Research Article

Fernando Shintate Galindo, Marcelo Carvalho Minhoto Teixeira Filho*, Salatiér Buzetti, Willian Lima Rodrigues, Guilherme Carlos Fernandes, Eduardo Henrique Marcandalli Boleta, Maurício Barco Neto, Antonio Leonardo Campos Biagini, Eduardo Bianchi Baratella, Jeferson Silva de Souza

Nitrogen rates associated with the inoculation of *Azospirillum brasilense* and application of Si: Effects on micronutrients and silicon concentration in irrigated corn

https://doi.org/10.1515/opag-2018-0056
received June 30, 2018; accepted October 8, 2018

Abstract: The aim of this study was to analyze whether there are differences between the inoculation with *Azospirillum brasilense* and the silicon application, thus enabling a higher efficiency of nitrogen fertilization, evaluating micronutrients and silicon concentration in shoots and roots of irrigated corn (*Zea mays*). The experiment was conducted in Selvíria, Brazil, under a no-till system, on a Typic Rhodic Hapludox. The experiment was set up as a randomized block design with four replications, in a 2 × 5 × 2 factorial arrangement consisting of two soil corrective sources (dolomitic limestone and Ca and Mg silicate as source of Si); five N rates (0, 50, 100, 150 and 200 kg ha^{-1}); with and without inoculation with *A. brasilense*. N rates increased B, Cu and Fe concentrations in shoots and B, Cu, Fe, Mn, Zn and Si in roots. Inoculation provided greater concentrations of B and Fe in shoots, and B in roots. Although inoculation with *A. brasilense* favored micronutrient uptake, it negatively affected Si concentration in shoots in 2015/16 crop. The use of Si in the form of Ca and Mg silicate promotes an increase in Mn, Si and Zn uptake in shoots and Mn and Si concentration in roots.

Keywords: *Zea mays*, biological nitrogen fixation in grasses, plant growth promoting bacteria, silicon application, efficiency of nitrogen fertilization

***Corresponding author: Marcelo Carvalho Minhoto Teixeira Filho,** Department of Plant Health, Rural Engineering, and Soils, Faculdade de Engenharia de Ilha Solteira, Universidade Estadual Paulista "Júlio de Mesquita Filho", Ilha Solteira, Sao Paulo State, Zip Code 15385-000, Brazil, E-mail: mcmteixeirafilho@agr.feis.unesp.br
Fernando Shintate Galindo, Salatiér Buzetti, Willian Lima Rodrigues, Guilherme Carlos Fernandes, Eduardo Henrique Marcandalli Boleta, Maurício Barco Neto, Antonio Leonardo Campos Biagini, Eduardo Bianchi Baratella, Jeferson Silva de Souza, Department of Plant Health, Rural Engineering, and Soils, Faculdade de Engenharia de Ilha Solteira, Universidade Estadual Paulista "Júlio de Mesquita Filho", Ilha Solteira, Sao Paulo State, Zip Code 15385-000, Brazil

1 Introduction

In order to achieve better plant nutrition and high corn grain yield, it is necessary to apply nitrogenous fertilizer, because soils, in general, do not supply the demand of the crop during its cycle (Teixeira Filho et al. 2014; Galindo et al. 2016; Galindo et al. 2017). In general, tropical soils are highly weathered and therefore are unable to supply macro and micronutrients, especially boron (B) and zinc (Zn), appropriately to the development of cereal crops (Galindo et al. 2018).

Corn is one of the oldest and most widespread crops in the world (Galindo et al. 2016). However, despite the technological advances available, the average Brazilian yield (e.g. 6,013 kg ha^{-1} in the Savannah region) is still low in terms of productive potential (Conab 2018, Follow-up of the Brazilian crop: fifth survey – February/2018, http://www.conab.gov.br/conteudos.php?a=1253). Consequently, nitrogen (N) fertilization is one of the highest costs of the production process of non-leguminous crops (Nunes et al. 2015). Wheat, corn, and rice crops utilize approximately 60% of all N fertilizer produced in the world (Espindula et al. 2014). Also, both nitrogen fertilizer production and application contribute to the emission of gases (CO_2 and NO_2) that contribute to the increase of the greenhouse effect on earth (Xu et al. 2012).

Due to high cost of fertilizers and awareness of sustainable and pollution free agricultural practices, one strategy to increase nitrogen fertilization efficiency, is the use of inoculants containing bacteria that promote growth and increase plant nutrition and productivity. The technology of inoculation of non-legumes with non-symbiotic plant growth-promoting bacteria (PGPB), whose main representative is *Azospirillum* spp., is also being increasingly adopted in several countries, mainly in Latin America, especially for crops such as corn and wheat (Hartmann and Bashan 2009; Marks et al. 2015). The analysis of results from a large number of field trials with various non-legume crops, conducted worldwide over 20 years under different soil and weather conditions, has demonstrated that yield increases of up to 30% could be obtained 70% of the time (Fukami et al. 2016; Fukami et al. 2017) in response to inoculation with *Azospirillum*.

They are believed to stimulate plant growth by an array of mechanisms including, but not restricted to, production and secretion of phytohormones such as indole-3- acetic acid (IAA), cytokinins, and gibberellins (Bashan and de-Bashan 2010; Meza et al. 2015), nitric oxide (Fibach-Paldi et al. 2012), increase of nutrient availability (Hungria et al. 2010), and biological nitrogen fixation (BNF) (Pankievicz et al., 2015). Due to the wide array of mechanisms proposed for stimulation of plant growth by *Azospirillum* spp., Bashan and de-Bashan (2010) proposed a theory of multiple mechanisms that might act either in a cumulative or sequential pattern.

The use of silicon (Si) in agriculture is another practice that exerts numerous benefits on grasses, especially when the plants are submitted to biotic and abiotic stresses, for example drought and salt stress (Crusciol et al. 2013a, 2013b), pathogens and insects (Bakhat et al. 2018) or strong wind and rain (Guntzer et al. 2012), which are common in adverse edaphoclimatic conditions such as in the Brazilian Cerrado (Savannah region). In addition, calcium (Ca) and magnesium (Mg) silicate, besides correcting soil acidity (Sarto et al. 2015), raise the levels of soluble phosphorus, calcium, magnesium and silicon and, consequently, bases saturation, reducing the toxic effects of iron, manganese, zinc, aluminum and cadmium (Reis et al. 2008; Guntzer et al. 2012; Camargo et al. 2014a, 2014b). Silicon can also stimulate plant growth and yield through the formation of upright leaves and better plant architecture which increase the photosynthetic rate (Ma and Yamaji 2008; Gong and Chen 2012), with consequent reduction of bedding due to the greater structural rigidity of the tissues, and still presents another important benefit related to reduction in the transpiration rate (Reis et al. 2008; Camargo et al. 2014a, 2014b). When accumulating in the cells of the epidermal layer, the Si can be a stable physical barrier in the penetration of some types of fungi (Guntzer et al. 2012; Bakhat et al. 2018).

Although one or more of these benefits are observed when seed inoculation with *Azospirillum* spp. and application of Si in the form of Ca and Mg silicate takes place, increased corn plant nutrition is not always observed. More experiments testing *Azospirillum* spp. associated with silicon application are needed to determine how to maximize its influence on plant nutrition and maximize plant development. Moreover, studies are still lacking to define how much N mineral needs to be applied in combination with *A. brasilense* and Si to achieve a better nutrient uptake. In addition, it would also be valuable to determine if Si application has any negative or positive interactions when corn is inoculated with *A. brasilense*.

Based on the above, we believe that there may be a synergetic effect between inoculation with *A. brasilense* and silicon application, thus allowing a higher efficiency of nitrogen fertilization and micronutrient uptake. Therefore, the objective of this study was to evaluate the effect of different nitrogen application rates associated with the inoculation of *A. brasilense* and application of Si, as a corrective acidity, on concentrations of micronutrients and silicon in shoots and roots of irrigated corn in Brazilian Cerrado (Savannah region).

2 Methods

2.1 Sites description

Field experiments were conducted during the cropping years of 2015/16 and 2016/17, in Selvíria, Mato Grosso do Sul (MS) state, Brazil (335 m above sea level) (Figure 1). The soil of the experimental area is classified as a Latossolo Vermelho distrófico of clayey texture, according to Embrapa (2013), and as Typic Rhodic Hapludox, according to the USDA (2010), which had been cultivated with annual crops for over 28 years, with the last 11 years under the no-till system, and the crops prior to corn were corn and wheat, respectively. Rainfall, air relative humidity, and maximum, mean, and minimum temperatures recorded during the experimental period are shown in Figure 2.

