

Rock phosphate fertilization harms *Azospirillum brasilense* selection by maize

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Abstract

Maize is the most important crop cultivated worldwide. It needs a significant amount of nitrogen and phosphorus fertilization to maintain high yields. However, the high cost of fertilization makes production more expensive and damages the environment. The present study used *Azospirillum brasilense* and *Bacillus subtilis* bacteria in an attempt to supply nitrogen and phosphorus fertilization to a maize crop. The experiment was carried out with maize plants under greenhouse conditions with a factorial scheme (4 x 2 x 2), where the first factor corresponded to bacterial inoculation: (a) control (no inoculation); (b) *A. brasilense* inoculation (AZ); (c) *B. subtilis* inoculation (BS) and (d) inoculation with a mixture of (AZ+BS), the second factor corresponded to the presence or absence of rock phosphate fertilization and the third factor corresponded to the presence or absence of top-dressed nitrogen fertilizer. Evaluated plant parameters were height, shoot dry matter (SDM), root dry matter (RDM), and soil parameters were total colony forming units of bacteria (CFU), nitrogen, soluble phosphorus and microbial biomass carbon (MBC). Although some parameters were improved with mineral fertilization in general, the findings showed that there were many adverse effects with the use of rock phosphate fertilization and *A. brasilense* inoculation. When both were applied together in treatments, there was reduction in plant height, microbial biomass carbon and total number of bacteria compared to treatments without rock phosphate fertilization. These results strongly suggest that rock phosphate harms the ability of *A. brasilense* to promote plant growth and demonstrate the necessity of new studies to verify whether this negative effect occurs under field conditions and could reduce yields in maize crop production.

Keywords: *Azospirillum brasilense*, *Bacillus subtilis*, nitrogen fixation, phosphate solubilization, *Zea mays*

Abbreviations: RDM = root dry matter; SDM = shoot dry matter; CFU = colony forming units; AZ = *Azospirillum brasilense*; BS = *Bacillus subtilis*; MBC = microbial biomass carbon

Introduction

Maize is an important crop, occupying a prominent position among agricultural species worldwide, being a food of high energetic value with relatively low cost. In addition, it can be used in a large number of products (Hussain et al., 2018). Maize is the most important crop, responsible for feeding millions of people around the world (Teixeira et al., 2018).

The essential nutrients for maize growth and development are phosphorus (P) and nitrogen (N). P is a vital compound of nucleic acids, phospholipids, and sugar phosphates and is an essential part of energy metabolism, photosynthesis, respiration and membrane transport in plants (Galindo-Castaneda et al., 2018; Xu et al., 2018), and nitrogen is found in the majority of macromolecules and in many secondary signaling compounds, including proteins, nucleic acids, cell wall components, hormones and vitamins (Romaniuk et al., 2018).

Although phosphorus is abundant in many soil types, most P is not readily available to plants due to leaching and/or immobilization. The reason for this is the formation of less soluble Fe, Al or Ca phosphates due to contact with phosphate anions in soil (Mendes et al., 2014). In

consequence, the frequent application of soluble inorganic forms of P are required to provide a satisfactory supply to plants. Such applications of P to the soil are usually provided in the form of chemical fertilizers. However, chemical fertilizer production is expensive and can potentially lead to environmental damage due to the use of energy intensive treatment with sulfuric acid at high temperature (Vassilev et al., 2006).

The direct application of rock phosphate to the soil could be one way to minimize pollution and reduce the costs of chemical treatment. It has been used by some farmers due to its advantages of being both low cost and a slow release phosphate fertilizer and the apparent positive results in terms of root development and plant uptake (Zapata and Zaharah, 2002). On the other hand, for other farmers, the low solubility presented by rock phosphate has discouraged its recommendation for direct use as a source of P for crops. It requires a long time for the P to become available (Vassilev and Vassileva, 2003).

