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*Article*

# Potential Use of Phosphate-Solubilizing Bacteria in Soybean Culture

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**Abstract:** Using microorganisms to enhance crop productivity is an active and increasing field of research, which encompasses the productive, environmental, and economic aspects of agricultural production to obtain high-quality crops with a reduction in the need for fertilizers. Among the nutrients necessary for plant growth, phosphorous is problematic due to its low availability and its susceptibility to convert into non-labile forms. In this regard, phosphate-solubilizing bacteria (PSB) can be an interesting tool to improve phosphorous availability and to reduce the requirements of phosphate fertilizers. This work aimed to evaluate the potential use of phosphate-solubilizing bacteria in the supply of phosphorus and soybean development. This study was conducted in the 2019/2020 and 2020/2021 harvests. The experimental design was in randomized blocks, containing seven treatments and six replicates. The treatments consisted of five doses of phosphate fertilization, using triple superphosphate fertilizer, associated with the application of *Bacillus subtilis* and *Bacillus megaterium* bacteria, and two treatments, with and without the use of phosphorous fertilizer and without the use of an inoculant. Plant tissue nutrients and biometric and productive parameters of the crop were assessed. According to the observed results, applying PSB associated with phosphate fertilization and phosphate fertilization alone did not influence soybean's nutritional, biometric, and productive parameters in the two harvests. Thus, the application of *B. subtilis* and *B. megaterium*, either associated or not associated with phosphate fertilization, does not contribute to the nutrition, development, and yield of soybean crops in soil with a naturally low P content, considering the climatic and soil conditions of the study.

**Keywords:** *Bacillus megaterium*; *Bacillus subtilis*; phosphate fertilization; bio-inputs

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## 1. Introduction

Recently, soybean (*Glycine max* (L.) Merrill) has become one of the most important crops on the world's agricultural scene. Soybean is responsible for producing more protein per area than any other large-scale crop species, and this crop has increased exponentially over the past 50 years. As a result, this species has had an impact on world food production, generating economic development for producing countries [1,2].

Soil chemical fertility is among the determining factors for the success of a crop, and it is strongly linked to agricultural productivity. Fertile soil contains sufficient and adequate amounts of nutrients so that plants can assimilate and absorb the nutrients [2]. The practice of fertilization involves a nutritional contribution to an established crop; the purpose of fertilization is to supplement the soil and make available nutrients to the plant. For this reason, to improve fertilization, nutrient levels in the soil must be measured, aiming at an economic balance and, consequently, making the process sustainable [3].

After nitrogen, phosphorus (P) is the second most important nutrient for plant development [4]. This element is classified as a macronutrient and has major roles in plant and animal metabolism. It is a component of the cell structure and organelles, intermediaries of respiration and photosynthesis, as well as the energy exchange currency of plants, adenosine triphosphate (ATP), participation in the formation of nucleic acids and phospholipids which integrate the membrane plasma cells of plant cells, among other functions [5].

Phosphorus is an essential element both for the natural biogeochemical cycle of the planet and for the anthropic cycle of food production, and it is obtained from non-renewable resources. In addition, P is not very mobile and tends to stick to the soil [6]. P mainly exists in two forms in the soil, i.e., organic phosphorus (Po) and inorganic fraction. Inorganic P is primarily represented by dihydrogen phosphate anion ($H_2PO_4^-$), which is present in slightly acidic media, and is mostly found in soil solution with a greater capacity of assimilation by the plants [7]. Plants absorb only soluble phosphorus in soil solution at chemical equilibrium with rocks and other P sources, called labile phosphorus. The phosphorus levels, regardless of its anionic form in the solution, are very low, causing it to be restored several times via chemical equilibrium between labile and non-labile sources during culture development [8]. Many soils can have very high phosphorus levels, but most phosphorus is in fixed form, occurring in non-soluble states or linked with clay particles, corresponding to non-labile sources for plants [6].

Microorganisms can act as plant nutrition facilitators that influence availability and promote a reduction in chemical and/or commercial fertilizers [9]. Richardson et al. [10] reported that fungi and bacteria performed phosphate mineralization and solubilization processes in the soil. Solubilization of inorganic phosphates adsorbed in clay fraction and iron and aluminum oxides in soil is primarily associated with releasing organic and inorganic acids produced by microorganisms. These acids can dissolve the phosphate bound to cations, releasing different phosphate anions into the soil solution and enabling their absorption by plants [11].

Bacteria such as *Bacillus subtilis* and *Bacillus megaterium* have excellent abilities to solubilize phosphorus, especially iron phosphate, due to the production of organic compounds such as siderophores, which, associated with other forms of dissolving phosphates, increase the bioavailability of this nutrient and associated cations [12]. In addition, *B. megaterium* produces the phosphatase enzyme, which can hydrolyze organic phosphorus, increasing its availability [13].

*B. subtilis* and *B. megaterium* also have the potential to produce indoleacetic acid (IAA). This phytohormone stimulates root development, increasing root length with more secondary roots and enhancing the root surface area for nutrient absorption [14].

