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Inoculation of Plant Growth Promoting *Bacillus* spp. in N-Fertilized Maize Crop in Soils With High Organic Matter Content in South Brazil

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Abstract

Inoculation of seeds with plant growth promoting bacteria (PGPB) often increases maize yields in N-deficient soils. However, would yields increase if inoculation with PBPB was made in soils with high organic matter contents receiving N-fertilizer? In this article, we report the results of four field-experiments performed in the Southern Brazil (Campo Largo-PR and Lapa-PR, Brazil) during the growing seasons of 2017/2018 and 2018/2019, including treatments with three doses of N (0, 50 and 100 kg of N ha^{-1}) supplied as $(NH_4)_2SO_4$, and inoculation of *Azospirillum brasilense* Abv05 and Abv06, or, of new strains of *Bacillus* sp. (LGMB 143, LGMB 152, LGMB 319 or LGMB 326). Application of 50 kg N ha^{-1} increased yields by 32% in Campo Largo, and 16% in Lapa, in relation to non-fertilized plots, but doubling the N fertilization or including PGPB inoculants did not affect crop growth and productivity. The average yields in the plots with N and bacterial inoculation was 8,255 kg ha^{-1} in Campo Largo and 11,311 kg ha^{-1} in Lapa. Increases of grain yields were related to increases in plant height, shoot dry matter, ear length and diameter and 1000 grains mass. This study adds to the fact that scientists and farmers should rethink the paradigm of excessive doses of N fertilization on maize. Furthermore, inoculation of maize seeds with PGPB do not increase yields when the N demand of the crop is satisfied via N-fertilization.

Keywords: ammonium sulfate fertilizer, inoculant development, maize yield, plant growth promoting bacteria

1. Introduction

Maize is a cereal crop planted worldwide, which grains are used for human and livestock nutrition, row matter for biofuels, and an abundant number of industrial uses. Brazil is one of biggest maize producers in the world, having produced in the season 2020/2021 more than 102 million metric tons, using 18.5 million hectares for this crop (CONAB, 2021). The average yield of maize is 6.4 t ha^{-1} in Brazil, whereas it is 8.2 t ha^{-1} in Argentina, and 11.7 t ha^{-1} in the United States (OECD, 2021). Several aspects historically contributed to increases in maize yields, but surely the use of hybrid seeds instead of open-pollinated varieties and the input of high doses of fertilizers combined with industrial phytosanitary control of plagues, and plant diseases are considered the most important (Edgerton, 2009). However, the N losses due to inefficient uptake by plants, leaching, volatization and erosion in well drained soils may vary from 50% to 85% of the N applied (Noor, 2017; Greer & Pittelkow, 2018; Coppess & Bullock, 2018). Thus, the prevailing maize production system based on excessive N fertilization may jeopardize agriculture sustainability. Improving the processes of crop production systems (Cassman et al., 2002), applying slow-releasing fertilizers (Zhu et al., 2020), splitting doses over time (Abbasi et al., 2012; Noor, 2017), increasing plant density and planting hybrids and varieties with improved capacity to absorb soil N (Noor, 2017) may help to increase N use efficiency by the crop, but it does not come without increasing monetary costs.

On the other hand, one may think about using Plant Growth Promoting Bacteria (PGPB) to stimulate maize growth and improve its nutrition, while it decreases the dependence on fertilizer inputs, reducing the risks of crop failure and the environment pollution (Santos et al., 2019; dos Santos et al., 2020; Sousa et al., 2021; Bomfim et al., 2021; Duarte et al., 2022). The PGPB are bacteria that colonize the rhizosphere and stimulate plant growth by several physiological mechanisms, such as hormone production, biological N fixation, soil phosphate solubilization, biological control of plant diseases, plant genetic expression up-regulation, production of induced systemic resistance, among others (Souza et al., 2015; Santos et al., 2019; dos Santos et al., 2020, Duarte et al., 2022). The inoculation of PGPB improves plant growth and increases nutrient acquisition by several crops, including wheat (Hungria et al., 2010), maize (Riggs et al., 2001; Hungria et al., 2010), sugarcane (Schultz et al., 2014; Rosa et al., 2020), grain legumes (Santos et al., 2019; Swarnalakshmi et al., 2020; Barbosa et al., 2021), vegetables and pastures (Santos et al., 2019) and possibly replace N fertilizers without harm crop yields or the environment.

