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CROP PRODUCTION

Inoculation with *Azospirillum brasilense* on corn yield and yield components in an integrated crop-livestock system

Paulo Eugênio Schaefer[1], Thomas Newton Martin[1*], Rodrigo Pizzani[2] and Elton Luiz Schaefer[1]

[1]Departamento de Fitotecnia, Universidade Federal de Santa Maria, Avenida Roraima, 1000, Cidade Universitária, 97105-900, Camobi, Santa Maria, Rio Grande do Sul, Brazil. [2]Sociedade Educacional Três de Maio, Três de Maio, Rio Grande do Sul, Brazil. *Author for correspondence. E-mail: martin.ufsm@gmail.com

ABSTRACT. Inoculation of corn with diazotrophic bacteria reduces the need for nitrogen fertilization and mitigates environmental contamination risks due to the bacteria's biological nitrogen-fixation capacity. The aim of the present study was to evaluate the effect of corn seed inoculation with *Azospirillum brasilense* under different nitrogen levels and post-grazing residual heights. The experiment was performed in two growing seasons and conducted in an integrated crop-livestock system for the 2014/15 and 2015/16. A factorial randomized block experimental design with sub-divided plots and three factors. The main plots varied in post-grazing residual height (0.10, 0.20, 0.30 m, continuous grazing, or no grazing), the subplots varied in inoculation (with or without seed inoculation), and the sub-subplots varied in nitrogen level (0, 75, 150, 225, or 300 kg ha^{-1} of N). The higher post-grazing residual height associated an *A. brasilense* and nitrogen fertilization resulted in increased corn biomass and production and yield. At the 300 kg dose of N, the highest grain yield was obtained under different post-grazing heights (10.15 Mg ha^{-1}) and in the absence of the bacterium (10.00 Mg ha^{-1}). *Azospirillum brasilense* helps plant growth and yield but does not replace the effect of N fertilization.

Keywords: forage; N fertilization; diazotrophic bacteria.

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Introduction

Corn (*Zea mays* L.) is the most produced cereal worldwide, ahead of important commodities such as wheat, rice, and soybean. A total 959.79 million tons of corn were produced in the 2015/16 harvest, with 70 million tons produced in Brazil, making Brazil the world's third largest corn producer (USDA, 2016). This production is associated with the high demand for corn for human and animal food, especially for birds, cattle, and pigs (Purwanto & Minardi, 2015).

The need to increase grain yield and production has led to the development of new technologies that constitute alternatives for grain production, such as integrated crop-livestock systems (ICL). These systems combine the production of grains, such as corn, with pastures, taking advantage of their mutual benefits (Sandini et al., 2011). The addition of large amounts of plant residues to the soil surface improves the physicochemical (Mendonça et al., 2013) and biological soil quality (Santos, Fontaneli, Spera, & Dreon, 2011).

The need to increase production has led to an increased use of nitrogen (N) fertilization because most of the soils present low N concentrations and do not meet plant growth demands (Spera, Santos, Fontaneli, & Tomm, 2009). However, the excessive use of N fertilizers, in addition to increasing production costs, has detrimental effects on the environment due to nitrate leaching into water courses (Walker et al., 2011) and volatilizations losses. The use of biological N fixation (BNF) aims to decrease the costs of using chemical nitrogen fertilizers, mitigate environmental impacts, and achieve higher plant growth and production gains (Filgueiras & Meneses, 2015).

The selection of bacteria more efficient for BNF (De-Bashan, Hernandez, & Bashan, 2012) and with bacterial characteristics such as the production of plant-growth-promoting or nutrient-solubilizing substances make diazotrophic bacteria an essential alternative for use in association with grasses such as corn (Hungria Campo, Souza, & Pedrosa, 2010). Bacteria from the genus *Azospirillum* are associated with several plant species, including corn (Piccinin et al., 2011), and have been observed to increase the plant production capacity between 12% and 14% (Kuss, Kuss, Lovato, & Flôres, 2007) and up to 30%, in the case of

A. brasilense, in grain yield or to reduce the need to use nitrogen fertilization on the order of 20 to 35% in relation to the non-inoculated control (Hungria et al., 2010). Plant characteristics other than productivity, such as plant height, stem diameter, chlorophyll index, stem and root dry weight (Okon & Vanderleyden, 1997), and ear length (Costa et al., 2015), are also affected by diazotrophic bacteria.