2.2 Experimental design

In both crops, a randomized-block design with four replicates was set up in a 2 × 5 × 2 factorial arrangement

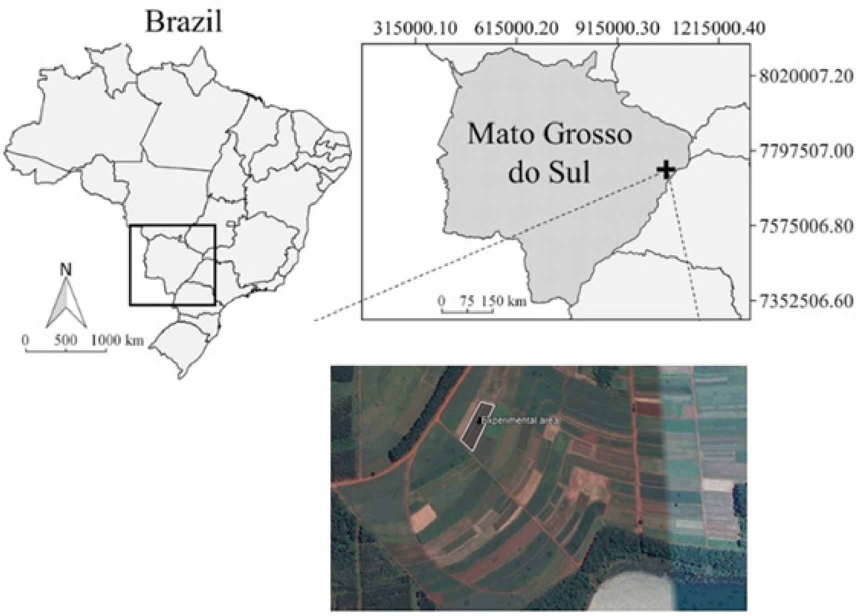

Figure 1. Study area at the Selvíria, Mato Grosso do Sul state, Brazil (20°22′S, 51°22′W, altitude of 335 m).

A

B

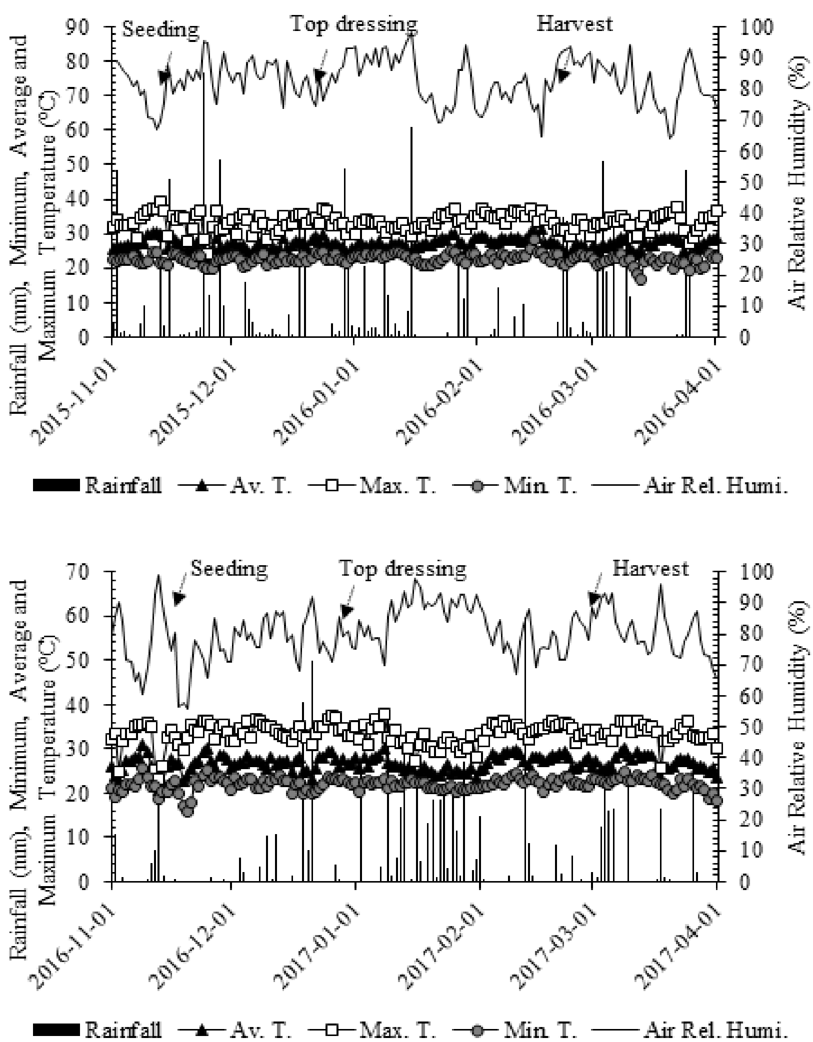

Figure 2. Rainfall, air relative humidity and maximum, average and minimum temperatures obtained from the weather station located in the Education and Research Farm of FE / UNESP during the corn cultivation in the period November 2015 to April 2016 (A) and November 2016 to April 2017 (B).

consisting of two soil corrective sources (*dolomitic limestone* with effective neutralizing power (ENP) = 80%, CaO = 28% and MgO = 20%; and *Ca and Mg silicate* as source of Si with ENP = 88%, Ca = 25%, Mg = 6% and Si total = 10%); five N application rates (0, 50, 100, 150 and 200 kg ha^{-1}, in the form of urea) applied as topdressing (fertilizer application without incorporation in the soil and between the crop lines); and with and without inoculation of the seeds with *A. brasilense*. The plots of the corn experiment were 5 m long with six lines spaced by 0.45 m, the plot area being the four central rows, with a 0.5 m margin from the extremities.

2.3 Trial establishment and management

The soil chemical properties of the top layer were determined before the start of the study in 2015, following the methodology proposed by Raij et al. (2001). The following results were obtained: the 0-0.20 m layer contained 1.04 g kg^{-1} Total N (determined by the regular Kjeldahl method using a block digester (Bremner 1996), followed by diffusion with NaOH (Stevens et al. 2000) and 9.4 mg dm^{-3} Si (Ca chloride 0.01 mol L^{-1}) according to methodology proposed by Korndörfer et al. (2004), 19 mg dm^{-3} P (resin); 10 mg dm^{-3} of S-SO_4; 21 g dm^{-3} organic matter; 5.0 pH ($CaCl_2$); K, Ca, Mg, H + Al and Al = 2.1; 19.0; 13.0; 28.0 and 1.0 $mmol_c$ dm^{-3}, respectively; Cu, Fe, Mn, Zn (DTPA) = 3.1; 20.0; 27.2 and 0.8 mg dm^{-3}, respectively; 0.17 mg dm^{-3} B (hot water) and 55% base saturation; and in the 0.20-0.40 m layer: 0.81 g kg^{-1} Total N, 10.2 mg dm^{-3} Si (Ca chloride 0.01 mol L^{-1}), 17 mg dm^{-3} P (resin); 30 mg dm^{-3} of S-SO_4; 16 g dm^{-3} organic matter; 4.8 pH ($CaCl_2$); K, Ca, Mg, H + Al and Al = 1.2; 11.0; 8.0; 28.0 and 2.0 $mmol_c$ dm^{-3}, respectively; Cu, Fe, Mn, Zn (DTPA) = 2.1; 10.0; 10.7 and 0.2 mg dm^{-3}, respectively; 0.11 mg dm^{-3} B (hot water) and 42% base saturation.

The granulometric analysis presented following results: 433, 471 and 90 g kg^{-1} of clay, sand and silt, and 447, 471 and 82 g kg^{-1} of clay, sand and silt, respectively on depth of 0-0.20 and 0-0.40 m.

The desiccation of the agricultural area was performed by the application of herbicides glyphosate (1800 g ha^{-1} of the active ingredient [a.i.]) and 2,4-D (670 g ha^{-1} of the a.i.). The corn straw (predecessor crop) was collected at sowing occasion to characterize and estimate the accumulation of nutrients: 78.2; 7.2; 68.8; 23.3; 21.1; 16.3 and 13.1 kg ha^{-1} of N, P, K, Ca, Mg, S and Si, respectively, and 260.4; 74.0; 1018.1; 709.6 and 185.1 g ha^{-1} of B, Cu, Fe, Mn and Zn, respectively and C/N ratio of 38.3.

With the aim of increasing the saturation by bases to 80% and based on the soil analysis and, the dose of 1.94 t ha^{-1} of dolomitic limestone and 1.76 t ha^{-1} of calcium and magnesium silicate was applied 30 days before sowing of corn, as top-dressing and without incorporation. During fertilization at planting, for both crop years of the experiment, 375 kg ha^{-1} of the 08-28-16 formulation was used, corresponding to 30 kg ha^{-1} N, 105 kg ha^{-1} P_2O_5, and 60 kg ha^{-1} K_2O, based on the soil analysis and the requirements of the corn crop.