Brazil has inherited much knowledge in selecting, promoting merchandise and using PGPB inoculants in annual crops due to successful case of rhizobial inoculants in grain legume crops (Santos et al., 2019; Bomfim et al., 2021). The use of inoculants in grain legumes and cereals in Brazil represented an economy of about 25 billions of Brazilian reais (circa of USD 5 billions) in the purchase of N fertilizers in 2020 (ANPII, 2021). After the inoculants containing legume nodule inducing rhizobia, the most remarkable PGPB inoculant is based on *Azospirillum brasilense* (strains Abv05 and Abv06), which is inoculated in seeds or in furrow or by spray in cereals, grain legumes, sugarcane and pastures (Hungria et al., 2010; Santos et al., 2019; Bomfim et al., 2021; Ribeiro et al., 2022). There are also evidences that *A. brasilense* inoculation increases tolerance to drought and salinity (Fukami et al., 2017, 2018a, 2018b; Azeem et al., 2022). Several studies reassure the ability of *A. brasilense* to favor crops in the face of adversities in the field (Santos et al., 2019; Bomfim et al., 2021). Furthermore, there are inoculants containing strains of *Bacillus subtilis* and *B. megaterium*, which have been proved to be very efficient in solubilizing soil phosphate and promoting plant growth and crop yields (Ribeiro et al., 2018; de Sousa et al., 2020; Duarte et al., 2022) and other ones using *Pseudomonas fluorescens*, *B. lichenniformis*, *B. amyloliquefaciens*, *B. pumilus* and *Nitrospirillum amazonense* (MAPA, 2021). In fact, the search for new strains and processes is mandatory, given that strains interact with the environment and may gain or lose efficiency along time (Torres et al., 2012; Bomfim et al., 2021). Moreover, it is advantageous to have several alternatives of commercial products based on PGPB available because farmers may choose those that best fit to their environment or substitute the one that is not available in the market.

In this article, we report the results of four field-experiments performed in the Southern Brazil (Campo Largo and Lapa, Paraná) during the growing seasons of 2017/2018 and 2018/2019, which evaluated the potential of inoculating *A. brasilense* (Abv05 and Abv06) and *Bacillus* (LGMB 143, LGMB 152, LGMB 319 and LGMB 326) on maize seeds, in order to verify whether N-fertilized maize crop responds to inoculation of PGPB *Bacillus* spp.

2. Method

2.1 Field Experiments

The experiments were carried out with maize (*Zea mays* L.) in two locations in the state of Paraná (Brazil), in the municipalities of Campo Largo and Lapa, in the 2017/2018 and 2018/2019 growing seasons. Details about location, climate and soil are presented in Table 1. At each site, 20 soil subsamples were collected at depth 0-0.2 m and homogenized, forming a single composite sample for chemical and textural characterization. Chemical analyzes were performed according to Santos et al. (2018) and texture analysis with the Bouyoucos Densimeter methodology (Gee & Bauder, 1986). Soil was fertilized according to these analyses following recommendations of Pauletti and Motta (2019).

The experiments were carried out under randomized block design with 8 treatments each year, 5 replications in 2017/2018 and 6 replications in 2018/2019. In Campo Largo, the plot had 6 rows of 6 m in length spaced 0.75 m apart, the seeds were sown at a distance of 0.2 meters from each other with a population of 66,666 plants ha^{-1}. In Lapa, the plot had 7 rows of 7.5 m long spaced 0.45 m apart, the seeds were sown at a distance of 0.25 meters with a population of 88,888 plants ha^{-1}.