A potential strategy to accelerate both the P solubilization process into soil and P uptake by roots is the use of plant

growth promoting rhizobacteria, such as *Bacillus subtilis* and *Azospirillum brasilense*, with the abilities to solubilize P and produce indole acetic acid, respectively (Santos et al., 2017; Santos et al., 2018).

PGPR can be found in the rhizosphere, on root surfaces and in root associations (Small and Degenhardt, 2018). The rhizosphere is the small volume of soil surrounding plant roots, which is under the direct influence of the roots (Morgan et al., 2005). It is a highly dynamic and diverse microenvironment and many of the processes that occur in the rhizosphere have been performed by microorganisms with the ability to promote plant growth (Di Salvo et al., 2018).

Among PGPR genera, *Azospirillum* and *Bacillus* are the most studied and characterized (Baldani and Baldani, 2005). *A. brasilense* is able to fix nitrogen in association with grass plants, in addition to producing and secreting a large amount of phytohormones, mainly auxins (indol-3- acetic acid) and gibberellins, as well as nitric oxide, that together, seem to contribute to root development thus improving the efficiency of the uptake of nutrients such as phosphorus and water (Santini et al., 2018).

Among *Bacillus* species, *B. subtilis* is well known as a plant growth promoter capable of mineral solubilization, nitrogen fixation, and the production of antibiotics, siderophores and secondary metabolites, which suppress plant pathogens (Chen et al., 2007), in addition to the capacity to increase nutrients in the soil and their absorption by host plants (Stamenkovic et al., 2018).

The utilization of plant growth promoting microorganisms requires in-depth knowledge of their ecology and how they are affected by agricultural practices including the application of fertilizers such as nitrogen and phosphorus (Berg, 2009; Small and Degenhardt, 2018). Additionally, the safe application of microorganisms to the soil to promote plant growth requires knowledge about their behavior and interaction with soil microbiota, including wild populations of microorganisms and nutrients in the rhizosphere (Small and Degenhardt, 2018).

In this context, this study aimed to elucidate the behavior of *A. brasilense* and *B. subtilis* in the presence or absence of rock phosphate and top-dressed nitrogen fertilizer in maize crops.

Results and discussion

The inoculation *versus* rock phosphate fertilization interaction significantly affected plant height (Figure 1a). In the treatment that received *A. brasilense* without P, the average plant height was 76.37 cm and in the treatment that received *A. brasilense* with rock phosphate fertilization, the average plant height was 64.12 cm. The difference between treatments was 16.0%

According to Mahmood and Ali, (2015), microorganisms used as biofertilizers can serve as potential tools to support agricultural production, without deteriorating soil and the environment. The soil microorganism composition is related to the presence of essential elements (Adesemoye and Kloepper, 2009). In addition, microorganisms are mainly responsible for nutrient cycling in plant-soil-microorganism systems (Ansari and Malik, 2007; Hussain et al., 2018; Small and Degenhardt, 2018) However, there is a lack of

information about the influence of rock phosphate fertilization on microorganism-plant-soil associations.

On the other hand, when *A. brasilense* was inoculated into plants without rock phosphate P fertilization, there was an increase in plant height, because *A. brasilense* was able to associate with plants and express their characteristics to promote plant growth, including IAA production and phosphate solubilization. Szilagy-Zecchin et al., 2014 and Kumar et al., 2017 reported that some bacteria species are able to solubilize P, previously present in the soil, and provide it to plants, but this action was significantly diminished when rock phosphate fertilization was applied.

As this study was carried out in a short period time, it is likely that the difference between the heights of plants that received rock phosphate fertilization compared with plants that did not, are more related to IAA produced by *A. brasilense* than to P release (Saengsanga, 2018). Most likely, rock phosphate harmed the plant-microorganism interaction, reducing IAA production by microorganisms and, as a result, the height of plants receiving rock phosphate was lower than the height of plants that did not receive rock phosphate. Moreover, for the same reason, plants inoculated with *A. brasilense* without P had SDM 28.8% higher than plants with P fertilization (Figure 1 b).