In Brazil, studies of the use of Azospirillum bacteria and N fertilization in corn crops in ICL are few or still without results. The use of Azospirillum bacteria in corn crops presents limitations, especially due to the inconsistency of the results, which vary depending on the cultivar, edaphoclimatic conditions, and experimental methods used (Bartchechen, Fiori, Watanabe, & Guarido, 2010). To clarify some results obtained for this system, the aim of the present study was to evaluate corn agronomical performance as a function of seed treatment with A. brasilense and different levels of N fertilization in an integrated crop-livestock system.

Material and methods

The experiment was conducted during the 2014/2015 and 2015/2016 harvests in the municipality of Mata, Rio Grande do Sul State (RS), Brazil. The study area is located at latitude 29°34'07"S and longitude 54°27'29"W and at an altitude of 103 m. The region's climate is Cfa (subtropical) (Peel, Finlayson, & McMahon, 2007). Rainfall during the experimental period was recorded using a field rain gauge (Figure 1).

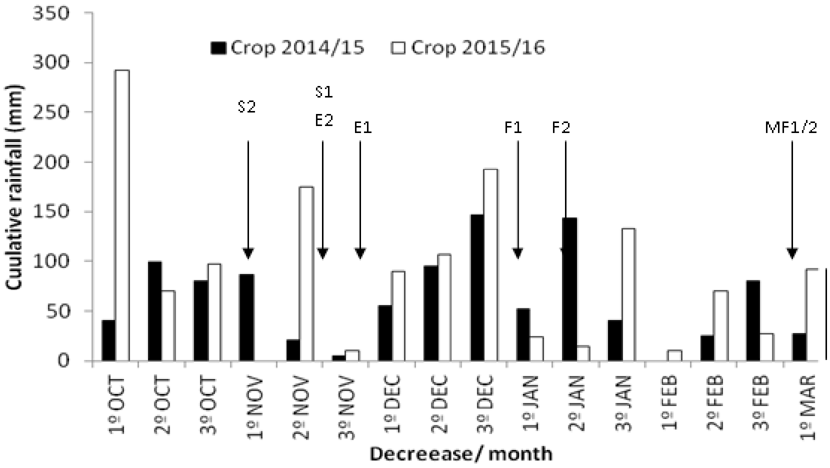

Figure 1. Cumulative rainfall for the 2014/15 and 2015/16 harvests. Mata, 2016. S: sowing, E: emergence, F: flowering and MF: physiological maturation. Season 1: 2014/15 and season 2: 2015/16.

The soil in the study area is classified as sandy Red Dystrophic Argisol (EMBRAPA, 2013) and has been cultivated under a no-till integrated crop-livestock system (ICL) since 2009, with corn (*Zea mays* L.) cultivation during the summer, in succession with black oat (*Avena strigosa*) and Italian ryegrass (*Lolium multiflorum* Lam.) in intercropping (100 kg ha^{-1} and 25 kg ha^{-1} of viable seeds, respectively), sown by broadcasting and incorporated into the soil by soft harrowing.

A factorial randomized block experimental design with subplots and three replicates per treatment was used. The treatments were arranged in a 5 x 2 x 5 factorial design. The factors evaluated were post-grazing residual height (winter), inoculation with *A. brasilense*, and N level. The main plots consisted of five post-grazing residual heights: 0.10 m (M-10); 0.20 m (M-20), 0.30 m (M-30), continuous grazing (CG; free-grazing area of 500 m^2), and a control treatment without grazing (NG). Subplots consisted of seed inoculation with *A. brasilense*. Inoculation was performed using *A. brasilense* strains AbV5 and AbV6, at 2.0 x 10^8 CFU mL^{-1} ("AzoTotal"- liquid) and 300 mL per 60,000 seeds. Sub-subplots consisted of five N levels: 0, 75, 150, 225, and 300 kg ha^{-1} of N. Twenty percent of the total N dose for each treatment was applied at sowing, and the remainder was applied as topdressing. The aimed productivity was 12 Mg ha^{-1}. Each sub-subplot consisted of fifteen 3-m long rows. All evaluations were performed in the central rows, and plants were collected along a 2-m line within each subplot.