Corn seeds inoculation with the bacterium *Azospirillum brasilense* strains Ab-V5 Ab-V6 (guaranteee of 2x10^{8} CFU mL^{-1} - Inoculants consisted of a mixture of strains CNPSo 2083 (=Ab-V5) and CNPSo 2084 (=Ab-V6) of *A. brasilense* from the Collection of Diazotrophic and Plant Growth- Promoting Bacteria of Embrapa Soja, WFCC # 1213, WDCM # 1054) was carried out at the dose of 300 mL of inoculant (liquid) per hectare of planted seeds, with the aid of a clean mixer for incorporation in the seeds and was carried out one hour before sowing the crop and after treatment of the seeds with insecticide and fungicide. For seed treatment, the fungicides pyraclostrobin + thiophanate-methyl (6 g + 56 g of a.i. per 100 kg of seed) and the insecticide fipronil (62 g of a.i. per 100 kg of seed) were used.

The mechanical sowing of the simple hybrid DOW 2B710 PW was carried out on 11/13/15 for the 2015/16 crop and 11/11/16 for the 2016/17 crop, being sown 3.3 seeds per meter and emergence of seedlings five days after sowing, on 11/18/2015 and 11/16/2016, respectively. The corn crop was irrigated using a center pivot sprinkling system, with a mean water depth of 14 mm and an irrigation interval of approximately 72 h. The herbicide tembotrione (84 g ha^{-1} of a.i.) and atrazine (1000 g ha^{-1} of a.i.) were applied for the control of post-emergence weeds, plus the addition of an adjuvant in the herbicide syrup, oil (720 g ha^{-1} of a.i.), on 12/04/2015 and 12/02/2016, respectively. Insect control was performed with methomyl (215 g ha^{-1} a.i.) and triflumurom (24 g ha^{-1} a.i.), on 12/20/2015 and 12/17/2016, respectively.

Nitrogen topdressing fertilization (treatments) was applied on hauls and without soil incorporation, between the corn lines, on 12/13/15 and 12/10/16 when the plants were with six leaves completely unfolded (V6 corn stage). The application was done manually, distributing the fertilizer on the soil surface (without incorporation), to the side and approximately 10 cm of the rows, in order to avoid the contact of the fertilizer with the plants. After cover fertilization, the area was irrigated by sprinkling (depth of 14 mm) to minimize losses by volatilization of ammonia, by dissolving the fertilizer, followed by the infiltration of the ammonia with the water in the soil. The harvest was carried out on 03/15/2016 and 03/21/2017, that is, at 117 and 125 days after corn emergence, respectively.

2.4 Evaluations

The following evaluations were performed: a) B, Cu, Fe, Mn, Zn and Si concentration in corn shoot and root, collecting the aerial part and roots of five corn plants per plot, in the female flowering (R1 corn stage). The micronutrients determination followed methodology described in Malavolta et al. (1997) and Si was determinate following methodology described by da Silva (2009).

2.5 Statistical analysis

All data were initially tested for normality using the Shapiro and Wilk (1965) test. All data were distributed normally (W≥0.90). Data were submitted to analysis of variance (F test). When a significant result was verified by the F test (p≤0.01 and p≤0.05), the Tukey test (p≤0.05) was used for comparison of means of acidity correctives sources and with or without *A. brasilense* inoculation, and adjusted to polynomial regression for the N rates using SAS program (SAS Inst. Inc., Cary, NC, 2015).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

The increment of N rates positively influenced B concentration in 2015/16 crop and Cu and Fe in shoot in 2016/2017 crop (Table 1). There was adjustment to the increasing linear function for both micronutrients (Figures 3a, b and c). There was also a positive influence on B and Cu concentrations in both crops, Fe in 2016/17 and Mn, Zn and Si in 2015/2016 crop in the roots, with adjustment to the linear function increasing for the aforementioned micronutrients and Si, except for the concentrations of B and Fe in 2016/17 crop that adjusted to the quadratic function with maximum point up to 144 and 152 kg ha^{-1} of N, respectively (Table 2 and Figures 3d, e and f and Figures 4a, b, c, d and e).

Regarding the sources of soil acidity correctives, the use of Ca and Mg silicate provided higher concentrations of Mn and Si in 2016/17 and Zn in 2015/16 crops in shoot, Si in both crops and Mn in 2016/17 crop in roots, however, the limestone application provided a higher concentration of B in roots in 2016/17 crop (Tables 1 and 2).

Concerning the seed inoculation with *Azospirillum brasilense*, there was increased B concentration in 2015/16 crop and Fe in both crops in corn shoot, B in both crops in roots, however, reduced Mn concentration in roots in 2016/17 crop (Tables 1 and 2).

The interaction between sources of acidity correctives and inoculation with *A. brasilense* was significant for the concentrations of B in 2016/17 crop, Si in 2015/16 crop in shoot and Cu in 2016/17 crop in roots (Figures 5a, b and c).

With the inoculation with *A. brasilense*, the Ca and Mg silicate increased the B concentration compared to the application of limestone. The use of Ca and Mg silicate when inoculated with *A. brasilense* increased B concentration when compared to the treatments without inoculation (Figure 5a).

Only in the absence of inoculation with *A. brasilense*, the Ca and Mg silicate application increased Si concentration compared to the application of limestone (Figure 5b).

In relation to the Cu concentration in roots, in the absence of inoculation with *A. brasilense*, the use of Ca and Mg silicate increased the concentration of this micronutrient compared to the application of limestone. The application of Ca and Mg silicate without seed inoculation increased Cu concentration when compared to treatments inoculated with *A. brasilense* (Figure 5c).

4 Discussion

The increment in N rates increased micronutrients absorption, with increase in B (2015/16 crop), Cu and Fe (2016/17) concentrations in shoot and B and Cu (both crops), and Fe (2016/17) and Mn, Zn and Si (2015/16) concentrations in roots and greater Si absorption, with numerically increase in Si concentration in 2.16 and 16.18% in shoot and 18.4 and 4.66% in root in 2015/16 and 2016/17 crops respectively. N is the nutrient that most interferes in the development and productivity of crops, especially grasses. This mineral nutrient is found in higher concentrations in vegetative tissues and grains, which characterizes it has been the element most demanded by the corn plant. Thus, the higher availability of this nutrient to the plants favored the development of the root system, which, by exploiting a larger volume of soil, may have uptake a greater number of micronutrients and water, reflecting the removal in roots and aerial part. Since N is involved in the synthesis of proteins, chlorophyll, coenzymes, phytohormones, nucleic acids and secondary metabolites (Marschner 2012).

The micronutrients and Si concentrations average in the corn shoot and root in descending order was Si>Fe>Mn>Zn>B>Cu in shoot and Si>Fe>Mn>B>Cu>Zn in root, considering the two years of cultivation. Increasing

Table 1. B, Cu, Fe, Mn, Zn and Si concentration in corn shoot as a function of N rates, sources of acidity correctives and seed inoculation with *Azospirillum brasilense*. Selvíria, MS – Brazil, agricultural crop 2015/2016[1] and 2016/2017[2]