These results are interesting and clearly show that the presence of rock phosphate fertilization might reduce the efficiency of *A. brasilense* to promote plant growth. To our knowledge, this is the first report concerning this issue, suggesting that in maize crops, rock phosphate fertilization with *A. brasilense* inoculation should not be used as this may result in lower microbial promotion of plant growth. The reasons for this reduction are unknown.

Usually, increasing SDM levels results in higher biomass and, consequently higher yield (Soares et al., 2016; Saengsanga, 2018). This increase in SDM is due to the inoculation of microorganisms able to produce phytohormones such as auxins, gibberellins and cytokinins (Small and Degenhardt, 2018; Stamenkovic et al.; 2018). Regarding the production of phytohormones, Cassan and Diaz-Zorita (2016), reported that plant growth promoted by *Azospirillum* spp. is not due to one growth promotion mechanism only, but many of them, which increase the probability of successful associations of plant and microorganisms in the absence of mineral fertilizers.

The interaction between rock phosphate fertilization and *A. brasilense* inoculation significantly affected soluble phosphorus content in the soil (Figure 1c). The treatment that received *A. brasilense* inoculation with rock phosphate fertilization showed an increase of 48.3% in the amount of soluble phosphorus compared to the treatment that received *A. brasilense* without rock phosphate fertilization. Certainly, this increase in soil available P was promoted by the ability of *A. brasilense* to solubilize P.

Once there is a high amount of insoluble P retained in the soil, it can become available and be provided to plants by the action of microorganisms, an ability for which *A. brasilense* is outstanding (Adesemoye and Kloepper, 2009). However, a high amount of soluble P in soil, solubilized by bacteria, does not result in an increase of P to plants. Usually, it indicates that plants will not suffer deficiency of this nutrient, which is desirable.

This study tested the potential for rock phosphate solubilization of *A. brasilense* and *B. subtilis* *in vitro* and in

field conditions. *B. subtilis* demonstrated greater ability to solubilize P from fluorapatite than *A. brasilense*, with the former providing approximately four times more soluble P (300.0 µg P per mL filtrate) than *A. brasilense* (22.50 µg P per mL filtrate) (Figure 2f). This result indicates that *B. subtilis* could have better performance in the field because its ability to solubilize P under test conditions was higher.

Although the *in vitro* P solubilization test suggested the use of *B. subtilis*, both strains were applied in the field. Unexpectedly, results opposite those of the *in vitro* results were obtained, and *A. brasilense* showed better performance in improving P solubilization in the soil in maize crops.

Although *A. brasilense* showed better performance for solubilizing P in the soil, the high P availability in the soil guarantees the supply of this nutrient to plants. However, it might not reflect extra benefits to plants, such as an increase in nutritional status compared to plants that received *B. subtilis*. Interestingly, plants that received *A. brasilense* without rock phosphate fertilization, although showing lower available P in the soil, had higher height and SDM than plants that received rock phosphate fertilization with *A. brasilense* inoculation (Figures 1a and 1b). An important research topic is determining which of these parameters are more important for the plant to increase its yield - the increase in height and SDM or the increase in available P in the soil. More studies are needed to clarify these questions. Meanwhile, in the presence or absence of rock phosphate fertilization, *B. subtilis* did not affect height, SDM, or P solubilization in the soil (Figures 1a, 1b, 1c). (Felici et al., 2008) showed that both *B. subtilis* and *A. brasilense* use independent signaling pathways, and the different characteristics of each bacteria could explain this behavior. In the control treatment, the presence of rock phosphate fertilization decreased the total nitrogen concentration in the soil. Most likely, phosphate fertilization inhibited the action of diazotrophic bacteria previously present in the soil, which are naturally responsible for nitrogen fixation. In addition, *A. brasilense* and *B. subtilis* had opposite behavior in the presence or absence of rock phosphate fertilization in relation to nitrogen fixation. Rock phosphate fertilization decreased the nitrogen concentration in the soil whereas *A. brasilense* increased the nitrogen concentration when applied in combination with *B. subtilis* (Figure 1d).