For the inoculants, the LGMB bacteria were grown in Luria-Bertani (LB) medium under continuous stirring (180 rpm) and temperature (28 ºC) for 24 hours. Subsequently, the concentration of bacterial was measured in a spectrophotometer at 600 nm to adjust the concentration as 1×10^8 cells ml^{-1}. Inoculation was performed with homogenization of 3 ml of bacterial suspension in 1800 maize seeds immediately before sowing.

Table 1. Experimental conditions

General information	Campo Largo	Lapa
Coordinates in 2017/2018	25°24′39.5″S; 49°29′42″W	25°50'38″S; 49°39'11.3″W
Coordinates in 2018/2019	25°19'31.6″S 49°28'44.8″W	by the side of previous year
Altitude (m)	987	869
Koppen-Geiger's Climate type	Cfb (Humid subtropical, oceanic, without dry season)	Cfb (Humid subtropical, oceanic, without dry season)
Accumulated precipitation during experiment (mm)	2017/2018: 1172.8 2018/2019: 765.8	2017/2018: 737.2
Average Temperature (°C)	2017/2018: 20.1 2018/2019: 21.3	2017/2018: 19.3
Soil characteristics		
Brazilian Classification [(1)]	Red latosol chernossolic	Cambisolic dystrophic red
USDA Classification	Eutrophic (Oxisol)	Latosol (Oxisol)
Clay:Silt:Sand, in g kg^{-1}	662:50:288	325:100:575
TOC (g dm^{-3})	2017/2018: 48.1 2018/2019: 33.9	2017/2018: 39.1
Available P (mg Kg^{-1})	2017/2018: 53.4 2018/2019: 56.5	2017/2018: 30.3
Available K (mg Kg^{-1})	2017/2018: 20.3 2018/2019: 54.7	2017/2018: 11.34
pH in $CaCl_2$	2017/2018: 4.96 2018/2019: 4.9	2017/2018: 4.92
pH in SMP	2017/2018: 5.2 2018/2019: 5.3	2017/2018: 6.1
Crop		
Maize cultivars	2017/2018: DKB 330 PRO3 2018/2019: AG8780	2017/2018: DKB 390 PRO3 2018/2019: XB4013
Seed treatment 2017/2018	Dermacor® (insecticide)	Dermacor® (insecticide)
Seed treatment 2018/2019	Vitavax® (fungicide) and Cropstar ®	Vitavax® (fungicide) and Cropstar®
Sowing/Harvest	1st November 2017 10 April 2018	25 October 2017 5 April 2018
Sowing/Harvest	21 November 2018 25 April 2019	14 November 2018 18 April 2019
Fertilizers in furrow at sowing	500 kg ha^{-1} of formulated fertilizer N-P-K: 8-20-20	500 kg ha^{-1} of formulated fertilizer N-P-K: 8-20-20

Note. [(1)] According to Santos et al. (2018).

Table 2. Bacterial strains

Bacteria	Year	Role in the experiment	Historic
Azospirillum brasilense Abv05 and Abv06	2017/2018 2018/2019	Control-inoculated as in the commercial inoculant AzoTotal®	They were first isolated from maize; it has been used in commercial inoculants for several crops in Brazil since 2009.
Bacillus sp. LGMB 143 (GenBank KJ667150)	2018/2019	Strain being tested as possible new inoculant	*In vitro* production of siderophore and root growth promotion
Bacillus sp. LGMB 152 (GenBank KJ667152)	2018/2019	Strain being tested as possible new inoculant	*In vitro* production of siderophore, control of plant pathogens
Bacillus sp. LGMB 319 (GenBank MF443192)	2017/2018 2018/2019	Strain being tested as possible new inoculant	When inoculated in maize in Leonard Jars, it increased growth as much as Abv05
Bacillus sp. LGMB 326 (GenBank MF443191)	2017/2018 2018/2019	Strain being tested as possible new inoculant	It was isolated from high yielding maize hybrids; when inoculated in maize in Leonard jars, it increased maize growth as much as Abv05, and accumulated higher N concentration in leaves.