Shoot	B		Cu		Fe		Mn		Zn		Si	
	(mg kg^{-1} D.M.)										(g kg^{-1} D.M.)	
N rates	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	26.33	19.20	19.00	21.38	325.78	180.75	78.88	65.75	54.63	30.63	1.16	2.41
50	26.31	20.00	19.75	22.75	369.98	203.12	74.63	64.75	58.38	33.38	1.19	2.85
100	27.59	20.59	21.50	23.63	323.90	221.13	87.75	73.50	58.50	30.50	1.19	2.53
150	29.70	18.74	22.13	23.63	316.80	248.75	78.75	67.00	53.75	28.63	1.17	2.90
200	30.16	19.85	22.38	27.63	342.40	235.38	96.38	73.75	53.00	27.63	1.19	2.91
Sources of acidity correctives												
Limestone	27.35 a	19.07	21.20 a	24.05 a	332.66 a	214.40 a	85.10 a	65.35 b	50.05 b	30.60 a	1.16	2.42 b
Ca and Mg silicate	28.69 a	20.28	20.70 a	23.55 a	338.89 a	221.25 a	81.45 a	72.55 a	61.25 a	29.70 a	1.19	3.02 a
L.S.D. (5%)	2.06	1.09	2.63	2.47	39.50	29.02	7.75	6.10	4.08	2.92	0.02	0.39
Inoculation												
Without *A. Brasilense*	26.97 b	18.63	20.30 a	22.35 a	302.54 b	201.45 b	83.00 a	68.65 a	54.75 a	29.35 a	1.18	2.78 a
With *A. Brasilense*	29.07 a	20.72	21.60 a	25.25 a	369.01 a	234.20 a	83.55 a	69.25 a	56.55 a	30.95 a	1.17	2.66 a
L.S.D. (5%)	2.06	1.09	2.63	2.47	39.50	29.02	7.75	6.10	4.08	2.92	0.02	0.39
Overall Mean	28.02	19.68	20.95	23.80	335.77	217.83	83.28	68.95	55.65	30.15	1.18	2.72
C.V. (5%)	11.11	8.36	19.00	15.65	17.77	20.13	14.06	13.38	11.07	14.65	2.82	21.52
P value												
RATES (R)	0.0050**	0.2333ns	0.3719ns	0.005**	0.4225ns	0.0050**	0.179ns	0.1759ns	0.2613ns	0.1363ns	0.1356ns	0.3155ns
SOURCES (S)	0.1869ns	0.0311*	0.6957ns	0.6760ns	0.7449ns	0.6269ns	0.3365ns	0.0232*	0.0000**	0.5270ns	0.0100**	0.0043**
INOCULATION (I)	0.0453*	0.0007**	0.3147ns	0.0235*	0.0023**	0.0290*	0.8835ns	0.8392ns	0.3673ns	0.2662ns	0.5741ns	0.5314ns
R X S	0.0577ns	0.4524ns	0.5634ns	0.1707ns	0.559ns	0.4879ns	0.3615ns	0.0589ns	0.4866ns	0.4326ns	0.1190ns	0.4583ns
R X I	0.1086ns	0.1147ns	0.4479ns	0.1974ns	0.1036ns	0.3113ns	0.7300ns	0.4976ns	0.5466ns	0.1758ns	0.5788ns	0.2442ns
S X I	0.5448ns	0.0206*	0.6957ns	0.1058ns	0.4648ns	0.1158ns	0.4828ns	0.2719ns	0.8003ns	0.1684ns	0.0410*	0.1401ns
R X S X I	0.1616ns	0.5604ns	0.8347ns	0.3936ns	0.8643ns	0.4346ns	0.2012ns	0.5516ns	0.2727ns	0.5547ns	0.1370ns	0.5977ns

Means followed by the same letters in the column do not differ by Tukey at 0.05 probability level.

**, * and ns: significant at $p<0.01$, $0.01<p<0.05$, and not significant, respectively

Table 2. B, Cu, Fe, Mn, Zn and Si concentration in corn root as a function of N rates, sources of acidity correctives and seed inoculation with *Azospirillum brasilense*. Selvíria, MS – Brazil, agricultural crop 2015/2016[1] and 2016/2017[2]

Root	B		Cu		Fe		Mn		Zn		Si	
	(mg kg^{-1} D.M.)										(g kg^{-1} D.M.)	
N rates	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	53.28	161.72	40.88	32.75	2306.25	5974.00	172.75	79.00	19.88	22.13	10.00	9.01
50	57.19	183.66	50.50	34.25	2296.25	7531.38	246.88	76.13	23.88	22.63	11.14	9.34
100	60.31	192.17	52.88	36.25	2326.25	8103.50	237.13	74.88	23.63	22.25	12.08	8.92
150	65.08	194.35	58.00	37.88	2327.88	7426.88	266.63	76.63	29.63	23.50	11.74	9.33
200	71.20	191.59	65.88	47.63	2297.50	8170.50	423.13	82.75	29.13	26.88	12.41	10.13
Sources of acidity correctives												
Limestone	60.98 a	193.20 a	53.05 a	37.05	2318.50 a	7277.20 a	245.55 a	71.05 b	27.05 a	25.05 a	9.59 b	7.23 b
Ca and Mg silicate	61.85 a	176.20 b	54.20 a	38.45	2303.15 a	7605.30 a	293.05 a	84.70 a	23.40 a	21.90 a	13.36 a	11.46 a
L.S.D. (5%)	3.79	9.15	8.53	5.83	66.44	571.81	78.07	7.31	5.64	4.68	0.79	0.79
Inoculation												
Without *A. brasilense*	58.54 b	177.30 b	53.30 a	40.35	2328.65 a	7414.75 a	278.00 a	81.70 a	24.75 a	24.85 a	11.19 a	9.37 a
With *A. brasilense*	64.28 a	192.09 a	53.95 a	35.15	2293.00 a	7467.75 a	260.60 a	74.05 b	25.70 a	22.10 a	11.76 a	9.32 a
L.S.D. (5%)	3.79	9.15	8.53	5.83	66.44	571.81	78.07	7.31	5.64	4.68	0.79	0.79
Overall Mean	61.41	184.70	53.63	37.75	2310.83	7441.25	269.30	77.88	25.23	23.48	11.47	9.35
C.V. (5%)	9.33	7.49	24.04	23.35	4.34	11.61	30.80	14.18	30.76	30.11	10.36	12.78
P value												
RATES (R)	0.0001**	0.0008**	0.0008**	0.0031**	0.9426ns	0.0004**	0.0065**	0.6443ns	0.0197*	0.6507ns	0.0006 **	0.3154ns
SOURCES (S)	0.6373ns	0.0010**	0.7810ns	0.6212ns	0.6342ns	0.2445ns	0.2182ns	0.0009**	0.1912ns	0.1750ns	0.0000**	0.0000**
INOCULATION (I)	0.0051**	0.0031**	0.8750ns	0.0776ns	0.2754ns	0.8482ns	0.6462ns	0.0411*	0.7282ns	0.2336ns	0.1455ns	0.8930ns
R X S	0.3616ns	0.2257ns	0.7581ns	0.7096ns	0.5150ns	0.7813ns	0.5434ns	0.4472ns	0.9560ns	0.9220ns	0.5192ns	0.4935ns
R X I	0.8269ns	0.1071ns	0.9360ns	0.3387ns	0.8269ns	0.0808ns	0.4504ns	0.6196ns	0.7276ns	0.7718ns	0.7955ns	0.2058ns
S X I	0.2589ns	0.5177ns	0.1023ns	0.0229*	0.2302ns	0.0742ns	0.6979ns	0.0547ns	0.3744ns	0.4182ns	0.0662ns	0.1013ns
R X S X I	0.7381ns	0.5833ns	0.2956	0.5156ns	0.7590ns	0.9667ns	0.5761ns	0.2031ns	0.8843ns	0.9713ns	0.5821ns	0.5408ns

Means followed by the same letters in the column do not differ by Tukey at 0.05 probability level.