Therefore, the use of phosphate-solubilizing bacteria (PSB) and plant growth promoters can reduce the need for fertilization with chemical fertilizers, making agriculture more economically and ecologically sustainable, especially in tropical soils with low to insufficient levels of available phosphorus for plant absorption [15].

Thus, the objective of the present work was to evaluate the use of phosphate-solubilizing bacteria (*B. megaterium* and *B. subtilis*) to improve the supply of phosphorus and investigate the impact of phosphate-solubilizing bacteria on the development of the soybean crop cultivated in an oxisol with low natural P availability.

## 2. Materials and Methods

### 2.1. Field Conditions and Soil Preparation

This research was carried out for two consecutive years at the University of Caxias do Sul, Vacaria Campus (RS) experimental field, with geographic coordinates 28°31′ S, 58°54′ W and an altitude of approx. 950 m above sea level.

The soil where the experiment was conducted was classified as oxisol. The soil had been cultivated in a no-tillage system since 2015, with a history of summer crops with two consecutive years of soybeans, later corn, and the last crop was soybeans. For winter crops, the first year was fallow. In the second year, wheat was grown. In the other years, oats were planted to maintain soil covering during rotation. In the implantation of the area with native grassland, correction of soil acidity and fertility was carried out through the application of 7.0 t·ha$^{-1}$ of dolomitic limestone and 14.0 t·ha$^{-1}$ of calcitic limestone, incorporated with 500 kg·ha$^{-1}$ of triple superphosphate (a source of soluble inorganic P).

The soil fertility conditions before the installation of the experiment, as well as their interpretation, are compiled in Table 1.

**Table 1.** Fertility parameters of the soil before the installation of the experiment and their interpretation regarding soybean crop requirements.

| Parameter | Unit | Result | Interpretation |
|---|---|---|---|
| pH | - | 6.5 | Weak acidity [16] |
| H + Al | $cmol_c \cdot dm^{-3}$ | 3.1 | Medium [16] |
| Clay | % *w/v* | 66.0 | Class I [17] |
| CTC (pH 7.0) | $cmol_c \cdot dm^{-3}$ | 21.4 | High [17] |
| MO | % *w/v* | 6.6 | High [17] |
| Al | $cmol_c \cdot dm^{-3}$ | <0.1 | Adequate [16] |
| Ca | $cmol_c \cdot dm^{-3}$ | 14.2 | High [17] |
| Mg | $cmol_c \cdot dm^{-3}$ | 3.4 | High [17] |
| K | $mg \cdot dm^{-3}$ | 285.8 | Very high [17] |
| P | $mg \cdot dm^{-3}$ | 5.8 | Low [17] |
| S | $mg \cdot dm^{-3}$ | 10.8 | High [17] |
| Zn | $mg \cdot dm^{-3}$ | 7.3 | High [17] |
| Cu | $mg \cdot dm^{-3}$ | 16.3 | High [17] |
| Mn | $mg \cdot dm^{-3}$ | 27.3 | High [17] |
| B | $mg \cdot dm^{-3}$ | 0.2 | Medium [17] |
| Saturation of bases | % | 86.1 | High [16] |

### *2.2. Treatments and Experimental Conditions*

The treatments consisted of five doses of phosphorus fertilization (zero, 25%, 50%, 75%, and 100% of the crop requirement [17]), combined with the application of *B. subtilis* and *B. megaterium* bacteria, and two treatments with and without phosphate fertilization (zero and 100% of the crop requirement, which was 150 t·ha$^{-1}$ $P_2O_5$ [17]), without the use of inoculants. The application of phosphate-solubilizing bacteria (PSB) was performed with the inoculant BiomaPhos®, composed of *B. subtilis* and *B. megaterium* bacteria, with $4 \cdot 10^9$ viable cells per milliliter of product. The BiomaPhos® (Bioma, Fazenda Rio Grande, Brazil) inoculant was applied in the first year via seed inoculation and jet driven into the sowing row in the second year. Both applications were used at a 100 mL·ha$^{-1}$ dosage.

Phosphate fertilization was carried out in the first year of cultivation with triple superphosphate fertilizer (TSP—monocalcium phosphate, $Ca(H_2PO_4)_2 \cdot H_2O$) containing 44 wt.% $P_2O_5$. The total phosphorus correction for the soybean crop was used as a reference, with an expected productivity of 5.0 t·ha$^{-1}$, according to the Liming and Fertilization Manual for the soils of Rio Grande do Sul and Santa Catarina [17], corresponding to 340 kg·ha$^{-1}$ of triple superphosphate in the sowing row. Only phosphate-solubilizing bacteria was applied in the second year in the respective plots. Potassium chloride (KCl) was added to all treatments, containing 60 wt.% $K_2O$, at a dose of 200 kg·ha$^{-1}$.