Corn cultivar "DEKALB 240" VT PRO 2 was used. Sowing was performed with 45-cm spacing, on the 15th of November, 2014 and the 3rd of November, 2015. When the plants reached stage V1 (one expanded leaf) (Ritchie, Hanway, & Benson, 1993), thinning was performed to a final density of 66,000 plants ha^{-1}. Maintenance fertilization was performed on the day of sowing, according to the expected productivity, with applications of 0, 15, 30, 45, or 60 kg ha^{-1} of N and 350 kg ha^{-1} of NPK 0-23-30. Topdressing fertilization was performed according to the N levels for each treatment, in the sowing rows and divided by two applications, when plants reached stages V3 - V4 and V7 - V8 according to the scale of Ritchie et al. (1993). Urea was used as the N source.

Different winter-pasture, post-grazing, residual heights were established through grazing by lactating Jersey cows of 350 kg average body weight. Grazed plots were 14-m wide and 15-m long. Three grazing events were performed for each harvest, the first beginning when an average 1.5 Mg ha^{-1} dry weight (DW) was reached. Animal withdrawal was determined depending on the intended post-grazing height, using the sward stick method, adapted from Barthram (1985).

Collections were performed manually when the grain moisture was between 20 and 25%, from two rows randomly selected in each experimental unit. Weight of 1,000 grains (WTG; g), grain yield (GY; Mg ha^{-1}), and harvest index (HI; %), i.e., ratio of dry matter production of grains to total dry matter production of the plant (less roots), were determined. All corn plants along 2 m of each selected row were collected and placed in a forced air oven at 65°C for 72 hours, and the shoot DW was determined.

The evaluated parameters were subject to the assumptions of the mathematical model. Analysis of variance was conducted using the F test with $p \leq 0.05$. When significant differences were found, averages were compared using the Scott-Knott test for qualitative factors or using polynomial regression analysis up to the third order for quantitative factors. Preliminary analyses were performed to ensure that the assumptions of each test were not violated. All analyses were performed using the SISVAR software (Ferreira, 2011).

Results and discussion

The effect of the factors tested (post-grazing residual height, inoculation with *A. brasilense*, and N level) on the variables analyzed was similar for the two harvests, with a significant three-way interaction between the factors. The values presented in the figures are therefore the overall averages for the two harvests: 2014/2015 and 2015/2016.

For the quantitative analysis of dry weight accumulation under the different N levels (Figure 2a) in the 2014/15 harvest, treatment M30 with 300 kg N ha^{-1} was 6% higher than the remaining treatments (20.32 Mg DW). This may be due to the improvement of physicochemical soil conditions and nutrient cycling because forage dry weight accumulation and root growth are higher with low grazing intensity (Barth Neto et al., 2013).

The highest dry weight production for the treatment without grazing occurred with 207 kg N ha^{-1}; this production was 8.8% higher than for the M10, which was the treatment with grazing that presented the highest dry weight at this N level. Grazing of winter forage decreases the soil cover, depending on the grazing intensity (Veiga, Pandolfo, Junior, & Durigon, 2016), which exposes the soil to nutrient losses due to erosion.

For treatments with higher post-grazing residual height, inoculation with N fixing bacteria increased the corn dry weight production with increasing levels of N fertilization (Figure 2b). For the highest N fertilization level (300 kg N), the largest increase in corn DW was observed for NG, followed by M30 (22.30 Mg ha^{-1} of DW), which was 5.7% lower. The high N demand for decomposition of forage residues with a high C/N ratio, together with the high level of N fertilization and BNF (De-Bashan et al., 2012), compensated the plant nitrogen demand for higher leaf and stem growth.

Corn seed inoculation with *A. brasilense* resulted in a 13% increase in the corn DW for the 2014/15 harvest (Figure 2c). This increase was related to N fixation, which met part of the plant N demands; plant hormone production, especially auxins and cytokinins (Hungria, 2011); and solubilization of nutrients such as phosphorus (Moreira, Silva, Nóbrega, & Carvalho, 2010) by the diazotrophic bacteria.

Significant differences in corn biomass production were observed among the different post-grazing heights, with production being highest for NG (18.5 Mg ha^{-1}) and lowest for CG (16.6 Mg ha^{-1}) (Figure 2c). A decrease of 10% in the corn biomass production was therefore observed for pastures subjected to intense grazing without control of the stocking rate. This decrease is related to higher nutrient export during the grazing period, lower soil cover, and lower nutrient cycling during corn establishment.