**, * and ns: significant at $p<0.01$, $0.01<p<0.05$, and not significant, respectively

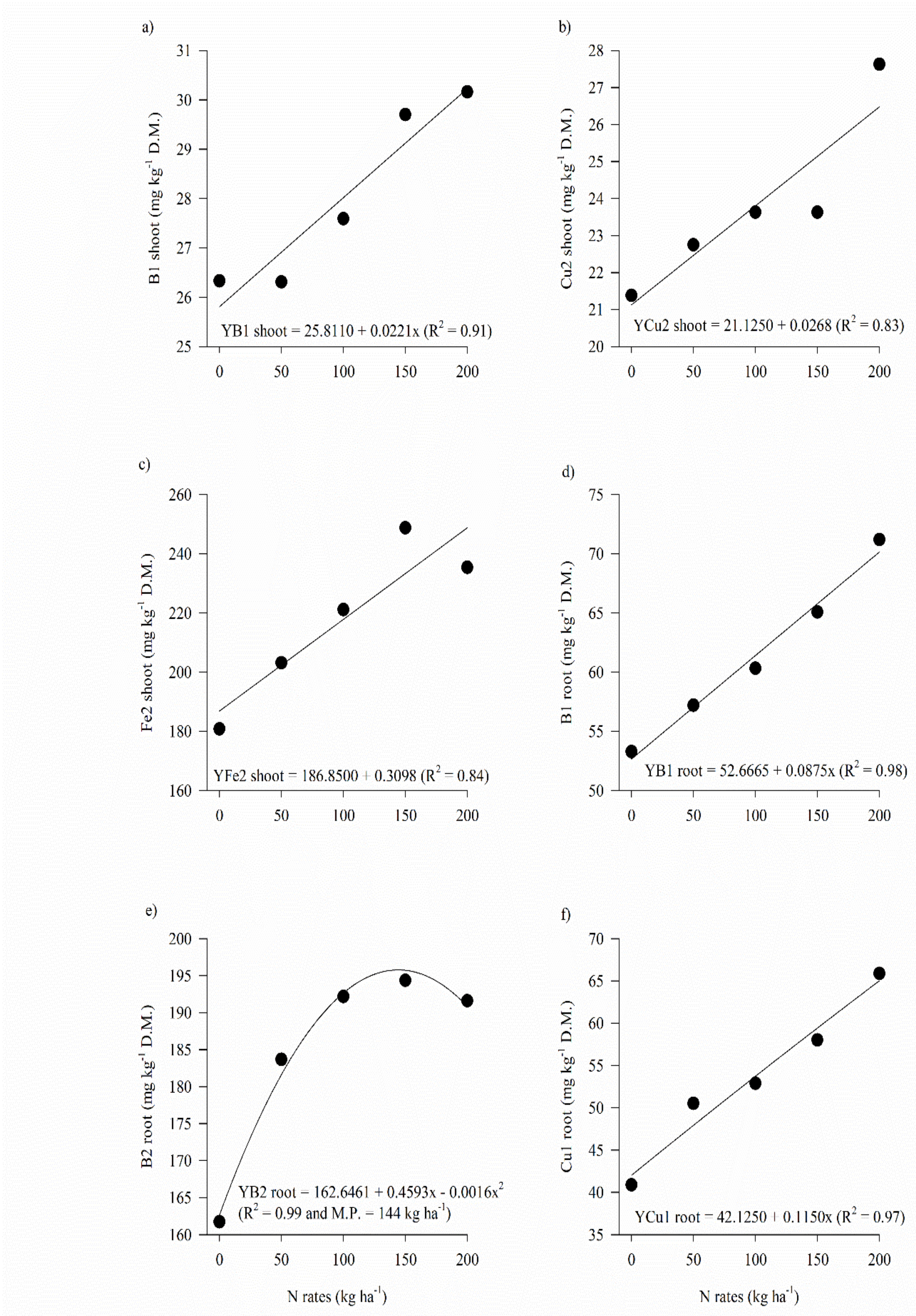

Figure 3. Effect of N rates in B (a), Cu (b) and Fe (c) concentration in shoot, and B (d and e) and Cu (f) concentration in root. Selvíria, MS – Brazil, agricultural crop 2015/2016[1] and 2016/2017[2]

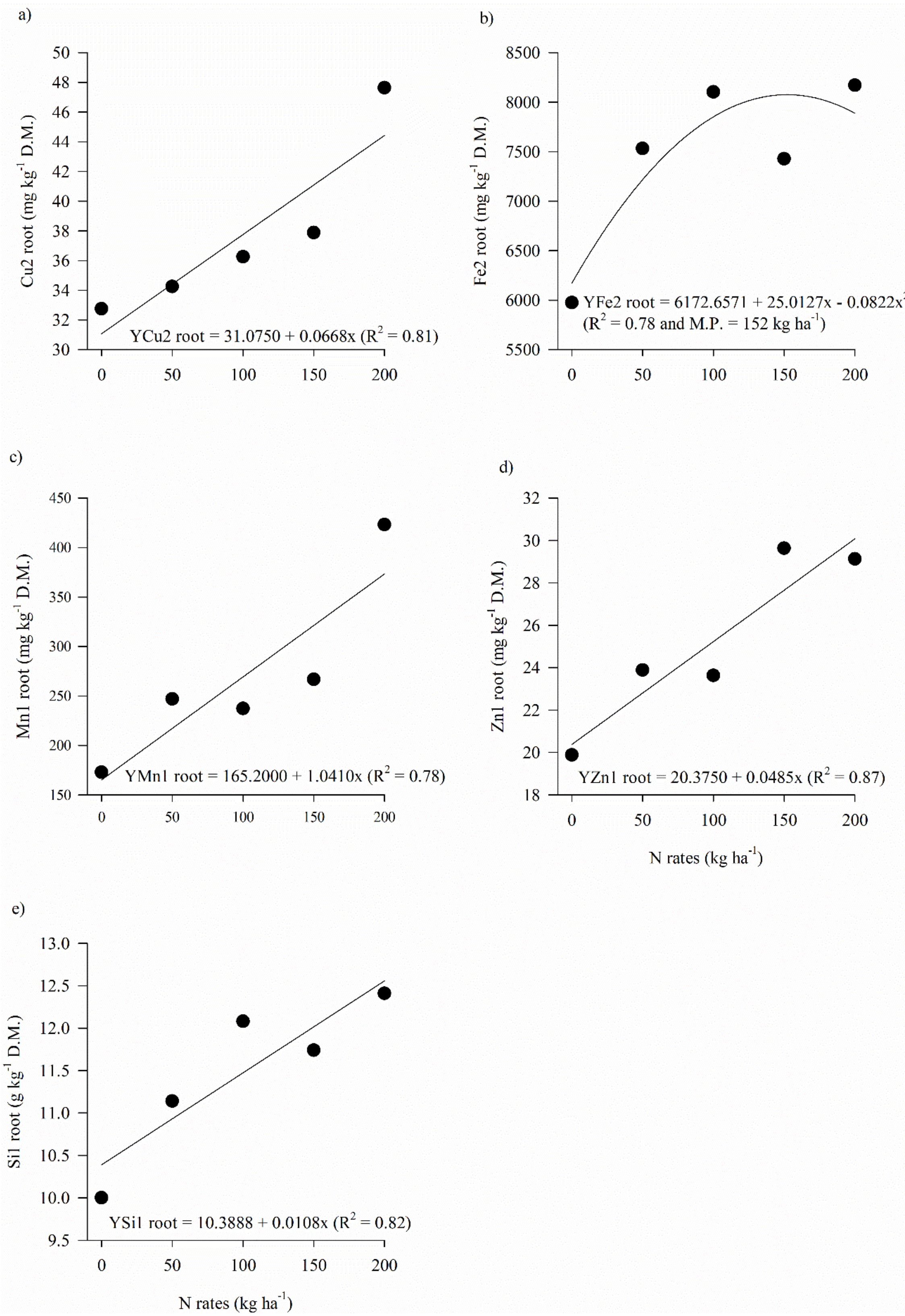

Figure 4. Effect of N rates in Cu (a), Fe (b), Mn (c) Zn (d) and Si (e) concentration in root. Selvíria, MS – Brazil, agricultural crop 2015/2016[1] and 2016/2017[2]