Soybean sowing was carried out following the Agricultural Zoning of Climatic Risk for the municipality of Vacaria, RS, using the cultivars Brasmax Raio IPRO (first crop) and Brasmax Ativa RR (second crop), with a seeder setting of 13 seeds per linear meter. The phytosanitary management of the soybean crop was carried out based on weekly monitoring for control of weeds, pests, and pathogens that could interfere with the data and productivity of the crop. In the second year of the experiment, 5.0 mm of water was irrigated via a sprinkler for two consecutive days after sowing in all treatments.

Rainfall was monitored through the Embrapa Grape and Wine Experimental Station meteorological station in Vacaria, RS, approximately 1.0 km from the experimental installation site. The data were analyzed in two years of experimenting, for five months (from

November 2019 to March 2020 and from November 2020 to March 2021), which were the field experiment periods. The rainfall in this period accumulated 690.2 mm (below the climatological normal for the same months, corresponding to 738 mm) for the first year and 796.2 mm for the second year of cultivation [18].

### 2.3. Evaluation of Soybean Biometric and Productive Parameters

The following parameters were evaluated: chlorophyll A, chlorophyll B, and total chlorophyll content; leaf nutritional content; root length; plant height; number of pods per plant; number of grains per legume; mass of thousand grains; and crop productivity. All parameters were assessed for two consecutive years.

For the evaluation of the chlorophyll A, chlorophyll B, and total chlorophyll indexes, leaves were collected in two stages of the plant: V5, that is, when the plant had four fully developed trifoliate leaves, and R1, the beginning of the plant's reproductive phase. The evaluation used a chlorophyll meter (Clorofilog, Falker®, Porto Alegre, Brazil).

For the evaluation of the nutritional indexes of the leaf, the 3rd trefoil developed from the upper third of the main stem of the plant, together without the petiole, were collected from 30 plants of each plot, specifically in R2 (full flowering), and stored in paper bags, as recommended by Seixas et al. [19]. Leaf macro- and micronutrient contents were determined by nitric-perchloric acid digestion, followed by colorimetry for the determination of P, S, and B; atomic-absorption spectrophotometry (AAS) for Ca, Mg, Mn, Cu, Zn, and Fe; and flame photometry for K. Nitrogen content in foliar tissue was determined by Kjeldahl determination after sulfuric digestion. The analyses were carried out following the procedures described by Malavolta et al. [20].

At the end of the physiological maturation of the crop (R7), the plants of the two central rows of each plot of the experiment were collected in four linear meters, totaling 4.0 $m^2$ per plot, for the evaluation of plant height, root length, number of pods per plant, number of grains per pod, mass of a thousand grains, and grain yield. Root length and plant height were determined using 20 plants per repetition, collected in sequence in one of the central rows of each plot. Subsequently, a ruler calibrated by Inmetro (Brasília, Brazil) was used to perform the measurements. The numbers of grains per pod and pods per plant were counted in the same plants. Then, all the collected plants were threshed, and the grains were homogenized at a moisture content of 13 wt.% to determine productivity.

### 2.4. Experimental Design and Statistical Analysis

The experimental design was carried out in randomized blocks, containing seven treatments with six replications. Due to the specificity of the treatments, three blocks were used, and each block was divided into two repetitions, encompassing six repetitions per treatment, with 10.0 $m^2$ each.

The results relative to each harvest were analyzed separately, following the same experimental design and statistical analysis. Data were analyzed for homoscedasticity and normality using the Levene and Shapiro–Wilk tests, respectively. The obtained results underwent one-way analysis of variance (one-way ANOVA), and the significant differences were assessed by Tukey's multiple range test at a 5% significance level ($p \leq 0.05$). All statistical analyses were conducted using the software AgroEstat® (São Paulo, Brazil).

## 3. Results and Discussion

The results of the leaf tissue analysis did not show significant differences between the treatments for applying phosphate-solubilizing bacteria (*B. subtilis* and *B. megaterium*) associated with phosphorus fertilization. In addition, the application of phosphorus fertilizer alone in the two years of the experiment did not significantly interfere with the crop's nutrition, as shown in Table 2.

**Table 2.** Macronutrient contents in the leaf tissue of soybean plants, conducted in a consolidated no-tillage system, with the application of *Bacillus magaterium* and *Bacillus subtilis* and different doses of phosphorus fertilization in two separate harvests, in the city of Vacaria, RS.