The treatments T1, T2 and T3 plots were not inoculated and received the doses of (T1) 0 (zero), (T2) 50 and (T3) 100 kg N ha^{-1}, respectively, supplied as $(NH_4)_2SO_4$ (20% N) at the vegetative stage V4 of the crop (four open leaves) on soil surface. The treatment T4 consisted of planting seeds inoculated with *Azospirillum brasilense* (Abv-05 and Abv-06) as formulated in the commercial inoculant AzoTotal® (Total Bioctenologia Ltd., Curitiba), containing 1×10^8 azospirilla cells ml^{-1}. The treatments T5, T6, T7 and T8 consisted of planting seeds inoculated respectively with *Bacillus* sp. LGMB 319, *Bacillus* sp. LGMB 326, *Bacillus* sp. LGMB 143 and *Bacillus* sp. LGMB 152, which information is given in Table 2. The treatments T9 and T10 were only included in 2017/2018 and consisted in the consortium of LGMB 319 + LGMB 326 or of Abv05 + Abv06 + LGMB 319 + LGMB 326. All inoculated treatments also received 50 kg N ha^{-1} supplied as $(NH_4)_2SO_4$ (20% N) at the vegetative stage V4, like in T2

2.2 Plant Measurements

The opposite leaves and below the ears of 5 plants were collected at the tasseling stage of the crop. They were harvested at random, rinsed with deionized water and dried in an oven with forced ventilation at 60 ºC for 72 hours. The leaves were ground (2 mm fragments) and their P contents were determined according to Martins & Reissmann (2007) and the N by dry combustion in the elemental analyzer (CNHOS, Vario EL III model elementar®, Germany). Plant height and diameter measurements were made on 10 plants per plot. Plant height was measured from the ground surface to the point of insertion of the flag leaf. The stem diameter was measured at the height of the first visible internode. The determination of the total dry weight of the shoot (including ears without grains) was made on 5 plants at the physiological maturity stage (R6), which were cut close to the ground, dried in a greenhouse with forced ventilation at 60 ºC for 14 days and weighed. Five ears were separated from each plot to determine their length and diameter. The ears were measured from the base to the apex. Ear diameter was measured at the middle of ear length.

2.3 Grain Yield

The ears were harvested manually from a useful area, discarding the two lateral lines, the first and the last meter of each planting line. The ears were threshed in a mechanical thresher. Grains were counted (for mass of one thousand grains) and weighed and their weights corrected to 13% moisture and extrapolated to kg ha^{-1}. A subsample of grains was removed for nutrient determination after milling in a 2 mm mesh, using the same procedure described above for shoot nutrient measurements.

2.3 Statistical Analyses

The data were submitted to the Shapiro-Wilk test to confirm the variance analysis (ANOVA) assumptions. ANOVA was performed with open source software R with the Agricolae package (de Mendiburu, 2009). Means were compared by TUKEY test at 5% probability.

3. Results and Discussion

There are several examples in the literature in which inoculation of seeds with PGPB improve plant growth and grain yields, reducing the rates of fertilizers that are applied to the crop (Adesemoye & Kloepper, 2009; Hungria et al., 2010; Ribeiro et al., 2018; Ribeiro et al., 2022; Santos et al., 2019; Ikeda et al., 2020). However, the development of an inoculant is continuous process that requires several steps of selection and experiments to confirm the recommendation for its use; and, it takes several years to decades to obtain a good candidate for inoculant production (Hungria et al., 2005; Santos et al., 2019; Bomfim et al., 2021; Duarte et al., 2022). For example, the strains Abv05 and Abv06 of *A. brasilense* was first indicated as potential PGPB many years earlier, but it was not until a network of field-experiments at national scale confirmed that they increase yields of wheat and maize (Hungria et al., 2010; Santos et al., 2019) that it was recommended for industrial inoculant production. Likewise, the recommendation of commercial inoculant based on *Bacillus* spp. for maize and soybeans took several years (Duarte et al., 2022).