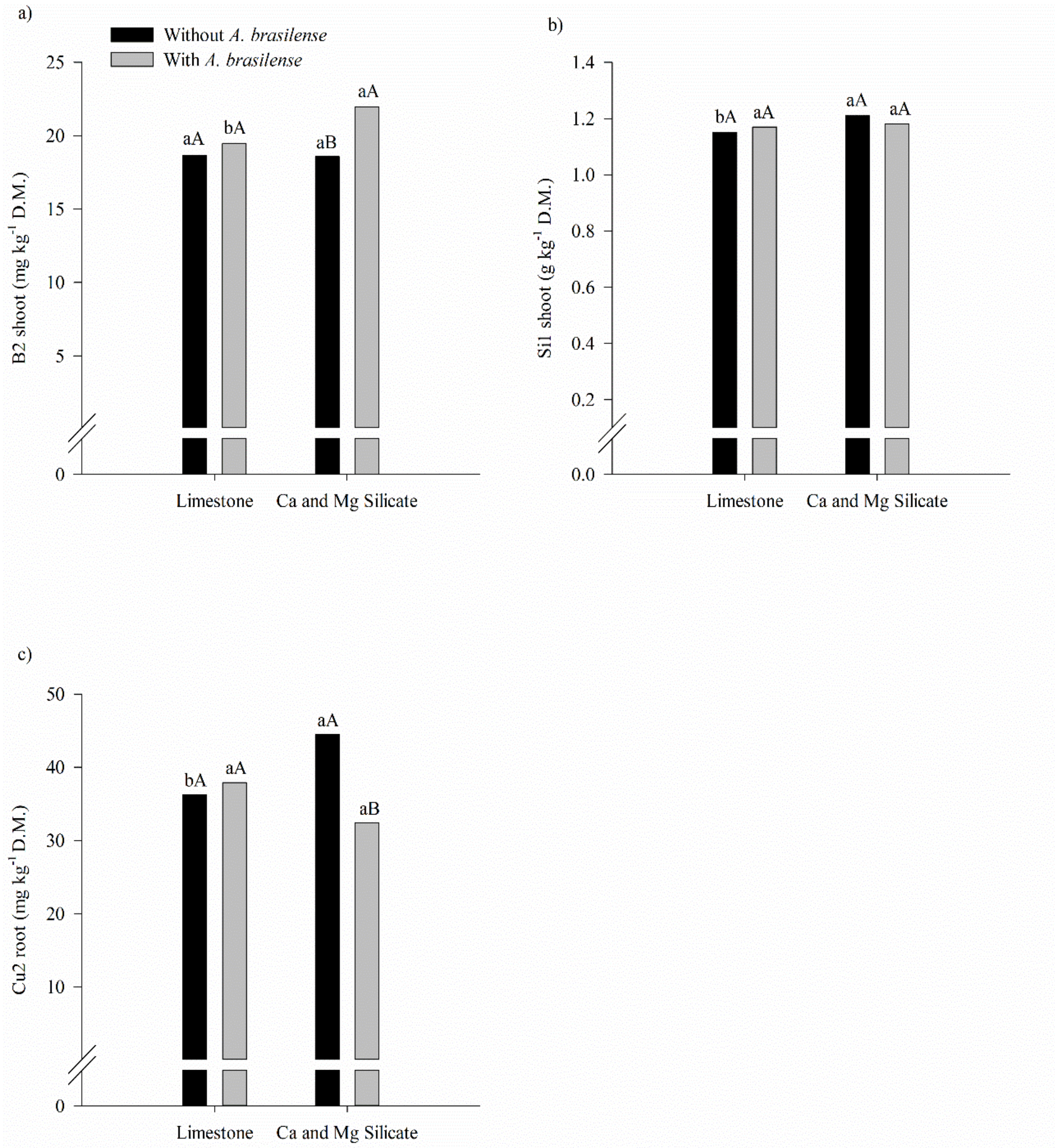

Figure 5. Interaction between sources of acidity correctives and inoculation with *Azospirillum brasilense* in B (a) and Si (b) concentration in shoot and Cu (c) in root. Selvíria, MS – Brazil, agricultural crop 2015/2016[1] and 2016/2017[2]. Means followed by the same letters tiny for sources of acidity correctives and uppercase for inoculation with *Azospirillum brasilense* do not differ by Tukey at 0.05 probability level.

concentrations of B, Cu, Fe, Mn and Zn as a function of the N rates is very interesting for corn nutrition, since Boron is an essential element to plant growth, participating in several processes, such as sugar transport, lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid (IAA) metabolism, phenolic metabolism, ascorbate metabolism, besides have function in cell wall synthesis and plasma membrane integrity (Galindo et al., 2018), influences the germination of the pollen grain and pollen tube growth, increases flower glue and granulation and causes less male sterility and less grain puffiness (Metwally et al. 2017). Cu in the plant is fundamental for the formation of pollen and for the biosynthesis of chlorophyll and the cell wall (lignification) of the plant. The deficiency of this micronutrient causes sterility of pollen in the ear, which leads to poor grain formation and loss of productivity (Barker and Pilbeam 2015). The Fe essentiality to plants is proven by action on the metabolism of proteins of heme and non-heme structure or in the coordination of protein structures, and these compounds act as enzymes (for example, catalases, peroxidases) or as electron carriers in the process of photosynthesis (ferredoxin, cytochrome) and in the metabolic routes of assimilation of other nutrients (N and S) in the plant (Barker and Pilbeam 2015). Mn is an essential element in plants and participates in the structure of phosphorylating proteins and enzymes. Its deficiency causes damage to chloroplasts, affecting the photolysis of water in photosystem II (Santos et al. 2017a), which provides the electrons necessary for photosynthesis (Fernando and Lynch 2015). In cereals, such as corn, Mn deficiency affects grain quality and yield, because the deficiency of this nutrient causes a reduction in the number and mass of the grains produced, possibly associated to the combination of low fertility pollen grains and decreased carbohydrate supply for the grain filling process (Marschner 2012). Zinc is one of the most important micronutrients for growth and development of higher plants. It is involved in completing many vital physiological functions such as protein synthesis, energy production and maintenance of membrane integrity (Hansch and Mendel 2009; Drissi et al. 2017). One of the most important functions of Zn in the plant is to participate as a component of a large number of enzymes such as dehydrogenases, proteinases, peptidases, and phosphohydrolases, and the basic functions in the plant are related to the metabolism of carbohydrates, proteins, and phosphates and in the formation of the structure of auxins, RNA, and ribosomes (Marschner 2012; Ojeda-Barrios et al. 2014). Zn is also related to the metabolism of phenols, formation of starch, increase in cell size and cell multiplication, and fertility of the pollen grains (Marschner 2012; Davarpanah et al. 2016).

The application of Ca and Mg silicate as a source of Si was effective in the availability of this element for corn crop as a function of the increase of Si concentration in shoot and root, mainly in 2016/17 crop, with higher Si uptake and concentration. This source of Si was also more effective in promote the absorption of Mn (2016/17 crop) and Zn (2015/16) in shoot and Mn (2016/17) in root, increasing their concentration.

In general, solutions of soils show liquid-phase Si levels varying between 2.8 and 16.8 mg dm^{-3}, and the dynamic balance of the element in the soil depends on the pH (Epstein 2009). In addition, the main sources of Si in soil solutions are the decomposition of plant residues, dissociation of polymeric H_4SiO_4, release of Si from Fe and Al oxides and hydroxides, dissolution of non-crystalline and crystalline minerals and addition of Si fertilizers and irrigation water (Korndörfer et al. 2004; Marafon and Endres 2013). The pH increases after application of the Ca and Mg silicate and together with corn straw decomposition in the first crop and wheat in second crop (predecessor to the corn), provided a higher amount of Si in the second year, justifying greater absorption and concentration of the element in 2016/17 crop. Most of the absorbed Si was concentrated in root. The Si root/Si shoot ratio was, in average, 11.2 and 3.8, for the two crops respectively.

Plants uptake Si directly from soil solution. It is transported up to the roots mainly via mass flow (Korndörfer et al. 2004), and its absorption in grass occurs quickly, however, translocation varies among species, being able to be retained in the roots and little translocated in aerial part (Isa et al. 2010; Agostinho et al. 2017). Si is transported by xylem and deposited on the cell wall in the form of hydrated amorphous silica or biogenic opals, also known as silica bodies or silica phytoliths ($SiO_2.nH_2O$). Once deposited, it becomes virtually immobile and no longer redistributes to the plant (Guntzer et al. 2012). The transport of monosilicic acid inside the plant takes place in the same direction as the mass flow (transpiration). Thus, Si deposits occur more frequently in regions where water is lost in large amounts (Isa et al. 2010), which explains the high concentration of Si in shoot and root.

Among plant species, rice (*Oryza sativa* L.) contains silicic acid at about 10% of above-ground dry weight (Isa et al. 2010). Rice accumulates Si as silica opal and silica bodies in the shoots, and the roots and can absorb several times more Si than common nutritional minerals such as N, P, and K mainly because silicon uptake through rice roots is mediated by specific transporters (Ma et al. 2006; Isa et al. 2010; Agostinho et al. 2017). These transporters were characterized and identified as low-Si genes (Lsi1 and Lsi2), and are responsible for transporting Si from the soil solution to the root cells (influx, Lsi1) and from inside to the outside of the root cells (efflux, Lsi2) (Ma et al., 2006, 2007). Recently, three Si transporters (Lsi2, Lsi3 and Lsi6) were also identified in the rice node (Yamaji et al. 2015). While Si transporters in the roots facilitate uptake of the element by the plant, Si transporters in the node are involved in intervascular transfer, which is required for the preferential distribution of Si to the leaves and grains (Yamaji et al. 2015; Agostinho et al. 2017), which may justify how some species of accumulating grasses such as corn can absorb and translocate large amounts of Si in shoot part.

The inoculation with *A. brasilense* provided greater B and Fe concentrations in shoot with increase in 2015/16 crop and B in roots in both crops, but it reduced the concentration of Mn in the roots in 2016/17 crop. Besides that, when associated with Si in the form of Ca and Mg silicate provided greater B concentration in shoot in 2016/17 crop.

Plant and soil bacteria participate in several molecular signaling events that establish specific symbiotic, endophytic, or associative relationships (Santos et al. 2017b). Such relationships differ according to plant genotypes, soil types, bacterial strains and abilities to improve plant growth (Philippot et al. 2013). *Azospirillum* sp. is one of the most studied genera of plant growth-promoting bacteria (PGPB) at present due its capacity to colonize many plant species (Cassan and Diaz-Zorita 2016). The plant growth-promotion by *Azospirillum* is mainly associated with its ability to fix nitrogen in grasses (Pankievicz et al. 2015; Santos et al. 2017b) but apparently, nitrogen fixation does not play a major role in plant growth promotion in most systems evaluated so far (Fibach-Paldi et al. 2012). On the other hand, *Azospirillum* are able to produce and secrete phytohormones (indole-3- acetic acid, cytokinins, and gibberellins), nitric oxide (Fibach- Paldi et al. 2012) which likely are key signals and components of plant growth promotion effects (Bashan and de-Bashan 2010), and with higher rates of absorption of water and nutrients by the plant (Dardanelli et al. 2008), higher tolerance to abiotic stresses, such as drought and salinity (Zawonski et al. 2011), that will promote greater uptake of micronutrients and water in corn crop, with a reflection on crop development.