| **Treatments** | | **N** | **Ca** | **Mg** | **P** | **K** | **S** |
|---|---|---|---|---|---|---|---|
| **PSB** | **Phosphate Fertilization** | **g·kg$^{-1}$** | | | | | |
| 2019/2020 Harvest | | | | | | | |
| Without | Zero | 61.6 ± 1.6 $^{ns}$ | 9.9 ± 1.0 $^{ns}$ | 2.9 ± 0.1 $^{ns}$ | 4.4 ± 0.3 $^{ns}$ | 18.3 ± 4.1 $^{ns}$ | 4.0 ± 0.3 $^{ns}$ |
| With | Zero | 56.5 ± 5.4 | 10.0 ± 0.4 | 2.8 ± 0.1 | 3.8 ± 0.1 | 16.2 ± 0.5 | 3.5 ± 0.5 |
| With | 25% | 65.1 ± 7.4 | 8.7 ± 2.1 | 2.6 ± 0.5 | 3.7 ± 0.7 | 15.0 ± 2.7 | 3.4 ± 0.2 |
| With | 50% | 65.1 ± 2.2 | 9.7 ± 0.3 | 2.9 ± 0.3 | 4.2 ± 0.3 | 16.7 ± 0.7 | 4.2 ± 0.6 |
| With | 75% | 58.0 ± 5.0 | 11.0 ± 0.8 | 2.9 ± 0.2 | 4.1 ± 0.2 | 20.7 ± 2.2 | 3.7 ± 0.4 |
| With | 100% | 61.7 ± 3.4 | 9.8 ± 0.5 | 2.7 ± 0.2 | 4.3 ± 0.3 | 14.5 ± 2.7 | 4.2 ± 0.4 |
| Without | 100% | 59.3 ± 2.6 | 9.5 ± 0.7 | 2.5 ± 0.1 | 3.9 ± 0.4 | 16.2 ± 1.4 | 3.9 ± 0.4 |
| Coefficient of variation (%) | | 1.8 | 25.9 | 26.9 | 28.3 | 12.1 | 14.8 |
| 2020/2021 Harvest | | | | | | | |
| Without | Zero | 56.7 ± 3.8 $^{ns}$ | 11.0 ± 1.3 $^{ns}$ | 2.9 ± 0.2 $^{ns}$ | 3.7 ± 0.5 $^{ns}$ | 16.7 ± 1.6 $^{ns}$ | 3.4 ± 0.4 $^{ns}$ |
| With | Zero | 53.3 ± 2.6 | 10.5 ± 1.2 | 3.0 ± 0.3 | 3.5 ± 0.5 | 18.1 ± 2.0 | 3.1 ± 0.5 |
| With | 25% | 55.0 ± 8.2 | 11.4 ± 1.6 | 2.9 ± 0.1 | 3.6 ± 0.4 | 17.9 ± 1.8 | 3.3 ± 0.2 |
| With | 50% | 51.4 ± 1.9 | 10.4 ± 1.4 | 2.9 ± 0.3 | 3.2 ± 1.0 | 18.6 ± 2.0 | 3.0 ± 0.5 |
| With | 75% | 51.9 ± 6.2 | 10.6 ± 1.7 | 3.0 ± 0.2 | 3.6 ± 0.7 | 18.9 ± 2.3 | 3.2 ± 0.2 |
| With | 100% | 46.5 ± 11.1 | 10.9 ± 1.7 | 3.0 ± 0.2 | 4.1 ± 0.5 | 18.5 ± 1.8 | 3.2 ± 0.5 |
| Without | 100% | 51.4 ± 3.0 | 9.7 ± 0.5 | 2.9 ± 0.1 | 3.8 ± 0.3 | 19.3 ± 0.8 | 3.2 ± 0.6 |
| Coefficient of variation (%) | | 26.4 | 2.6 | 8.4 | 36.3 | 6.63 | 18.6 |

$^{ns}$ not significant by the F-test (ANOVA) at a 5% error probability; PSB, phosphate-solubilizing bacteria.

The levels of foliar macronutrients in the soybean crop were sufficient for the crop phase, according to Seixas et al. [19], in both years of the experiment. In line with the present work, Araujo [21] observed that applying *B. subtilis* in seed inoculation did not cause an increase in soybean foliar nutritional levels. The experiment was conducted in a greenhouse, and no statistical differences were observed for the nutrients N, P, K, Ca, and S compared to the control (non-inoculated plants). In addition, there was no significant difference in N content among the treatments, which indicated that the protein content also remained similar with and without PSB and phosphate fertilization.

The foliar micronutrient contents in the soybean crop were also not influenced by the treatments with *Bacillus* spp. and/or phosphate fertilization, according to the data compiled in Table 3.

The levels of the micronutrients Zn, Cu, Mn, and Fe were sufficient for an adequate crop supply. However, in the second year of cultivation, the micronutrient boron (B) showed low values (<40 mg·kg$^{-1}$) in all treatments [19]. Boron contents are higher in more superficial layers in well-drained soils, linked to soil organic matter. For this reason, in conditions of water deficit, the absorption of B by plants can be hampered [22]. This fact could be associated with poorly distributed rainfall in November and December 2020, which reached the municipality of Vacaria, RS, during the initial phase of crop development [18].