Following those examples, the strains *Bacillus* sp. LGMB 143, *Bacillus* sp. LGMB 152, *Bacillus* sp. LGMB 319 and *Bacillus* sp. LGMB 326 (Table 2) were isolated from rhizosphere of maize hybrids (Ikeda et al., 2013), characterized, and selected based on *in vitro* capacity of siderophores production, phosphate solubilization, biological N fixation and acid indolacetic production (Ikeda, 2014), identified by 16S rDNA sequencing (cf. GeneBank deposit; Table 2), and evaluated regarding their potential to promote maize growth in Leonard jars (Ikeda et al., 2020). In this study, field-experiments in Campo Largo and Lapa analyzed the effects of these "LGMB" strains to support plant height, shoot dry matter, ear length and diameter, and 1000 grains mass in relation to non-fertilized plot fields (Tables 3, 4, and 5). The results showed that application of 50 kg N ha^{-1} increased yields by 32% in Campo Largo, and 16% in Lapa when compared with non-fertilized plots, but also indicated that doubling the N fertilization or including PGPB inoculants did not affect productivity (Table 5).

Table 3. Plant growth indicators in maize inoculated or not with *Bacillus* sp LGMB 143, LGMB 319, LGMB 326 and *Burkholderia* sp. LGMB 152

Measurement	**Plant height (m)**				**Shoot dry matter (g plant^{-1})**			
Field	Campo Largo		Lapa		Campo Largo		Lapa	
Harvest	2018	2019	2018	2019	2018	2019	2018	2019
Zero N fertilizer	2.12b*	2.53	2.60b	n.d.	94.3b	n.d.	107.1	169
50 kg N ha^{-1}	2.25a	2.61	2.67ab	n.d.	125.2a	n.d.	117.1	166
100 kg N ha^{-1}	2.19ab	2.56	2.68ab	n.d.	108.5ab	n.d.	116.9	167
Azospirillum Abv05/Abv06	2.20ab	2.54	2.69ab	n.d.	106ab	n.d.	113.7	180
Bacillus LGMB 319	2.21a	2.56	2.67ab	n.d.	110.9ab	n.d.	113.1	146
Bacillus LGMB 326	2.23a	2.55	2.64ab	n.d.	108.7ab	n.d.	109.4	153
Bacillus LGMB 143	n.d.	2.56	n.d.	n.d.	n.d.	n.d.	n.d.	155
Burkholderia LGMB 152	n.d.	2.58	n.d.	n.d.	n.d.	n.d.	n.d.	144
Bacillus LGMB 319 + LGMB 326	2.19ab	n.d.	2.69ab	n.d.	108.4ab	n.d.	117.1	n.d.
Azospirillum Abv05 + Abv06 + *Bacillus* LGMB 319/LGMB 326	2.22ab	n.d.	2.7a	n.d.	120.6ab	n.d.	117.3	n.d.
CV(%)	2.47	3.2	1.90	n.d.	13.37	n.d.	8.71	28

Note. * Different letters indicate significant differences by the Tukey test at $p < 0.05$. n.d. = not determined.

Table 4. Plant growth indicators in maize inoculated or not with *Bacillus* sp LGMB 143, LGMB 319, LGMB 326 and *Burkholderia* sp. LGMB 152

Measurement	**Ear length (cm)**				**Ear diameter (cm)**			
Field	Campo Largo		Lapa		Campo Largo		Lapa	
Harvest	2018	2019	2018	2019	2018	2019	2018	2019
Zero N fertilizer	16b*	n.d.	15.7	n.d.	45.3b	n.d.	54.2	n.d.
50 kg N ha^{-1}	18.6a	n.d.	17.2	n.d.	48.4a	n.d.	55.7	n.d.
100 kg N ha^{-1}	18.3a	n.d.	16.9	n.d.	47.2ab	n.d.	55.2	n.d.
Azospirillum Abv05/Abv06	17.7a	n.d.	16.5	n.d.	46.6ab	n.d.	55.3	n.d.
Bacillus LGMB 319	18.2a	n.d.	16.6	n.d.	46.8ab	n.d.	54.8	n.d.
Bacillus LGMB 326	18a	n.d.	16.2	n.d.	47.1ab	n.d.	54.5	n.d.
Bacillus LGMB 143	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Burkholderia LGMB 152	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bacillus LGMB 319 + LGMB 326	17.1ab	n.d.	16.9	n.d.	46.2b	n.d.	54.7	n.d.
Azospirillum Abv05 + Abv06 + *Bacillus* LGMB 319/LGMB 326	18.7a	n.d.	17.3	n.d.	47.2ab	n.d.	55.6	n.d.
CV(%)	6.21	n.d.	5.17	n.d.	2.63	n.d.	2.18	n.d.