The results obtained demonstrate benefits in corn nutrition, elucidating the need for new research related to the beneficial effects of inoculation with *A. brasilense* associated with nitrogen fertilization and calling attention to the possibility of wide use of this technology in the field due to low economic cost, non-toxic and with high potential of response of corn crop, even with the application of N rates considered high for BNF. For this reason, this technique is likely increasingly adopted by rural farmers.

The use of silicon (Si) in agriculture exerts numerous benefits on grasses, especially when the plants are submitted to biotic and abiotic stresses, common in adverse edaphoclimatic conditions such as in the Brazilian Cerrado (Reis et al. 2008), but as ponder Camargo et al. (2014a, 2014b), this element will provide benefits mainly to the plants considered as hiperaccumulators, which include some grasses such as rice and sugar cane and contains concentration of SiO_2 above 4% (Lima et al. 2011), and that some factors will influence the uptake of Si by plants, such as genotype, type of soil and plant species, which could explain the slight response of the use of Si in the form of Ca and Mg silicate in corn crop in the present study, even with a high absorption of Si, further elucidating the importance of new silicon studies in potentially accumulating crops such as corn in Brazilian Cerrado.

Besides that, according to Korndörfer et al. (2010) and Sarto et al. (2015), when the levels of soil available Si are greater than 10.0 mg kg^{-1} increased grain yield of grass species to the Si supply is unlikely. Although the soil content in the 0-0.20 m layer was slightly below this range (9.4 mg kg^{-1}) the 0.20-0.40 m layer provided 10.2 mg kg^{-1}, in addition to the potentially available amount of Si over the corn crop as a function of straw decomposition (13.1 kg ha^{-1} Si and C/N ratio 38.3).

Tropical and subtropical soils generally have low levels of Si because of de-silication due to leaching and weathering processes (Epstein 2009). In addition, intensive cultivation removes Si from the soil, as it is estimated that annually 210–224 million tons of Si is taken out from the world's arable soils (Meena et al. 2014). In cultivated areas, the Si removal of crops does not allow the recycling of Si by plants, and the biogeochemical cycle of Si is disturbed (Bakhat et al. 2018). In this way the decrease of bioavailable Si may have significant impacts on cereal yields if not properly restored (Guntzer et al. 2012). Besides that, silicon may affect the global carbon turnover by changing the plant cellulose, lignin and phenol content in biomes with grasses as main vegetation. Resulting from this, the effect of silicon on the terrestrial and semiterrestrial part of the global carbon turnover is a neglected research field and has to be investigated (Schaller et al. 2012).

In conclusion, the increment of N rates increased B, Cu and Fe concentrations in shoot and B, Cu, Fe, Mn, Zn and Si in roots.

The inoculation with *A. brasilense* alone provided greater concentrations of B and Fe in shoot, and B in roots. Besides that, when associated with Si in the form of Ca and Mg silicate favored B concentration in shoot in 2016/17 crop. Although it favored micronutrients uptake, the inoculation with *Azospirillum brasilense* negatively affected Si concentration in shoot in 2015/16 crop.

The use of Si in the form of Ca and Mg silicate promote an increase in Mn, Si and Zn uptake in shoot and Mn and Si uptake in root, being necessary new researches seeking the better understanding of the Si use associated to growth promoting bacteria aiming to improve the management of nitrogen fertilization and micronutrients uptake in potentially Si accumulating crops such as corn.

Acknowledgements: The authors are gratefull to Fundação de Amparo à Pesquisa do Estado de São Paulo-FAPESP, process number 2017/06002-6.

Conflict of interest: Authors declare no conflict of interest.

References

Agostinho F.B., Tubana B.S., Martins M.S., Datnoff L.E., Effect of different silicon sources on yield and silicon uptake of rice grown under varying phosphorus rates, Plants 2017, 6, 1-17

Bakhat H.F., Bibi N., Zia Z., Abbas S., Hammad H.M., Fahad S., et al., Silicon mitigates biotic stresses in crop plants: A review, Crop Protec., 2018, 104, 21–34

Barker A.V., Pilbeam D.J., Handbook of plant nutrition, 2nd ed., CRC Press, Boca Raton, USA, 2015

Bashan Y., de-Bashan L.E., How the plant growth-promoting bacterium *Azospirillum* promotes plant growth - a critical assessment, Adv. Agron., 2010, 108, 77-136

Bremner J.M., Keeney D.R., Determination and isotope-ratio analysis of different forms of nitrogen in soils: 3. Exchangeable ammonium, nitrate, and nitrite by extraction-distillation methods, Soil Sci. Soc. Amer. Proc., 1966, 30, 577–582

Camargo M.S., Korndörfer G.H., Foltran D.E., Silicon absorption and stalk borer incidence by sugarcane varieties in two ratoons, Bioscience J., 2014b, 30, 1304-1313, (in Portuguese)

Camargo M.S., Korndörfer G.H., Wyler P., Silicate fertilization of sugarcane cultivated in tropical soils, Field Crops Res., 2014a, 167, 64-75

Cassán B., Diaz-Zorita M., *Azospirillum* sp. in current agriculture: from the laboratory to the field, Soil Biol. Biochem., 2016, 103, 117-130

Crusciol C.A.C., Soratto R.P., Castro G.S.A., Costa C.H.M., Neto J.F., Foliar application of stabilized silicic acid on soybean, common bean, and peanut, Rev. Ciênc. Agron. 2013a, 44, 404-410

Crusciol C.A.C., Soratto R.P., Castro G.S.A., Neto J.F., Costa C.H.M., Leaf application of silicic acid to upland rice and corn, Semina: Ciênc. Agrár., 2013b, 34, 2803-2808

Dardanelli M.S., de Córdoba F.J.F., Espuny M.R., Carvajal M.A.R., Díaz M.E.S., Serrano A.M.G., et al., Effect of *Azospirillum brasilense* coinoculated with *Rhizobium* on *Phaseolus vulgaris* flavonoids and Nod Factor production under salt stress, Soil Biol. Biochem., 2008, 40, 2713–2721

Davarpanah S., Tehranifar A., Davarynejad G., Abadía J., Khorasani R., Effects of foliar applications of zinc and boron nano-fertilizers onpomegranate (*Punica granatum* cv. Ardestani) fruit yield and quality, Sci. Hort., 2016, 210, 57-64

Drissi S., Houssa A.A., Bamouh A., Benbella M., Response of corn silage (*Zea mays* L.) to zinc fertilization on a sandy soil under field and outdoor container conditions, J. Saudi Soc. Agric. Sci., 2017, 16, 145-153

Duca D., Lorv J., Patten C.L., Rose D., Glick B.R., Indole-3-acetic acid in plantmicrobe interactions, Antonie van Leeuwenhoek, 2014, 106, 85-125

Empresa Brasileira de Pesquisa Agropecuária - Embrapa. Centro Nacional de Pesquisa de Solos, Brazilian System of soil classification, 3rd ed., DF: Embrapa, Brasília, 2013, (in Portuguese.)

Epstein E., Silicon: its manifold roles in plants, Ann. Appl. Biol., 2009, 155, 155-160

Espindula M.C., Rocha V.S., Souza M.A.D., Campanharo M., Pimentel A.J.B., Urease inhibitor (NBPT) and efficiency of single or split application of urea in wheat crop, Rev. Ceres, 2014, 61, 273-79

Fernando D.R., Lynch J.P., Manganese phytotoxicity: new light on an old problem, Ann. Bot., 2015, 116, 313–319

Fibach-Paldi S., Burdman S., Okon Y., Key physiological properties contributing to rhizosphere adaptation and plant growth promotion abilities of *Azospirillum brasilense*, FEMS Microbiol. Lett., 2012, 326, 99-108

Fukami J., Nogueira M.A., Araujo R.S., Hungria M., Accessing inoculation methods of maize and wheat with *Azospirillum brasilense*, AMB Express, 2016, 6, 3-16

Fukami J., Ollero F.J., Megías M., Hungria M., Phytohormones and induction of plant-stress tolerance and defense genes by seed and foliar inoculation with *Azospirillum brasilense* cells and metabolites promote maize growth, AMB Express, 2017, 7, 153-163

Galindo F.S., Teixeira Filho M.C.M., Buzetti S., Santini J.M.K., Alves C.J., Nogueira L.M., et al., Corn yield and foliar diagnosis affected by nitrogen fertilization and inoculation with *Azospirillum brasilense*, Rev. Bras. Ciênc. Solo, 2016, 40, e015036