The effect of N fertilization on the corn shoot biomass production for the two harvests evaluated is presented in Figure 2d. For both harvests, shoot biomass production was best fitted by a quadratic equation but was more uniform for the 2014/15 harvest, varying almost linearly with the increasing N level (Figure 2d). For the 2015/16 harvest, the shoot biomass accumulation increased starting at 75 kg ha^{-1} of N, reaching the highest values with 280 kg ha^{-1} of N. The different responses observed for the two harvests were related to the different climate conditions during the two harvests, with higher rainfall occurring during the leaf and stem formation stage for the second harvest (Figure 1). In addition, soil moisture is essential for plant N uptake (Fageria, 1998).

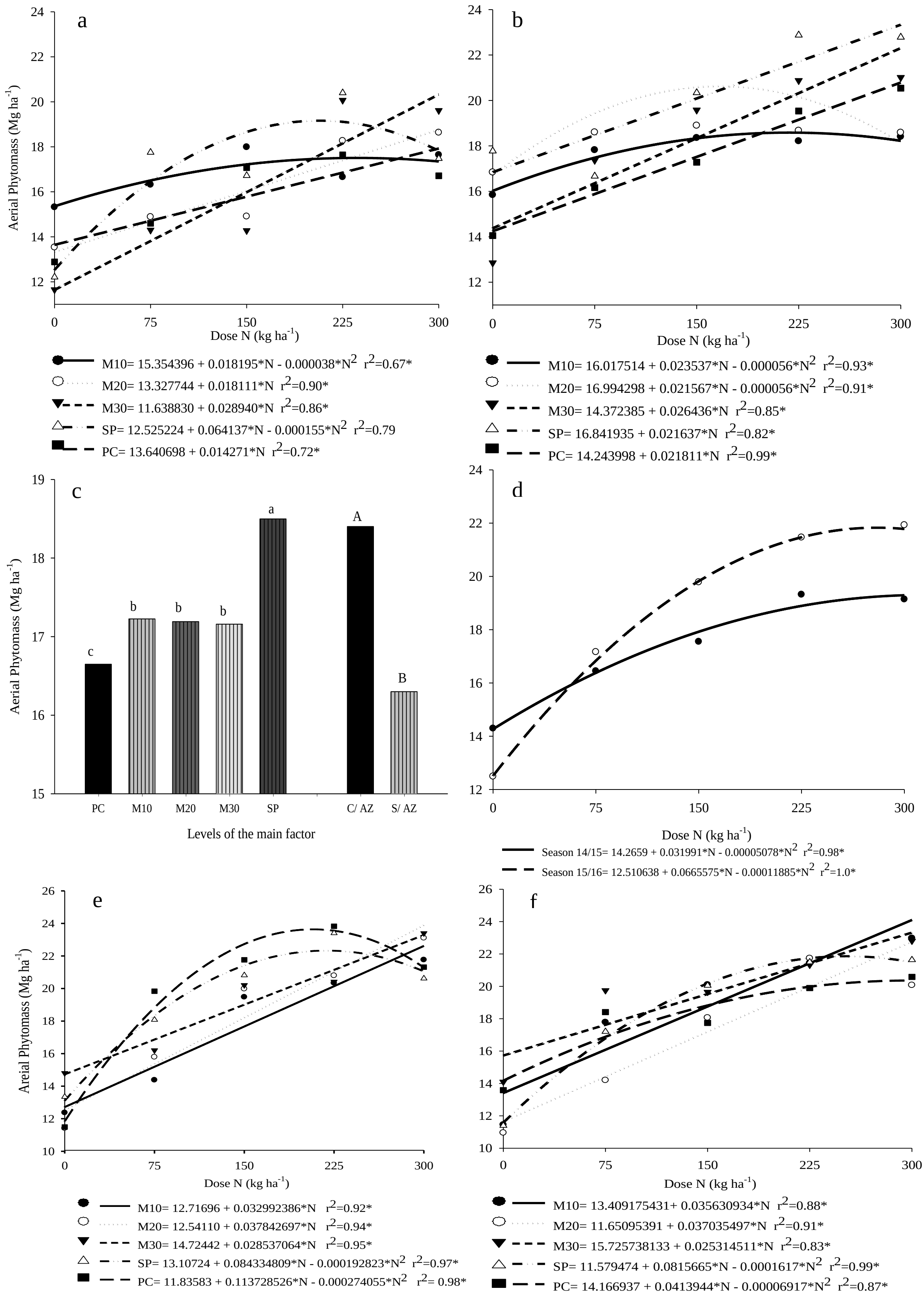

Figure 2. Aerial phytomass production of corn crops for the 2014/15 season without (a) and with inoculation of *A. brasilense* (b); main isolated factors (residual height and inoculation of seeds [c], nitrogen dose [d]); 2015/16 crop without (e) and with inoculation (f) in the ILP system with no-till. M10, post-grazing residual height 0.10 m; M20, residual height 0.2 m; M30, residual height 0.3 m; SP, without grazing; PC, continuous grazing; C/AZ, with seed inoculation; S/AZ, without seed treatment with inoculant. * Distinct letters

(lowercase for residual height and upper case for seed inoculation) indicate significance at 5% probability.

For the second harvest (2015/16), the responses to the N fertilization level for the different post-grazing residual heights were best fitted by quadratic and linear equations (Figure 2e). The highest shoot production for treatment CG was observed with 208 kg N; the highest shoot production for treatment NG was observed with 219 kg N. For the remaining treatments, plant biomass production linearly increased with the increasing N fertilization level. The response of plant biomass