**Table 3.** Micronutrient contents in the leaf tissue of soybean plants grown in a consolidated no-tillage system, with the application of *Bacillus magaterium* and *Bacillus subtilis* and different doses of phosphorus fertilization in two separate harvests, in the city of Vacaria, RS.

| **Treatments** | | **Zn** | **Cu** | **B** | **Mn** | **Fe** |
|---|---|---|---|---|---|---|
| **PSB** | **Phosphate Fertilization** | | | **mg·kg$^{-1}$** | | |
| | | | 2019/2020 Harvest | | | |
| Without | Zero | 40.9 ± 9.6 $^{ns}$ | 11.5 ± 1.8 $^{ns}$ | 42.7 ± 3.8 $^{ns}$ | 130.3 ± 66.0 $^{ns}$ | 171.3 ± 16.5 $^{ns}$ |
| With | Zero | 33.3 ± 2.4 | 10.6 ± 0.3 | 54.4 ± 1.7 | 108.1 ± 40.4 | 162.0 ± 10.6 |
| With | 25% | 32.3 ± 7.5 | 9.3 ± 2.3 | 44.1 ± 8.4 | 97.0 ± 51.3 | 148.8 ± 32.1 |
| With | 50% | 38.9 ± 2.8 | 11.1 ± 0.9 | 42.5 ± 11.0 | 122.5 ± 38.0 | 166.4 ± 15.0 |
| With | 75% | 34.7 ± 5.2 | 10.8 ± 0.3 | 57.3 ± 4.7 | 111.3 ± 58.0 | 151.8 ± 21.8 |
| With | 100% | 37.0 ± 3.2 | 10.7 ± 0.3 | 40.9 ± 8.2 | 108.8 ± 42.8 | 163.0 ± 7.8 |
| Without | 100% | 34.2 ± 2.8 | 10.1 ± 0.4 | 50.4 ± 13.5 | 112.6 ± 27.9 | 148.4 ± 17.6 |
| Coefficient of variation (%) | | 2.8 | 21.0 | 31.4 | 6.0 | 19.8 |
| | | | 2020/2021 Harvest | | | |
| Without | Zero | 40.7 ± 6.9 $^{ns}$ | 10.3 ± 0.8 $^{ns}$ | 24.2 ± 6.7 $^{ns}$ | 116.6 ± 56.9 $^{ns}$ | 122.8 ± 10.1 $^{ns}$ |
| With | Zero | 34.8 ± 2.6 | 10.5 ± 0.4 | 24.1 ± 4.5 | 101.2 ± 34.6 | 115.5 ± 4.9 |
| With | 25% | 38.7 ± 6.7 | 11.3 ± 0.5 | 26.8 ± 1.9 | 103.6 ± 41.7 | 123.7 ± 14.1 |
| With | 50% | 41.6 ± 2.0 | 10.9 ± 0.9 | 28.4 ± 1.5 | 114.4 ± 31.5 | 120.1 ± 5.1 |
| With | 75% | 33.8 ± 4.8 | 10.2 ± 1.2 | 22.7 ± 4.2 | 85.8 ± 35.6 | 121.5 ± 28.6 |
| With | 100% | 38.0 ± 5.1 | 11.5 ± 1.3 | 26.0 ± 1.0 | 91.5 ± 24.5 | 120.9 ± 16.6 |
| Without | 100% | 39.0 ± 3.2 | 11.1 ± 1.0 | 26.9 ± 1.9 | 111.5 ± 26.9 | 120.7 ± 4.4 |
| Coefficient of variation (%) | | 1.4 | 8.7 | 29.2 | 3.2 | 2.1 |

$^{ns}$ not significant by the F-test (ANOVA) at a 5% error probability; PSB, phosphate-solubilizing bacteria.

According to a study by Silva et al. [23], the B content in soybean leaves was affected, so soil water availability was limited. According to the same authors, this fact occurred due to the movement of B in the soil being predominantly regulated via mass flow, which was carried by water, making it difficult for the interception of this nutrient by the plant root. The study was carried out in a greenhouse and aimed to evaluate the response of the soybean crop to the application of B doses correlated with soil water availability.

Regarding the chlorophyll index in soybean leaves, no significant differences were observed in the contents of chlorophyll A, chlorophyll B, and total chlorophyll, regardless of the application of phosphate-solubilizing bacteria (*B. megaterium* and *B. subtilis*) and the application of phosphorus fertilization, evaluated in the vegetative and reproductive stages of the crop, according to the data in Table 4.

Aquino et al. [24] did not observe any influence on the chlorophyll content of plants with the inoculation of corn seeds with *B. subtilis* and *B. megaterium* applied separately in an experiment conducted in a greenhouse.

The root length and plant height of soybean were also not influenced by the application of *B. megaterium* and *B. subtilis*, as well as by different doses of phosphorus fertilization on the soil surface, as shown in Table 5.