Note. * Different letters indicate significant differences by the Tukey test at $p < 0.05$. n.d. = not determined.

Maize is a very responsive crop to N fertilization (Cassman et al., 2002; Abbasi et al., 2012; Noor, 2017; Coppess & Bullock, 2018; Ferreira et al., 2021), but there were no increases in yields from 50 to 100 kg N ha^{-1} in the experiments reported here (Table 5) neither there were increases in total N and total P in shoot (Table 6). For the four experiments, doses of 50 kg of N ha^{-1} were sufficient to improve yields in relation to zero N application. The completion of the demand of N required for the crop may have resulted from mineralization of soil organic matter (Osterholz et al., 2017; Ferreira et al., 2021), particularly because the areas used for this study have been managed under no-tillage for many years, had high contents of total organic carbon and had little limitation of other nutrients (Table 1). Additionally, in the inoculated treatments without application of N fertilizer, N could have been supplied from mineralization of soil organic matter (Osterholz et al., 2017; Ferreira et al., 2021), from biological nitrogen fixation by the inoculated bacteria and by the positive side-effect of increasing root growth (Ikeda, 2014; Hungria et al., 2010; Souza et al., 2015; Santos et al., 2019; Bomfim et al., 2021; Ribeiro et al., 2022). It is possible that the biological processes regarding N uptake and assimilation were more synchronized with the plant demand than when it is supplied by application of N fertilizer. For example, *Bacillus amyloliquefaciens* (Nautiyal et al., 2013) and *A. brasilense* (Fukami et al., 2017, 2018b) are capable to activate plant gene expression once they colonize the rhizosphere of rice and maize, respectively; and from that, to increase plant tolerance to drought and salinity stress by changing plant physiology (Fukami et al., 2017, 2018a, 2018b). Several PGPB stimulate root growth, realize biological nitrogen fixation and solubilize soil phosphate (Ikeda, 2014; Souza et al., 2015; Santos et al., 2019; Bomfim et al., 2021), which probably act systematically to improve plant nutrition by fostering plants to exploit better the soil resources (Souza et al., 2015; Sousa et al., 2020; Ribeiro et al., 2022). For this reason, inoculating PGPB on seeds may seem as good as applying more N, but actually, it is better, because it is cheaper and more environmental-friendly.

Table 5. Mass of 1000 grains and yield of maize inoculated or not with *Bacillus* sp LGMB 143, LGMB 319, LGMB 326 and *Burkholderia* sp. LGMB 152