production to the amount of N applied was 11.8 to 23.9 Mg ha^{-1}. The response to N fertilization under the different soil cover conditions therefore varied greatly, being very dependent on the edaphoclimatic conditions for each crop year. However, the dry weight production increased with increasing N fertilization levels. This result is due to the close relation of N with plant growth, due to its role in protein synthesis, photosynthesis, respiration, and cell division and differentiation (Okumura, Mariano, & Zaccheo, 2011).

Regarding the effect of *A. brasilense* inoculation at the different N fertilization levels and with the different forage managements for the 2015/16 harvest, the behavior of the treatment NG was best fitted by a quadratic equation, with the highest efficiency being observed for 252 kg N and inoculation with *A. brasilense* (Figure 2f). For the remaining treatments, although a decrease in efficiency was observed for CG, the shoot biomass production increased with the increasing N fertilization level. Shoot biomass production presented the same behavior for both treatments, with an average 11.6 to 24.1 Mg ha^{-1}. For the second harvest, inoculation with *A. brasilense* did not result in increased shoot biomass production when compared to the treatment without seed inoculation, except for M30, for which a 6.8% increase was observed without N fertilization.

Of the yield components, WTG was affected by the tested factors. For the 2014/15 harvest, WTG presented a linear response for M20, M30, NG, and CG, reaching higher values with 300 kg N, and with the highest WTG being observed for NG (284.79 g) (Figure 3a). M10 presented a higher WTG with 241 kg N. Higher straw soil cover and corn dry biomass production may therefore be associated with a higher WTG. Lower water loss via evapotranspiration and higher leaf area contribute to higher photo-assimilation allocations to grain (Yang & Grassini, 2014).