Soil physicochemical properties and plants can directly determine the specific rhizosphere microbial composition by changing the rhizospheric environment, with some microorganisms benefiting. Plants modulate their exudate composition to select microorganisms of their interest (Lareen et al., 2016). Indeed, rock phosphate fertilization was a determining factor in creating an unfavorable environment for *A. brasilense* once this bacterium was not able to express its characteristics for promoting plant growth. These results strongly suggest that studies should verify the influence of the chemical composition in the rhizosphere on the behavior of microorganisms, as in (Dumbrell et al., 2010; Long et al., 2018), who demonstrated that P availability affects the ability of plant exudates to solubilize microorganisms.

Phosphate fertilization *versus* inoculation significantly affected MBC, where *A. brasilense* inoculation in the absence of rock phosphate resulted in an increase of 72.42% in MBC compared to the treatment that received *A.*

brasilense with rock phosphate fertilization. Interestingly, the same did not occur with *B. subtilis* inoculation. MBC measurement can show the active and dynamic reservoir of soil organic matter, which is mainly composed of fungi, bacteria, and actinomycetes (Ma et al., 2011). In the present study, both inoculums were composed of bacteria, and the expectation was that with the inoculation of these bacteria, the MBC levels would have increased. However, there was a strong reduction in MBC when *A. brasilense* was applied with rock phosphate fertilization, whereas for *B. subtilis*, no effect was observed (Figure 1e).

This result shows that although MBC reflects the presence of many microorganisms present in the soil, in this study, MBC levels were determined mainly by the presence of the two inoculated bacteria. Most likely, it shows the great influence of bacterial populations on the MBC measurement, at least in vase conditions. Another interesting result is that for microorganisms to release soilborne nutrients for plant use, these microorganisms need to grow in the rhizosphere first (Jacoby et al., 2017); however, when rock phosphate was released in the soil with *A. brasilense*, the number of total bacteria and MBC were reduced. On the other hand, the phosphorus bioavailability increased (Figures 1e, 1f and 1c). The reasons for this effect are not clear yet.

Plants and microorganisms have developed a close relationship, since this relationship allowed them to coexist many years ago (Nihorimbere et al., 2011; Small and Degenhardt, 2018; Etesami and Maheshwari, 2018; Stamenkovic et al., 2018). However, when the soil composition is changed, this relationship is also changed. The plant-soil-microorganism system works together contributing to plant health and yield, but for this to occur, the soil composition needs to offer a favorable environment for the majority of microbial populations present in the soil (Chaparro et al., 2014).

Interestingly, the inoculation of only one bacterium species as well as *A. brasilense* in the absence of rock phosphate was enough to increase the MBC level. On the other hand, the inoculation of *A. brasilense* with rock phosphate fertilization decreased this level.

Nitrogen fertilization versus inoculation significantly affected plant height. The mixture of *A. brasilense* and *B. subtilis* in the absence of top-dressed nitrogen fertilization (nitrogen fertilization was made during sowing) increased plant height by 9.8% compared to the treatment that received the same mixture of microorganisms with top-dressed nitrogen fertilization (Figure 2a). Similarly, when nitrogen fertilization was carried out without the inoculation of microorganisms, the root dry matter increased by 30.1% compared to the absence of both microorganisms and nitrogen fertilization (Figure 1b).