Measurement	**1000 grains mass (g)**				**Grain yield (kg ha^{-1})**			
Field	Campo Largo		Lapa		Campo Largo		Lapa	
Harvest	2018	2019	2018	2019	2018	2019	2018	2019
Zero N fertilizer	149b*	310.0	276.8b	274a	6,180.7b	9,342.3	9,912.3b	n.d.
50 kg N ha^{-1}	185.2a	330.1	298.2a	294a	9,131.3a	10,093.8	11,801.2a	n.d.
100 kg N ha^{-1}	166.4ab	331.2	279.0ab	292a	8,105.4a	10,352.7	11,123.5ab	n.d.
Azospirillum Abv05/Abv06	165.4ab	312.9	289.6ab	304a	8,119.4a	9,866.1	11,426.5ab	n.d.
Bacillus LGMB 319	160.2ab	312.9	292.0ab	279a	8,420.6a	9,446.4	11,336.4ab	n.d.
Bacillus LGMB 326	167.9ab	328.1	285.5ab	284a	8,402.5a	9,922.6	11,559.9ab	n.d.
Bacillus LGMB 143	n.d.	324.5	n.d.	280a	n.d.	9,623.5	n.d.	n.d.
Burkholderia LGMB 152	n.d.	320.0	n.d.	293a	n.d.	9,943.5	n.d.	n.d.
Bacillus LGMB 319 + LGMB 326	149.6b	n.d.	292.6ab	n.d.	7,887.8a	n.d.	11067.3ab	n.d.
Azospirillum Abv05/Abv06 + *Bacillus* LGMB 319/LGMB 326	158.5b	n.d.	290.6ab	n.d.	8,464.6a	n.d.	11164.2ab	n.d.
CV(%)	9.75	6.78	3.99	6.0	13.71	12.91	8.05	n.d.

Note. * Different letters indicate significant differences by the Tukey test at $p < 0.05$. n.d. = not determined.

Table 6. Total N and P (g kg^{-1}) in shoot dry matter and grains in maize inoculated or not with *Bacillus* sp LGMB 143, LGMB 319, LGMB 326 and *Burkholderia* sp. LGMB 152

Measurement	**Total N in Shoot Dry Matter**				**Total P in Shoot Dry Matter**			
Field	Campo Largo		Lapa		Campo Largo		Lapa	
Harvest	2018	2019	2018	2019	2018	2019	2018	2019
Zero N fertilizer	19.9b*	20.0	27c	10.7b	2.4	2.6	2.2	2.9
50 kg N ha^{-1}	27.4a	24.5	31a	24.4a	2.6	2.8	2.4	3.9
100 kg N ha^{-1}	25.8a	22.9	29.7ab	24.3a	2.5	2.9	2.3	4.2
Azospirillum Abv05 + Abv06	26.4a	20.9	29.3abc	24.1a	2.5	2.6	2.3	3.8
Bacillus LGMB 319	25.3a	23.5	28.6bc	22.0a	2.6	2.6	2.3	3.8
Bacillus LGMB 326	26.8a	22.9	27.9bc	22.8a	2.5	2.9	2.3	4.3
Bacillus LGMB 143	n.d.	23.6	n.d.	22.4a	n.d.	2.6	n.d.	3.6
Burkholderia LGMB 152	n.d.	22.5	n.d.	22.5a	n.d.	2.8	n.d.	4.0
Bacillus LGMB 319 + LGMB 326	25.3a	n.d.	28.8abc	n.d.	2.5	n.d.	2.4	n.d.
Azospirillum Abv05 + Abv06 + *Bacillus* LGMB 319 + LGMB 326	27.4a	n.d.	28.7abc	n.d.	2.6	n.d.	2.5	n.d.
CV (%)	10.21	12.0	5.41	9.0	6.28	15.15	5.94	23

Note. * Different letters indicate significant differences by the Tukey test at $p < 0.05$. n.d. = not determined.

Future studies should include treatments only with PGPB without topdressing N fertilization to confirm the hypothesis that PGPB may supply N sufficiently to sustain high grain yields. Additionally, it should be mention that the paradigm of increasing doses of N steadily increases grain yields should be revisited with the new technological advances obtained in the field of agricultural microbiology. In this experiment, either N or PGPB inoculation increased maize growth, probably because soil physical, chemical and biological attributes were satisfactory for plant growth. On the other hand, there is an increasing body of evidences that selected microbes can support plant growth as much as conventional fertilizers (Adesemoye & Kloepper, 2009; Souza et al., 2015; Santos et al., 2019; Barbosa et al., 2021; Ribeiro et al., 2022) and agriculturists should consider that fact in the formulation of recommendation tables of N fertilization in the future. Therefore, this study adds to the fact that scientists and farmers should rethink the paradigm of excessive doses of N fertilization on maize, and gather more knowledge to adjust doses, combining reduced doses of N fertilizers with biological inputs.

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