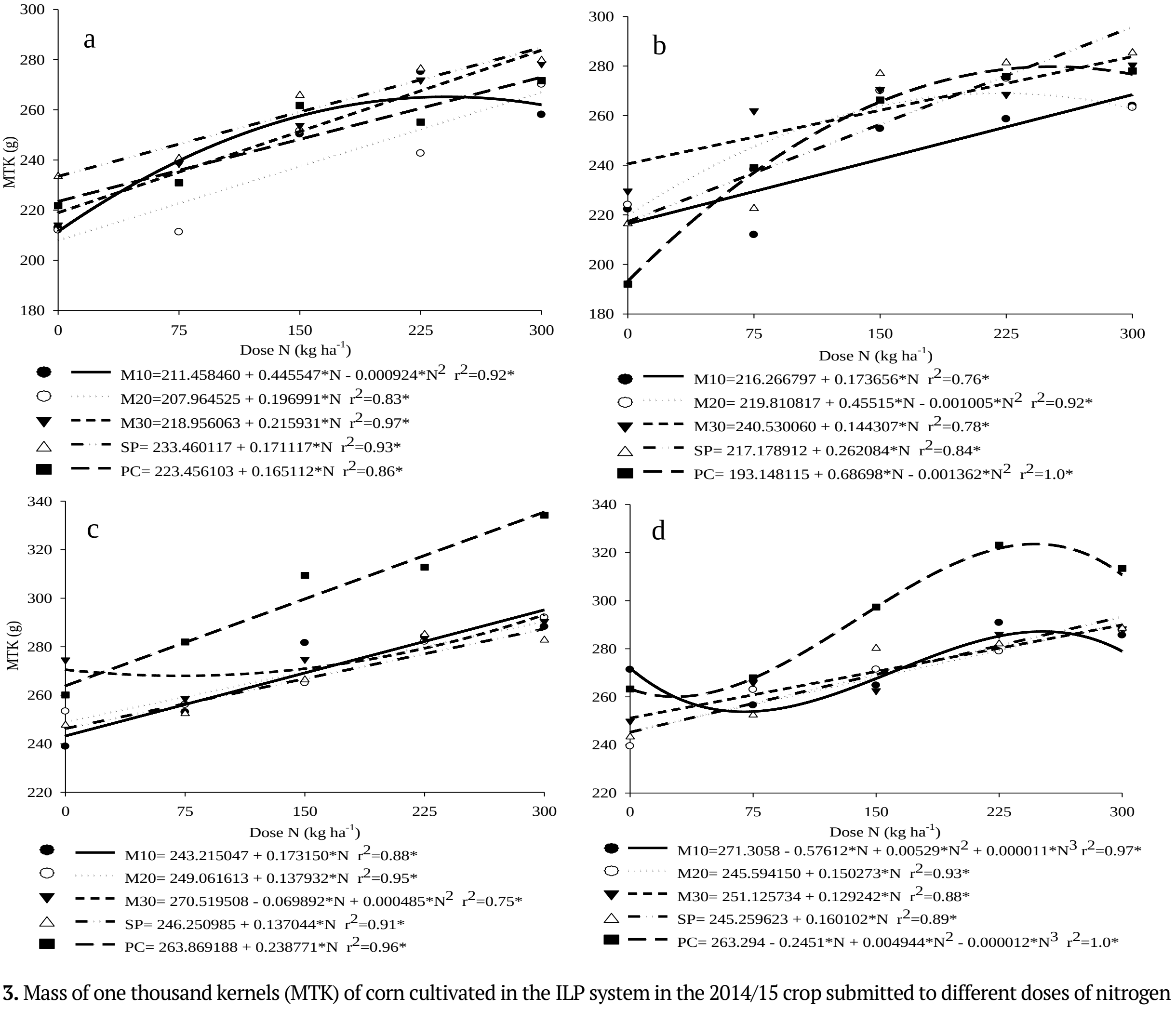

Figure 3. Mass of one thousand kernels (MTK) of corn cultivated in the ILP system in the 2014/15 crop submitted to different doses of nitrogen

fertilization and on different post-grazing heights, without and with seed treatment with *A. brasilense* for the 2014/15 (a, b) and 2015/16 (c, d) seasons.

Regarding the N level x *A. brasilense* interaction, behavior was best fitted by a linear equation for M10, NG, and M30 and by a quadratic equation for treatments M20 and CG. The latter reached their highest efficiency with 221 and 252 kg N, respectively (Figure 3b). Costa et al. (2015) observed that WTG responded linearly and positively to increasing N levels, with or without seed inoculation.

Regarding the post-grazing residual height x N level interaction for the 2015/16 harvest, WTG presented a linear response for both treatments, except for M30, which presented a negative response, with N levels up to 80 kg ha^{-1}, followed by an increased WTG, with higher N levels (Figure 3c). The treatment with lower soil cover, resulting from higher grazing pressure (CG), presented the highest WTG with the different N levels, with an increase of 13.66% being observed with 300 kg N.

With *A. brasilense* inoculation, the highest WTG was also observed for CG with 247 kg ha^{-1} of N, with this WTG being 11.30% higher than for M20 with 300 kg N, which showed the second highest WTG. In addition to CG, M10 was also best fitted by a cubic equation, with lower efficiency observed with low soil cover of 28 and 69 kg N, respectively. For the remaining post-grazing residual heights in the N x *A. brasilense* interaction, a linear response to the increasing N level was observed for NG, M30, and M20 (Figure 3d). The observed increasing WTG with increasing N level agrees with the results of Santos et al. (2011), who also observed increases of 8.1 and 15% with 200 kg N for two harvests.

