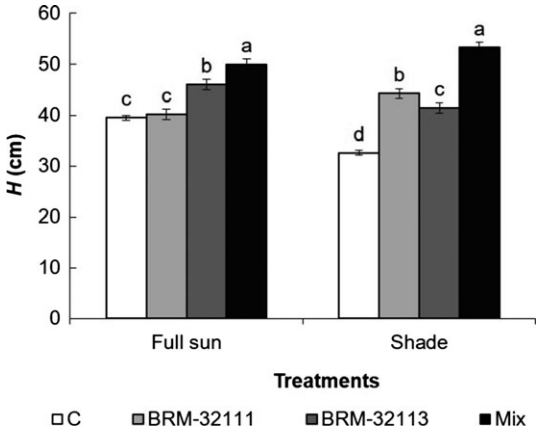

FIGURE 2 Effect of light intensity and plant growth-promoting rhizobacteria (PGPR) on plant height (H) of *Brachiaria brizantha*. Columns with different letters within each light condition denote significant differences ($p < .05$, Duncan's test) among PGPR inoculation treatments. Mean ± *SE* ($n = 5$). C = Non-inoculated; BRM-32111 = *Pseudomonas fluorescens*; BRM-32113 = *Burkholderia pyrrocinia*; MIX = BRM-32111 + BRM-32113

4 | DISCUSSION

When inoculation was performed individually, the benefit varied with the type of bacteria and light intensity. Growth of plants inoculated only with *B. pyrrocinia* was positively related to high light intensities. A possible cause for this response was a likely higher demand in photoassimilates for the symbiosis of this micro-organism (Aguilar-Chama & Guevara, 2016; Konvalinková & Jansa, 2016). A contrasting response occurred in plants inoculated only with *P. fluorescens*, which fostered higher growth mainly under restricted light intensities.

It could be inferred that the higher radiation intensity might have modified the quantity and chemical composition of root exudates, affecting rhizodeposition processes and disturbing rhizosphere functioning (Haichar et al., 2008; Venturi & Keel, 2016; Vimal et al., 2017), especially in *P. fluorescens*. This could have interfered in the quorum sensing, inhibiting the mutual interaction under full sun (Goh et al., 2013; Venturi & Keel, 2016). This response supports the evidence that light can interfere with mutualistic interaction, which in turn varies with hosts and beneficial micro-organisms (Konvalinková & Jansa, 2016). Under limited light conditions, microbial root symbionts can create additive costs, resulting in decreased plant fitness, as observed in *Vatica albiramis* (Saner et al., 2011) and in Datura stramonium (Aguilar-Chama & Guevara, 2016), where growth promotion is positively related to light intensity.

When co-inoculated, *B. pyrrocinia* and *P. fluorescens* promoted growth of *B. brizantha*, irrespective of light intensity. The rhizosphere microbiota is known to extend the adaptive capacity of plants (i.e., phenotypic plasticity) to environmental stresses (Venturi & Keel, 2016). The overall beneficial effect of the interaction between micro-organisms and plant growth, under contrasting light environments, has also been reported in studies with mycorrhizal growth promoters (Aguilar-Chama & Guevara, 2016; Casierra-Posada, Peña-Olmos, Peñaloza, & Roveda, 2013; Konvalinková & Jansa, 2016).

For instance, in rice, from which the rhizobacteria used in this study were originally isolated, growth is higher with *B. pyrrocinia*

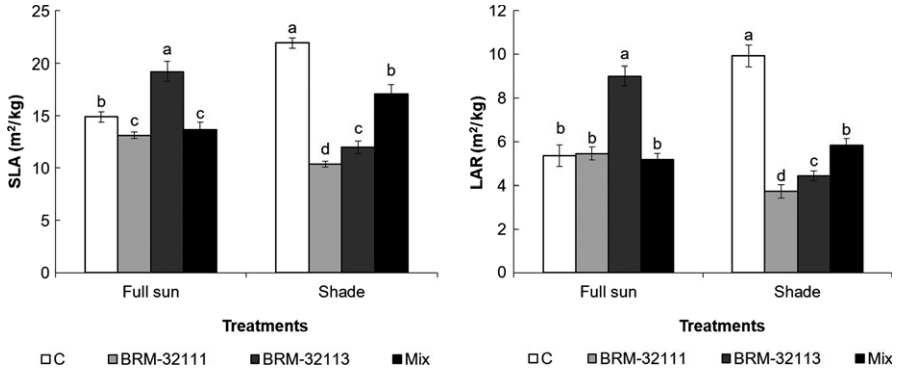

FIGURE 3 Effect of light intensity and plant growth-promoting rhizobacteria (PGPR) on specific leaf area (SLA) and leaf area ratio (LAR) of *Brachiaria brizantha*. Columns with different letters within each light condition denote significant differences ($p < .05$, Duncan's test) among PGPR inoculation treatments. Mean ± *SE* ($n = 5$). C = Non-inoculated; BRM-32111 = *Pseudomonas fluorescens*; BRM-32113 = *Burkholderia pyrrocinia*; MIX = BRM-32111 + BRM-32113