Galindo F.S., Teixeira Filho M.C.M., Buzetti S., Boleta E.H.M., Rodrigues W.L., Santini J.M.K., et al., Technical and economic viability of wheat with forms of application and doses of boron, J. Agric. Sci., 2018, 10, 306-315

Galindo F.S., Teixeira Filho M.C.M., Buzetti S., Santini J.M.K., Alves C.J., Ludkiewicz M.G.Z., Wheat yield in the Cerrado as affected by nitrogen fertilization and inoculation with *Azospirillum brasilense*, Pesq. Agropec. Bras., 2017, 52, 794-805

Gong H., Chen K., The regulatory role of silicon on water relations, photosynthetic gas exchange, and carboxylation activities of wheat leaves in field drought conditions, Acta Phys. Plant, 2012, 34, 1-6

Günes A., Turan M., Güllüce M., Sahin F., Nutritional content analysis of plant growth-promoting rhizobacteria species, Eur. J. Soil Biol., 2014, 60, 88-97

Guntzer F., Keller C., Meunier J., Benefits of plant silicon for crops: A review, Agron. Sustain. Dev., 2012, 32, 201-213

Hansch R., Mendel R.R., Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl), Curr. Opin. Plant Biol.., 2009, 12, 259-266

Hartmann A., Bashan Y., Ecology and application of *Azospirillum* and other plant growth-promoting bacteria (PGPB), Eur. J. Soil Biol., 2009, 45, 1–2

Hungria M., Campo R.J., Souza E.M., Pedrosa F.O., Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil, Plant Soil, 2010, 331, 413-425

Isa M., Bai S., Yokoyama T., Ma J.F., Ishibashi Y., Yuasa T., et al., Silicon enhances growth independent of silica deposition in a low-silica rice mutant, lsi1. Plant Soil 2010, 331, 361-375

Korndörfer G.H., Pereira H.S., Nolla A., Silicon analysis: soil, plant and fertilizer, Boletim Técnico, 2, Uberlândia, GPSi/ ICIAG/UFU, 2004, (in Portuguese)

Korndörfer G.H., Silva G.C., Teixeira I.R., Silva A.G., Freitas R.S., Effect of silicon fertilizer on forage grasses and soil chemical characteristics, Pesq. Agropec. Trop., 2010, 40, 119-125

Lima M.A., Castro V.F., Vidal J.B., Enéas-Filho J., Silicon application on plants of maize and cowpea under salt stress, Rev. Cienc. Agron., 2011, 42, 398-403, (in Portuguese)

Ma J.F., Tamai K., Yamaji N., Mitani N., Konishi S., Katsuhara M., et al., A silicon transporter in rice. Nature, 2006, 440, 668-691

Ma J.F., Yamaji N., Mitani N., An efflux transporter of silicon in rice, Nature, 2007, 448, 209-212

Ma J.F., Yamanji N., Functions and transport of silicon in plants, Cell. Mol. Life Sci., 2008, 65, 3049-3057

Malavolta E., Vitti G.C., Oliveira S.A., Evaluation of the nutritional status of plants: Principles and applications, 2nd .ed., Potafos, Piracicaba, 1997, (in Portuguese)

Marafon A.C., Endres L., Silicon: fertilization and nutrition in higher plants, Amaz. J. Agric. Environ. Sci., 2013, 56, 380-388

Marks B.B., Megías M., Ollero F.J., Nogueira M.A., Araujo R.S., Hungria M., Maize growth promotion by inoculation with *Azospirillum brasilense* and metabolites of *Rhizobium tropici* enriched on lipo-chitooligosaccharides (LCOs), AMB Express, 2015, 5, 71-82

Marschner P., Marschner´s mineral nutrition of higher plants, 3rd ed., Academic Press, New York, 2012

Meena V., Dotaniya M., Coumar V., Rajendiran S., Kundu S., Rao A.S., A case for silicon fertilization to improve crop yields in tropical soils, Proc. Natl. Acad. Sci. India, Sect. B Biol. Sci., 2014, 84, 505–518

Mercado-Blanco J., Prieto P., Bacterial endophytes and root hairs, Plant Soil, 2012, 361, 301-306

Metwally A.M., El-Shazoly R.M., Hamada A.M., Physiological responses to excess boron in wheat cultivars, Eur. J. Biol. Res., 2016, 7, 1-8

Meza B., de-Bashan L.E., Bashan Y., Involvement of indole-3-acetic acid produced by *Azospirillum brasilense* in accumulating intracellular ammonium in *Chlorella vulgaris,* Res. Microbiol., 2015, 166, 72-83

Nunes P.H.M.P., Aquino L.A., Santos L.P.D.D., Xavier F.O., Dezordi L.R., Assunção N.S., Yield of the irrigated wheat crop subjected to nitrogen application and to inoculation with *Azospirillum brasilense,* Rev. Bras. Cienc. Solo, 2015, 39, 174-182, (in Portuguese)

Ojeda-Barrios D.L., Perea-Portillo E., Hernández-Rodríguez O.A., Martínez-Téllez J., Abadía J., Lombardini L., Foliar fertilization with zinc in pecan trees, HortScience, 2014, 49, 562-566

Pankievicz V.C.S., Amaral F.P., Santos K.F.D.N., Agtuca B., Xu Y., Schueller M.J., et al., Robust biological nitrogen fixation in a model grass-bacterial association, Plant J., 2015, 81, 907-19

Philippot L., Raaijmakers J.M., Lemanceau P., van der Putten W.H., Going back to the roots: the microbial ecology of the rhizosphere, Nat. Rev. Microbiol., 2013, 11, 789-799

Raij B., van Andrade J.C., Cantarella H., Quaggio J.A., Chemical analysis for fertility evaluation of tropical soils, IAC, Campinas, 2001, (in Portuguese)

Reis M.A.R., Arf O., da Silva M.G., de Sá M.E., Buzetti S., Silicon application in upland rice under sprinkler irrigation, Acta Sci. Agron., 2008, 30, 37-43, (in Portuguese)

Santos A.R.S., Etto R.M., Furmam R.W., Freitas D.L., Santos K.F.D.N., Souza E.M., et al., Labeled *Azospirillum brasilense* wild type and excretion-ammonium strains in association with barley roots, Plant Physiol. Biochem., 2017b, 118, 422-426

Santos E.F., Santini J.M.K., Paixão A.P., Furlani Júnior E., Lavres J., Campos M., et al., Physiological highlights of manganese toxicity symptoms in soybean plants: mn toxicity responses, Plant Physiol. Biochem., 2017a, 113, 6–19

Sarto M.V.M., Lana M.C., Rampim L., Rosset J.S., Wobeto J.R., Effects of silicate application on soil fertility and wheat yield, Semina: Cienc. Agrar., 2015, 36, 4071-4082

SAS Institute, Procedure Guide for Personal Computers, Version 9.4., Cary, 2015

Schaller J., Brackhage C., Dudel E.G., Silicon availability changes structural carbon ratio and phenol content of grasses, Environ. Exp. Bot., 2012, 77, 283-287

Shapiro S.S., Wilk M.B., An analysis of variance test for normality (complete samples), Biometrika, 1965, 52, 591–611

da Silva F.C., Manual of chemical analyzes of soils, plants and fertilizers, In: da Silva F.C. (Ed.), 2nd ed., Brasília, Embrapa Informação Tecnológica, Embrapa Solos, Rio de Janeiro, 2009, (in Portuguese)

Stevens W.B., Mulvaney R.L., Khan S.A., Hoeft R.G., Improved diffusion methods for nitrogen and ^{15}nitrogen analysis of Kjeldahl digests, J. AOAC Int., 2000. 83, 1039-1046

Teixeira Filho M.C.M., Buzetti S., Andreotti M., Benett C.G.S., Arf O., Sá M.E., Wheat nitrogen fertilization under no till on the low altitude Brazilian Cerrado, J. Plant Nutr., 2014, 37, 1732-1748

United States Department of Agriculture – USDA. Keys to soil taxonomy, 11th ed., USDA, NRCS, Washington, 2010

Xu G., Fan X., Miller A.J., Plant nitrogen assimilation and use efficiency, Ann. Rev. Plant Biol., 2012, 63, 153-182

Yamaji N., Sakurai G., Mitani-Ueno N., Ma J.F., Orchestration of three transporters and distinct vascular structures in node for intervascular transfer of silicon in rice, Proc. Nat. Acad. Sci. U.S.A., 2015, 112, 11401-11406

Zawoznik M.S., Ameneiros M., Benavides M.P., Vázquez S., Groppa M.D., Response to saline stress and aquaporin expression in *Azospirillum*-inoculated barley seedlings, Appl. Microbiol. Biotechnol., 2011, 90, 1389–1397