Significant interactions between factors were also observed for corn grain yield. For the 2014/15 harvest, a significant positive linear relation with the N fertilization level was observed ($p \leq 0.05$; Figure 4a). Treatment M30 presented a higher yield with 176 kg ha^{-1} of N, with a grain yield of 11.20 Mg ha^{-1} for the highest N level. NG presented a higher grain yield from 67 to 175 kg ha^{-1} of N. This may be related to the higher shoot biomass accumulation, number of grains per row, WTG, and level of N fertilization used (Amaral Filho, Fornasieri Filho, Farinelli, & Barbosa, 2005). Regarding the linear equations fitted, Amaral Filho et al. (2005) also observed higher grain yields at N levels higher than 280 kg ha^{-1} of N.

Regarding inoculation with A. brasilense, for the first harvest, the corn yield presented a uniform trend for all treatments, best fitted by quadratic equations (Figure 4b). Treatment M30 presented the highest yield with 300 kg ha^{-1} of N, but yield increases resulting from bacteria inoculation were observed with up to 292 kg ha^{-1} of N and was more pronounced with 139 kg ha^{-1} of N (12.6% higher than without inoculation).

The overall average grain yield for treatments with or without inoculation revealed that inoculation resulted in an increase in grain yield of 613 kg. This was due to BNF and plant hormone synthesis (Filgueiras & Meneses, 2015), which resulted in higher plant growth (root and shoot) and photosynthetic pigments. The present grain yield results agree with Vogt, Balbinot Junior, Galotti, Padolfo, and Zoldan (2014), who also evaluated the application of an *Azospirillum* spp.-based inoculum to corn crops under field conditions and found no consistent positive effect of diazotrophic bacteria on grain yield. The 613 kg increase in yield was reduced by 5.5% with the use of mineral N for the same productivity, that is, an economic gain with the reduced contribution of mineral N.

For the 2015/16 harvest, corn yield increased linearly with the increasing N fertilization level and was highest for the CG with 300 kg ha^{-1} of N, with an increase of 68.67% compared to the highest grain yield without N fertilization (Figure 4c). These results were fitted by the same type of equation as the previous harvest, with a different response being observed only for the factor of residual height. This response may be due to the higher production of photo-assimilates because, due to the edaphoclimatic conditions during the second harvest, CG presented higher shoot biomass accumulation, NFG, NGF, and WTG in response to the different nitrogen levels.

Regarding the N level x *A. brasilense* interaction, the grain yield behavior for treatments M10, M20, and M30 were best fitted by linear equations; the treatments NG and CG were best fitted by quadratic equations. The highest yield observed was 10.11 Mg ha^{-1} of grain for treatment M10 (Figure 4d). The highest technical efficiency was observed with 215 kg ha^{-1} of N for NG and CG, with a grain yield increase of 3.2% compared to the treatment without grazing. Without N fertilization (0 kg ha^{-1} of N), inoculation with *A. brasilense* resulted in a yield increase of 4.5% compared to the treatment without inoculation. This agrees with the results of Lana, Dartora, Marini, and Hann (2012), who observed that inoculation with *A. brasilense* resulted in increases of 7 to 14% in corn grain yield, even without the addition of N.

The harvest index (HI) represents the ratio of harvested grain to total plant shoot dry matter and thus the biological yield and grain yield. It is an indication of the efficiency of the transport of photoassimilates produced in the leaves for the process of grain filling, i.e., the conversion of the partially harvested aboveground biomass or commercialized crop (Martins & Costa, 2003). In the experiment, values ranging from 0.35 to 0.60% were observed. With respect to seed inoculation with *A. brasilense*, this index value was 0.50 and 0.47, respectively, for the absence and presence of inoculant in the 2014/2015 harvest. The lower HI is associated with higher aerial dry matter production in the presence of the bacteria, as can be observed in Figure 2c and compared with grain yield in Figure 4.