**Table 4.** Chlorophyll A, chlorophyll B, and total chlorophyll indexes in soybean leaves, evaluated in the vegetative and reproductive stages in a consolidated no-tillage system with the application of *Bacillus megaterium* and *Bacillus subtilis* and different doses of phosphorus fertilization in two different harvests in the city of Vacaria, RS.

| **Treatment** | | **Vegetative Stage (V5)** | | | **Reproductive Stage (R1)** | | |
|---|---|---|---|---|---|---|---|
| **PSB** | **Phosphate Fertilization** | **Chlorophyll A** | **Chlorophyll B** | **Total Chlorophyll** | **Chlorophyll A** | **Chlorophyll B** | **Total Chlorophyll** |
| 2019/2020 Harvest | | | | | | | |
| Without | Zero | 41.3 ± 1.2 $^{ns}$ | 9.8 ± 2.0 $^{ns}$ | 51.1 ± 3.9 $^{ns}$ | 44.0 ± 1.6 $^{ns}$ | 9.8 ± 0.4 $^{ns}$ | 53.7 ± 3.0 $^{ns}$ |
| With | Zero | 41.7 ± 1.2 | 9.1 ± 0.6 | 50.8 ± 2.8 | 45.0 ± 1.0 | 10.2 ± 0.6 | 55.2 ± 2.5 |
| With | 25% | 44.2 ± 4.5 | 10.2 ± 1.6 | 52.5 ± 6.5 | 44.5 ± 1.9 | 9.8 ± 0.5 | 54.4 ± 3.4 |
| With | 50% | 42.1 ± 2.2 | 10.1 ± 2.6 | 52.2 ± 5.2 | 43.8 ± 1.5 | 9.9 ± 0.7 | 53.7 ± 3.2 |
| With | 75% | 43.7 ± 1.8 | 10.7 ± 2.9 | 55.4 ± 5.2 | 43.9 ± 0.7 | 9.9 ± 0.2 | 53.8 ± 2.4 |
| With | 100% | 42.2 ± 3.0 | 9.6 ± 2.7 | 53.0 ± 5.7 | 44.8 ± 1.4 | 10.1 ± 0.4 | 54.9 ± 3.0 |
| Without | 100% | 40.3 ± 5.8 | 9.1 ± 1.0 | 47.8 ± 7.3 | 44.7 ± 1.8 | 10.0 ± 0.6 | 54.7 ± 3.4 |
| Coefficient of variation (%) | | 1.03 | 3.27 | 10.59 | 0.55 | 1.03 | 0.53 |
| 2020/2021 Harvest | | | | | | | |
| Without | Zero | 33.1 ± 3.6 $^{ns}$ | 8.3 ± 1.5 $^{ns}$ | 41.4 ± 5.1 $^{ns}$ | 44.6 ± 4.6 $^{ns}$ | 12.4 ± 2.7 $^{ns}$ | 56.7 ± 7.4 $^{ns}$ |
| With | Zero | 34.8 ± 5.4 | 8.6 ± 1.4 | 43.4 ± 6.3 | 46.2 ± 4.0 | 12.5 ± 1.3 | 58.9 ± 5.3 |
| With | 25% | 33.4 ± 4.7 | 8.2 ± 1.9 | 41.2 ± 6.2 | 44.8 ± 4.9 | 11.8 ± 2.9 | 56.1 ± 7.6 |
| With | 50% | 35.3 ± 3.0 | 8.6 ± 1.1 | 43.9 ± 4.0 | 46.5 ± 3.4 | 12.8 ± 2.9 | 59.4 ± 6.0 |
| With | 75% | 34.1 ± 4.1 | 8.1 ± 1.5 | 42.2 ± 5.2 | 46.9 ± 4.6 | 12.9 ± 2.9 | 58.9 ± 7.5 |
| With | 100% | 33.7 ± 2.5 | 8.0 ± 1.2 | 41.8 ± 3.6 | 44.6 ± 4.4 | 11.9 ± 2.5 | 56.2 ± 6.9 |
| Without | 100% | 35.8 ± 2.9 | 9.5 ± 1.0 | 46.0 ± 3.8 | 45.9 ± 4.5 | 12.5 ± 2.1 | 58.4 ± 6.4 |
| Coefficient of variation (%) | | 0.44 | 5.06 | 2.27 | 11.44 | 7.83 | 10.03 |

$^{ns}$ not significant by the F-test (ANOVA) at a 5% error probability; PSB, phosphate-solubilizing bacteria.

**Table 5.** Root length and height of soybean plants in a consolidated no-tillage system according to the application of *Bacillus megaterium* and *Bacillus subtilis* and different doses of phosphorus fertilization on the soil surface in two different harvests in the city of Vacaria, RS.