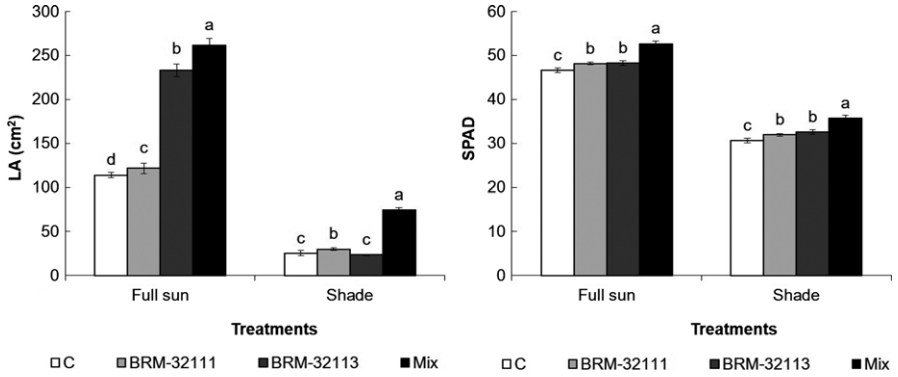

FIGURE 4 Effect of light intensity and plant growth-promoting rhizobacteria (PGPR) on leaf area (LA) and SPAD index (chlorophyll content) of *Brachiaria brizantha*. Columns with different letters within each light condition denote significant differences ($p < .05$, Duncan's test) among PGPR inoculation treatments. Mean ± *SE* ($n = 5$). C = Non-inoculated; BRM-32111 = *Pseudomonas fluorescens*; BRM-32113 = *Burkholderia pyrrocinia*; MIX = BRM-32111 + BRM-32113

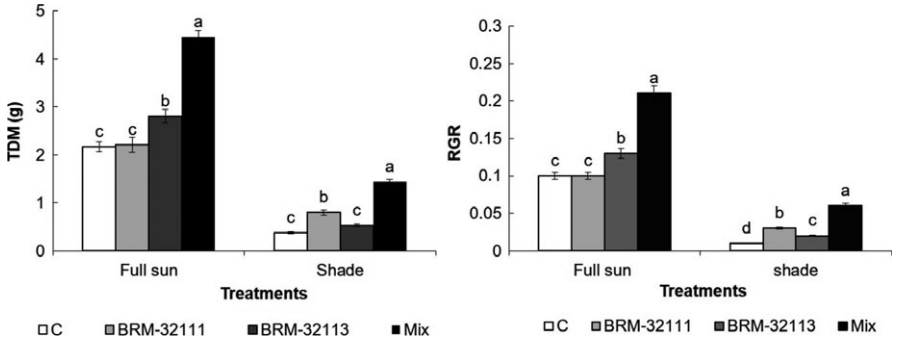

FIGURE 5 Effect of light intensity and plant growth-promoting rhizobacteria (PGPR) on total dry mass production (TDM) and relative growth rate (RGR) of *Brachiaria brizantha*. Columns with different letters within each light condition denote significant differences ($p < .05$, Duncan's test) among PGPR inoculation treatments. Mean ± *SE* ($n = 5$). C = Non-inoculated; BRM-32111 = *Pseudomonas fluorescens*; BRM-32113 = *Burkholderia pyrrocinia*; MIX = BRM-32111 + BRM-32113

inoculation than with *P. fluorescens* inoculation (Nascente et al., 2016; Rêgo et al., 2014), or with co-inoculation with these rhizobacteria (Rêgo et al., 2014). The synergistic effect of *B. pyrrocinia* and *P. fluorescens*, promoting growth in *B. brizantha*, was possibly a response of increased nutrient flow (Casierra-Posada et al., 2013; Paredes & Lebeis, 2016), phytohormone production, such as auxin or cytokinin, or decreased plant ethylene levels, through the action of the enzyme ACC deaminase (Ahemad & Kibret, 2014; Vimal et al., 2017).