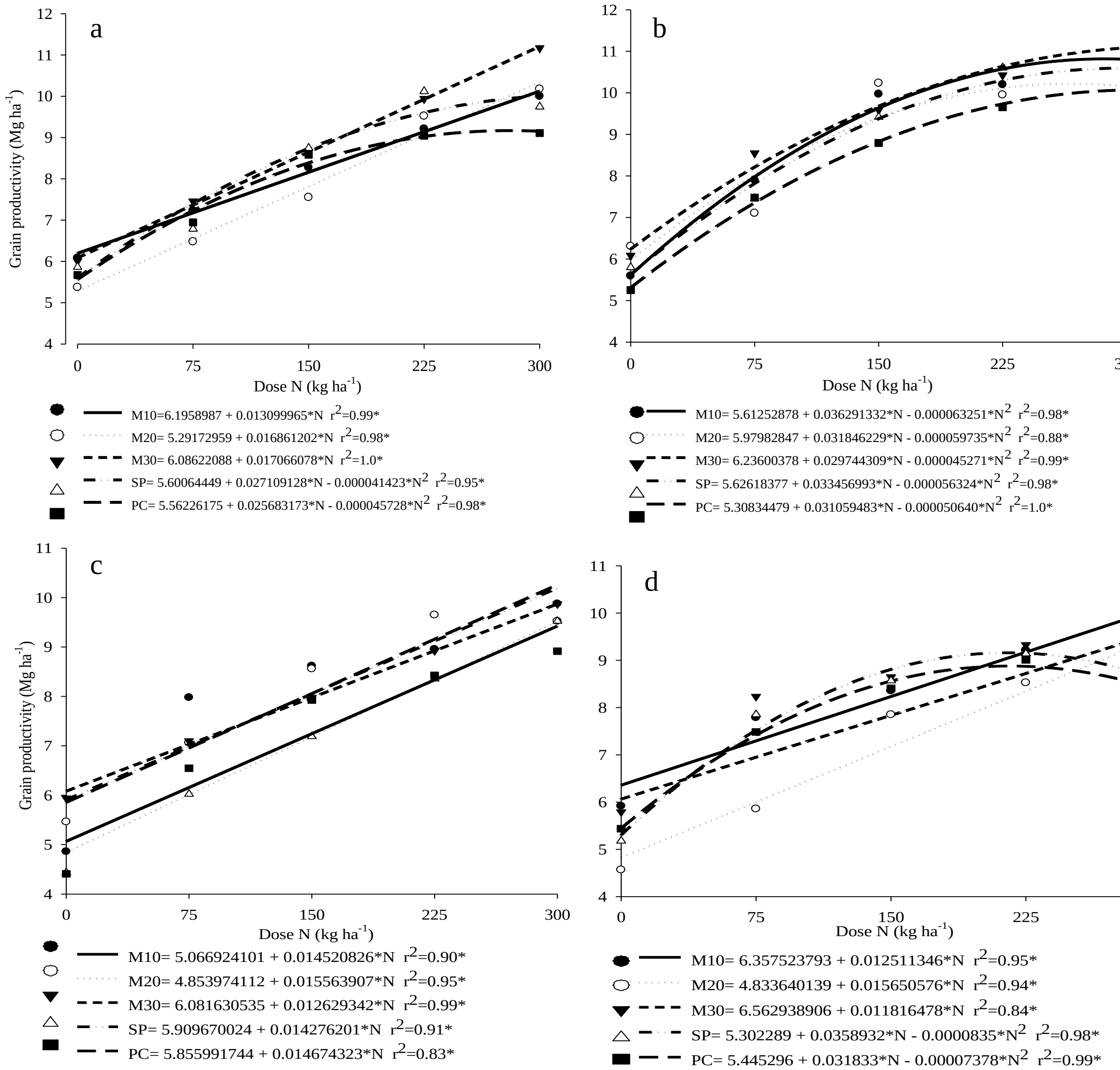

Figure 4. Grain productivity of corn in the ILP system with the absence and presence of *A. brasilense*, respectively, for the 2014/15 (a, b) and 2015/16 (c, d) seasons under a Rhodic Paleudalf.

Conclusion

Higher soil cover at the end of the grazing period resulted in higher corn plant growth and grain yield.

Nitrogen fertilization resulted in increased corn grain yield, yield components, and shoot biomass in the different post-grazing, residual-height treatments. Under the tested experimental conditions, grain accumulation was highest with 300 kg ha^{-1} of N in the different post-grazing residual height treatments.

Corn seed inoculation with *A. brasilense* resulted in increased corn plant growth, WTG, and grain yield.

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