| **Treatment** | | **Root Length** | **Plant Height** |
|---|---|---|---|
| **PSB** | **Phosphate Fertilization** | **cm** | |
| 2019/2020 Harvest | | | |
| Without | Zero | 14.98 ± 0.41 $^{ns}$ | 69.59 ± 2.25 $^{ns}$ |
| With | Zero | 15.42 ± 0.76 | 66.87 ± 2.78 |
| With | 25% | 15.60 ± 1.09 | 69.86 ± 2.53 |
| With | 50% | 16.01 ± 1.81 | 69.32 ± 3.03 |
| With | 75% | 15.11 ± 1.10 | 67.75 ± 3.98 |
| With | 100% | 15.50 ± 0.63 | 70.58 ± 2.93 |
| Without | 100% | 15.44 ± 1.11 | 69.37 ± 4.58 |
| Coefficient of variation (%) | | 2.51 | 11.28 |
| 2020/2021 Harvest | | | |
| Without | Zero | 13.00 ± 1.59 $^{ns}$ | 89.76 ± 5.39 $^{ns}$ |
| With | Zero | 12.99 ± 0.94 | 91.85 ± 6.34 |
| With | 25% | 13.80 ± 1.01 | 96.39 ± 4.66 |
| With | 50% | 13.15 ± 0.97 | 93.16 ± 3.01 |
| With | 75% | 13.10 ± 1.11 | 90.08 ± 5.93 |
| With | 100% | 13.66 ± 1.35 | 92.85 ± 6.18 |
| Without | 100% | 12.32 ± 0.76 | 95.51 ± 5.20 |
| Coefficient of variation (%) | | 2.49 | 14.17 |

$^{ns}$ not significant by the F-test (ANOVA) at a 5% error probability; PSB, phosphate-solubilizing bacteria.

Differences in the height of plants observed between the two years of conducted work occurred due to the different cultivars, considering that in the first harvest, the cultivar Brasmax Raio® was used, and in the second harvest, the cultivar Brasmax Activa® was used.

The results relative to the biometric parameters of the plants showed similar behavior to a study by Lima et al. [25] who evaluated corn crops and reported that applying *B. subtilis*, with or without nitrogen fertilization, did not promote significant differences between treatments and control (non-inoculated plants) for plant height. However, the same authors commented that plant height may not directly correlate with crop productivity.

The crop yield components did not express significant responses with the inoculation of *B. subtilis* and *B. megaterium*, either associated or not associated with phosphorus fertilization, and not even with isolated phosphorus fertilization, according to the results shown in Table 6.

**Table 6.** Yield components of the soybean crop in a consolidated no-tillage system as a function of applying *Bacillus magaterium* and *Bacillus subtilis* and different doses of phosphorus fertilization in two different harvests in the city of Vacaria, RS.

| **Treatment** | | **Grain Productivity** | **Mass of 1000 Grains** | **Pods per Plant** | **Grains per Pod** |
|---|---|---|---|---|---|
| **PSB** | **Phosphate Fertilization** | **kg·ha$^{-1}$** | **g** | **Number** | |
| 2019/2020 Harvest | | | | | |
| Without | Zero | 3708 ± 74 $^{ns}$ | 177.6 ± 3.2 $^{ns}$ | 37.2 ± 1.5 $^{ns}$ | 2.4 ± 0.1 $^{ns}$ |
| With | Zero | 3501 ± 102 | 178.1 ± 3.8 | 35.8 ± 5.2 | 2.3 ± 0.1 |
| With | 25% | 3635 ± 220 | 181.0 ± 5.0 | 34.6 ± 2.4 | 2.3 ± 0.1 |
| With | 50% | 3672 ± 237 | 180.3 ± 3.9 | 40.0 ± 6.4 | 2.3 ± 0.2 |
| With | 75% | 3621 ± 117 | 180.9 ± 2.8 | 38.4 ± 4.2 | 2.3 ± 0.1 |
| With | 100% | 3577 ± 98 | 177.6 ± 2.9 | 36.2 ± 2.8 | 2.4 ± 0.1 |
| Without | 100% | 3518 ± 210 | 177.7 ± 3.6 | 37.4 ± 3.0 | 2.3 ± 0.2 |
| Coefficient of variation (%) | | 0.69 | 0.35 | 2.67 | 7.23 |
| 2020/2021 Harvest | | | | | |
| Without | Zero | 3286 ± 569 $^{ns}$ | 172.4 ± 10.8 $^{ns}$ | 47.0 ± 5.7 $^{ns}$ | 2.2 ± 0.1 $^{ns}$ |
| With | Zero | 3134 ± 242 | 166.8 ± 7.0 | 49.1 ± 2.7 | 2.3 ± 0.1 |
| With | 25% | 3197 ± 446 | 168.7 ± 10.2 | 46.8 ± 7.9 | 2.3 ± 0.1 |
| With | 50% | 3044 ± 547 | 174.8 ± 8.3 | 46.7 ± 6.0 | 2.2 ± 0.2 |
| With | 75% | 3167 ± 381 | 171.5 ± 12.7 | 53.3 ± 13.7 | 2.2 ± 0.2 |
| With | 100% | 3164 ± 583 | 172.4 ± 12.2 | 51.3 ± 5.7 | 2.3 ± 0.1 |
| Without | 100% | 2867 ± 671 | 171.4 ± 4.5 | 43.5 ± 5.7 | 2.2 ± 0.2 |
| Coefficient of variation (%) | | 1.75 | 1.05 | 2.68 | 16.37 |

$^{ns}$ not significant by the F-test (ANOVA) at a 5% error probability; PSB, phosphate-solubilizing bacteria.