Many studies have shown that nitrogen fertilization harms *Rhizobium* nodulation in soybean (Zhou et al.; 2006; Yong et al.; 2018; Nyoki and Ndakidemi, 2018). This phenomenon occurs because nitrogen inhibits the ability of legumes to recruit *Rhizobium*, once the necessary amount of this nutrient has been supplied. However, *Rhizobium* is associated with plant roots and both *A. brasilense* and *B. subtilis* are usually rhizospheric, even though in specific conditions they can become endophytes. Likewise, the presence of nitrogen from nitrogen fertilization also inhibits the action of rhizospheric microorganisms related to biological nitrogen fixation. This is an indication of

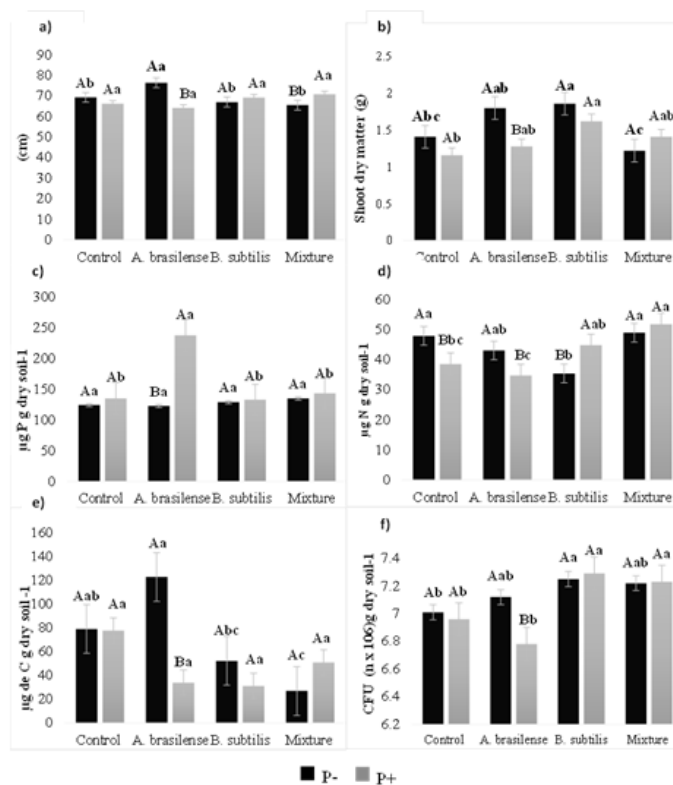


Fig. 1. Results of inoculation and rock phosphate fertilization for: a) plant height; b) shoot dry matter (SDM), c) phosphorus content in the soil, d) nitrogen content in the soil, e) microbial biomass carbon and f) total number of bacteria in colony forming units (CFU).

antagonistic movement of these nutrients as shown by Nyoki and Ndakidemi (2018).

Plants that received the mixture of bacteria had their root dry matter increased by 30.1% compared to plants that did not receive the mixture of bacteria without nitrogen fertilization (Figure 2c). Dissimilarly, (Felici et al., 2008) showed no effect of *B. subtilis* and *A. brasilense* coinoculation in tomato. Compounds released by roots act as chemical attractants for a large number of microbial communities. The composition of these compounds depends on the physiologic status of the plant species and microorganisms (Kang et al., 2010). The microorganisms inoculated in this study have the ability to fix nitrogen and produce IAA. Most likely, the absence of nitrogen fertilization promoted the selection of these microorganisms by plants to supply their needs. As these microorganisms are IAA producers, this phytohormone is probably responsible for root growth. Interestingly, the same effect did not occur when nitrogen was applied. Likewise, the presence of nitrogen inhibited the selection of microorganisms and their effects on root growth.

Plants that received *A. brasilense* inoculation without top-dressed N fertilization increased P concentration by 41.6% in the soil compared to plants that received the same bacterium with N fertilization. The absence of N fertilization provided *A. brasilense* with the ability to solubilize more P. The reasons for this are not fully clear, and further studies are needed to understand the relationship between nitrogen fertilization and phosphorus solubilization with *A. brasilense* inoculation. For *B. subtilis* inoculation, nitrogen

fertilization did not have significant effects on any parameters studied (Figure 2c).