It can be inferred that co-inoculation increased phenotypic plasticity in various key traits as a strategy to mitigate the effect of shade in *B. brizantha*. This was achieved by increasing plant height, culm and leaf elongation (i.e., etiolation) (Goh et al., 2013; Martins et al., 2014; Valladares et al., 2016), hence increasing total plant biomass. As tree

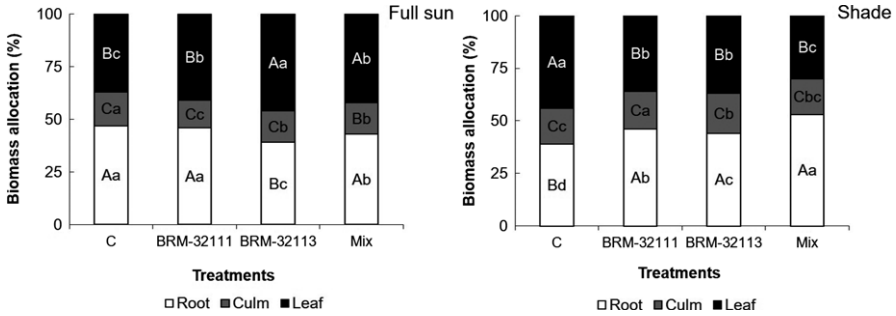

FIGURE 6 Effect of light intensity and plant growth-promoting rhizobacteria (PGPR) on biomass allocation pattern of *Brachiaria brizantha*. At each light condition, columns with different lower-case letters are significantly different among treatments ($p < .05$, Duncan's test). Different upper-case letters within columns indicate significant differences among plant organs ($p < .05$, Duncan's test). Mean ± *SE* ($n = 5$). BRM-32111 = *Pseudomonas fluorescens*; BRM-32113 = *Burkholderia pyrrocinia*; MIX = BRM-32111 + BRM-32113

shading (Santos et al., 2016) and crop shading (Neves Neto, Santos, Alexandrino, & Santos, 2015) are known to reduce forage production in *B. brizantha* cv. BRS Piatã pastures, it could be inferred that co-inoculation would be a suitable strategy to counteract limited pasture growth in integrated systems. Beneficial bacteria also mitigated the deleterious effects of drought stress in sorghum, wheat and maize plants (Ngumbi & Kloepper, 2016), and also salt stress in wheat plants (Arshadullah, Hyder, Mahmood, Sultan, & Naveed, 2017), promoting increased shoot length, dry biomass and chlorophyll content.

Specific leaf area (SLA) and leaf area ratio (LAR) vary with light, temperature, moisture and nutrient availability (Barbero et al., 2013; Daniagry and Dang, 2014). Increased SLA and LAR under low light are a strategy to improve light capture in *B. brizantha* (Dias-Filho, 2000), and this response was observed in non-inoculated plants in our study. Our results also indicate that the ability of *B. brizantha* to modify SLA and LAR under low light can be influenced by plant growth-promoting rhizobacteria. A possible cause for this response is the potential ability of the rhizobacteria to supply the needed soil nutrient resources for shaded *B. brizantha*. This is attained through increased root growth, making it unnecessary for the shaded plants to increase SLA and LAR to those levels observed in uninoculated plants. This indirect effect could make inoculated plants more tolerant to additional stresses such as grazing, increasing pasture performance and productivity (Pimentel et al., 2016).

Considering that the greater the SPAD index, the higher the chlorophyll and nitrogen content of *B. brizantha* leaves (Lima, Nascente, Leandro, & Silveira, 2016; Martuscello, Jank, Gontijo Neto, Laura, & Cunha, 2009), presumably co-inoculated rhizobacteria also acted synergistically to increase the nitrogen content of leaves. Under restricted sunlight, a high light capture capacity is required for C fixation, and this was achieved in shaded plants by increasing leaf area and chlorophyll content with co-inoculation.

The general increase in root biomass and biomass allocation to roots with co-inoculation observed in this study may increase the water and nutrient use efficiency under full-sun and shade conditions. Therefore, the observed responses of co-inoculated plants would be useful to increase the fitness of *B. brizantha* in water-limited environments. In grazed pastures, resource-use efficiency ensures the persistence of plants over time and longevity of pastures (Dias-Filho, 2000; Martuscello et al., 2009; Pimentel et al., 2016). Also, the increased relative growth rate, tillering, leaf area and biomass production in co-inoculated plants would be a desirable trait in intensively managed systems, such as rotational grazing systems, reducing grazing intervals.

In conclusion, our results attest the potential of co-inoculated *P. fluorescens* and *B. pyrrocinia* to increase biomass production in *B. brizantha* and the expression of those plant characters that may enhance persistence under reduced light availability. In addition, these findings set up the basis for additional exploratory studies, particularly on the ability of this beneficial interaction to remain fully active under grazing, in integrated crop-livestock-forestry systems.

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CONFLICT OF INTEREST

None.

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