With behavior similar to the present study, Schwaab and Aguiar [26] did not observe significant effects of the application of *B. megaterium* and *B. subtilis* bacteria on soybean crop productivity, as well as on yield components such as the mass of a thousand grains, legumes per plant, and grains per plant. The same authors reported that the negative results may have been attributed to the interaction of abiotic factors with the bacteria, such as, for example, edaphoclimatic conditions during the cultivation period.

Unlike this work, Paiva et al. [27] observed an increase in the productivity of a corn crop with inoculation via seed treatment with *B. subtilis* and *B. megaterium*. The study analyzed different doses of phosphate fertilization added to the PSB strains, in which treatments with 50% of the recommendation for phosphate fertilization associated with PSB showed better results than the control and isolated phosphate fertilization. However, it is worth emphasizing that the organic matter contents described by the authors were low, and the phosphorus contents in the soil were variable compared to those found in the present work.

Elkoca et al. [28] observed an increase in bean crop productivity with the inoculation of *B. megaterium* and *B. subtilis*. The experiment was conducted in field conditions and showed a significant response for leaf chlorophyll contents and crop yield components. The authors also reported that the association of PSB outperformed the non-inoculated control treatment and the treatment with phosphorus fertilization. However, the soil in the experiment had lower clay and organic matter contents and a high phosphorus content, distinguishing it from the present study.

Pinto et al. [29] observed behavior similar to that seen in this study and reported that the best way to understand the dynamics of phosphorus in the soil is to quantify its fractions, from the most soluble to the most complexed, especially in soils that bring in their origins a high degree of phosphorus from weathering. In these soils, the organic form gains space and supplies phosphorus to the plants, mainly where the levels of inorganic phosphorus (Pi) are low. Santos et al. [30] reported that organic phosphorus played a fundamental role in supplying the nutritional demand of the plant when Pi levels were low, and this process was carried out through the decomposition and mineralization of the labile fraction of Po in the soil.

The recommendation for phosphate fertilization established by CQFS [17] for the states of Rio Grande do Sul and Santa Catarina (South Brazil) uses the P content extracted by the Mehlich method (extraction of soluble phosphate by using an extractor solution of diluted strong acid) to estimate phosphorus availability to plants. This estimate may not reflect the dynamics of less labile forms of the nutrient, which are not extracted by the method, but may also contribute to plant supply in the medium and long term. For this reason, a good correlation is not always found between the phosphorus content extracted from the soil and crop productivity [31].

According to Nogueirol et al. [32], extractors commonly used in routine laboratories, such as the Mehlich I, have been developed for conventional cultivations. These extractors do not seem effective in systems with a high level of organic matter, as these extractor solutions cannot extract from the soil the amounts of P correlated with those accumulated in the plants. A possible reason for this variation is that while extracting inorganic phosphorus, they underestimate the influence of organic forms of phosphorus on plant nutrition.

Therefore, the high availability of organic phosphorus, not quantified by soil phosphorus assessments used by routine laboratories (Mehlich), may result in an underestimation of soil capacity to supply phosphorus to plants. Thus, the need for phosphate fertilization should be lower in soils with high levels of organic matter, such as those in the present study, which may lead to lesser importance of the contribution of phosphate-solubilizing bacteria in the supply of phosphorus to the plants. Still, according to Oliveira-Paiva et al. [33], the bacteria *B. magaterium* and *B. subtilis* also contribute to the enlargement of the root system, which could improve water absorption in years with water deficit and, consequently, increase crop productivity. However, this experiment did not observe these responses in the two years evaluated under water-deficient field conditions.

In addition, when microorganisms are applied under field conditions, the effects are highly variable and often lack consistency, which restricts their applicability. Inoculated microorganisms must compete with highly diverse microflora. Depending on the size, diversity, and interactions with native populations, an introduced inoculant strain can establish itself successfully or with difficulty [34]. Kavamura et al. [35] pointed out that the efficiency of microorganism inoculation in agriculture is a challenge and is related to the inherent susceptibility of the microorganism to stress and its ability to colonize different niches in the face of competition with the native microbiota, as well as the field expression of the desired function. Thus, a better understanding of the interactions of these inoculants when applied to the soil is necessary, aiming to develop strategies to help the establishment and persistence of beneficial microorganisms in agricultural systems, guaranteeing their safety for native organisms and the environment.

## 4. Conclusions

Applying *B. subtilis* and *B. megaterium* did not affect the soybean crop's nutritional, biometric, and productive parameters in the soil and climatic conditions studied. In addition, neither of the phosphate-fertilizing treatments contributed to the soybean crop's nutrition, development, and yield components grown in an oxisol, with low phosphorus levels in the layer from zero to 20 cm. It is important to point out the importance of the interference of edaphoclimatic conditions associated with soil microbiology on the effectiveness of the use of bio-inputs, such as phosphorus solubilizing bacteria, to establish it as a more sustainable and ecologically friendly alternative compared to conventional methods of soil nutrition management.

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