Interestingly, treatments that received nitrogen fertilization with *A. brasilense* or *B. subtilis* inoculation showed an increase in the nitrogen content in the soil. However, these nitrogen levels in the soil were not significantly different from those of the treatment that did not receive nitrogen fertilization. On the other hand, control treatment without microbial inoculation resulted in higher nitrogen content in the soil in the absence of nitrogen fertilization. These results suggest that the nitrogen content in the soil in the control treatment was provided from diazotrophic bacteria previously present in the soil, and its capacity to increase the nitrogen content in the soil created N levels higher than those seen with nitrogen fertilization (Figure 2d).

Nitrogen fertilization increased the microbial biomass carbon when *A. brasilense* was inoculated, but the same effect did not occur for *B. subtilis* inoculation. Usually, nitrogen fertilization increases the microbial communities when there is nutritional stress for this nutrient, but this behavior depends on the bacterial species (Figure 2e).

Chu et al. (2007), reported an increase in MBC related to nitrogen fertilization due to organic matter mineralization, thus the total number of microorganisms increased as a result of the root biomass increase and high production of exudates.

Figure 2f shows the IAA levels produced by both *A. brasilense* and *B. subtilis*, showing that *A. brasilense* has a greater ability to produce IAA than *B. subtilis*.

Figure 2g shows the rock phosphate solubilization *in vitro* test for *A. brasilense* and *B. subtilis*. As mentioned above,

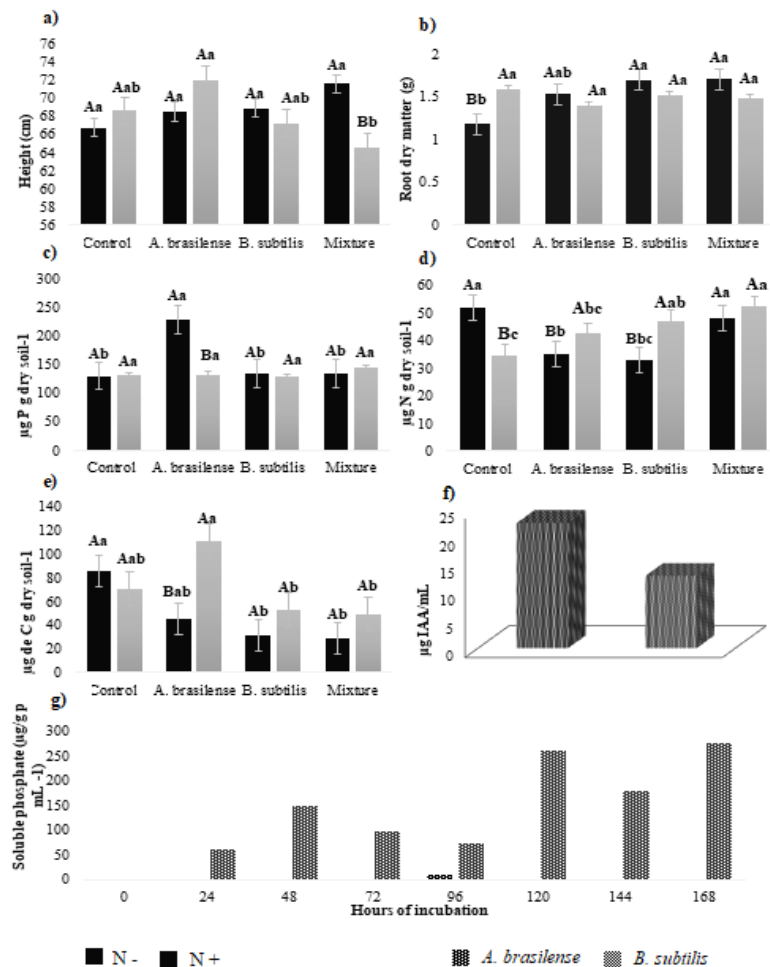


Fig. 2. Results of the interaction between inoculation and nitrogen fertilization: a) plant height; b) root dry matter (RDM), c) phosphorus content in the soil, d) nitrogen content in the soil; e) microbial biomass carbon; f) indole acetic acid concentration and g) daily concentration of soluble phosphorus *in vitro*

the *B. subtilis* strain solubilized higher amounts of P compared to *A. brasilense*, and unexpectedly, the soil results were the opposite.

Materials and methods

Seedling and fertilization

The study was carried out in a greenhouse located at the State University of São Paulo, Campus of Jaboticabal – SP. Four maize seeds (*Zea mays* L) of the 2B707 PW commercial hybrid (Dow Agro Sciences) were planted in plastics vases of 12 dm³ filled with eutrophic red Latosol soil (USDA, 2018) with clay (55 g of clay kg⁻¹ of soil). The chemical characterization of the soil at 20 cm in depth was measured according to (Raj and Quaggio 1983).

One week after plant emergence, thinning was carried out, keeping two plants per vase. All plants received regular irrigation daily to maintain the water capacity at 70% and plants were kept under natural light, with an average temperature of 25± 2° C.

All plants received 70 kg ha⁻¹ of both N and K as base fertilizers, and urea (45% of N) 30 days after emergence (DAE) as a topdressing. For treatments that received rock phosphate fertilization, 98 kg ha⁻¹ of Araxa phosphate was

applied at sowing, which belongs to the fluorapatite group, with low solubility and slow assimilation.

Experimental design

The experimental design used randomized blocks with treatments arranged in a factorial scheme (4x2x2). The first factor corresponded to bacterial inoculation: a) control (no inoculation); b) *A. brasilense* inoculation (AZ); c) *B. subtilis* inoculation (BS); d) inoculation with a mixture of bacteria (AZ+BS), the second factor corresponded to the presence or absence of phosphate fertilization and the third factor corresponded to the presence or absence of top-dressed nitrogen fertilizer. Treatments were carried out with four replicates.

Inoculum preparation and inoculation

For *A. brasilense* inoculation, strains Ab-V5 and Ab-V6 were used; for *B. subtilis* inoculation, the strain used was isolated from a maize plant. All of the strains belong to the Laboratory of Microbiology collection. Isolates were stocked in test tubes containing PDA medium according to (Kucey, 1983) and were kept at 4° C.

A 0.2 mL aliquot from each isolate was inoculated into 125 mL Erlenmeyer flasks containing 50 mL of nutrient broth at

pH 7.0 and were kept at 28° C for 24 hours. Then, samples were homogenized and suspensions were diluted in sterilized distilled water and the concentrations were adjusted to 1×10^7 and 3×10^8 CFU mL⁻¹ for *B. subtilis* and *A. brasilense*, respectively.

Inoculation was carried out during sowing with the aid of a pipette, in which 20 mL of bacterial inoculum were applied, according to each treatment, to the soil near the seeds. The control treatment received 20 mL of sterilized distilled water only. The treatment that received the mixture of *A. brasilense* and *B. subtilis* received 10 mL of each bacteria at their respective concentrations.

Evaluations

Height, shoot and root dry matter

At 60 days after sowing, plants were harvested, and their heights were measured from the soil surface to the plant apex with the aid of a measuring tape. For determination of shoot (SDM) and root dry matter (RDM), plants were recollected and sectioned into shoots and roots. Then, plants were washed three times with deionized water. Then, they were conditioned in paper bags and identified and placed in a closed air circulation oven at $62 \pm 2^\circ$ C for 72 hours. Dry matter evaluation was performed with a semi-analytical scale.

Soil analysis

Vases were dismantled and 200 g of rhizospheric soil was collected and divided into two parts for microbiological and chemical analyses. The first portion of the soil was kept at 4°C and the second was dried and maintained at room temperature at 28°C.

For total bacterial counting, 10 g of rhizospheric soil was added to 95 mL of 0.1% pyrophosphate solution (w/v) and subjected to serial dilution (Wollum, 1982) up to a 10^{-4} concentration. A 0.1 mL aliquot from the dilution (10^{-4}) was transferred to Petri plates containing (Bunt and Rovira, 1955), pH 7.4. Then, plates containing the bacterial inoculum were kept at 30°C for 72 hours and, after this period, the number of colonies was counted (Vieira and Nahas, 2005) with the aid of a magnifying glass with a 6x magnification.

For microbial carbon biomass, the irradiation-extraction method was used, adapting the methods proposed by Islam and Weil (1998), which consisted of the use of electromagnetic energy (microwave) to promote cell disruption and release the intracellular compounds for later carbon extraction and quantification.

Total nitrogen was measured according to Bremner and Mulvaney (1982). Digestion was performed with concentrated sulfuric acid, followed by distillation with NaOH and titration with sulfuric acid. The nitrogen level was calculated based on a standard curve determined with ammonium sulfate solution.

For soluble phosphate quantification, extraction was carried out through a sodium bicarbonate solution, and determination was made with sulfuric acid, and a reagent mainly composed of ascorbic acid and ammonium molybdate and potassium ammonium tartrate, followed by incubation in water at 45° C for 20 minutes. The reading of

samples was performed with spectrophotometric absorbance (Watanabe and Olsen, 1965).

Indole acetic acid production and fluorapatite solubilization

Indole acetic acid production was measured according to the methodology of (Kuss et al., 2007) with a few modifications. Isolates were inoculated in 20 mL of dextrose yeast glucose sucrose (DYGS) supplemented with 5 mM of L-tryptophan, incubated for 48 hours at 28° C under constant agitation of 120 rpm and in the darkness. Subsequently, 5 mL of each culture was centrifuged at 10,000 rpm for 10 minutes and 2 mL of supernatant was transferred to a test tube containing 2 mL of 2% Salkowski reagent (w/v) (0.5 M FeCl₃ in 35% perchloric acid) (Sarwar and Kremer, 1995) and incubated in darkness for 30 minutes. IAA production was determined by spectrophotometer at 530 nm and values were obtained through a standard curve with known concentrations of commercial IAA. This assay was performed in triplicate.

The solubilization activity of fluorapatite was determined by the transfer of a 0.2 mL suspension at a concentration of 1×10^7 CFU mL⁻¹ to Erlenmeyer flasks containing medium described by Nahas et al., (1994), supplemented with 5 g L⁻¹ fluorapatite (Araxá apatite). After inoculation, the bacterial solution was incubated with no agitation at 28°C for seven days. Four flasks of each bacterial solution were removed daily. Solutions were centrifuged to 9,000 rpm for 15 minutes and the supernatant was collected to determine the phosphate content according to methods described by Ames (1966).

Statistical analysis

Analysis of variance was carried out by F test, and the average was compared by Duncan's test at 5%. Data regarding bacterial counting were transformed into log (x) values. Analyses were performed with Agroestat software version 1.0 (Barbosa and Maldonado, 2010).

Conflict of interest

The authors declare that they have no conflicts of interest.

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Research involving human participants and/or animals

This article does not contain any studies with animals performed by any of the authors.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Conclusion

Although some parameters were improved with mineral fertilization in general, the findings showed that there were many adverse effects from using rock phosphate fertilization with *A. brasilense* inoculation. When both were applied together in treatments, there was a reduction in plant height, microbial biomass carbon and total numbers of bacteria compared to treatment without rock fertilization. These results strongly suggest that rock phosphate harms the interaction of plant microorganisms and the ability of *A. brasilense* to promote plant growth and show the necessity

for new studies to verify whether this negative effect occurs in field conditions, reducing yields in maize crop production.

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