


## Article

# Impact of the Application of Rhizobacteria in Bean Cultivars: Potential for Sustainable Management

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**Abstract:** The use of bacterial inoculants has great potential to improve yield and sustainability; however, application forms still face bottlenecks, such as the standardization of methods and compatibility with different varieties of bean. The objective of this study was to evaluate the growth and yield of bean cultivars subjected to inoculation and co-inoculation with rhizobacteria. The experiments were carried out using a randomized block design, with three replicates. Treatments consisted of four bean cvs. (BRS FC 402, BRS Estilo, BRS Pitanga, and BRS Esteio), subjected to inoculation with *Rhizobium* and co-inoculation with *Azospirillum*, applied to seed or soil, plus eight additional treatments with a mineral N source and its absence (control) for each cvs. The use of co-inoculation of *R. tropici* with *A. brasilense* promoted an improvement in the morphophysiological and agronomic characteristics, attributed to the ability of rhizobial bacteria to supply nitrogen to plants and to *Azospirillum* through hormonal action. The cvs. BRS Estilo and BRS Esteio had the best grain yields when co-inoculated via soil, 2049 and 1831 kg ha<sup>-1</sup>, respectively. Co-inoculation with *R. tropici* + *A. brasilense* applied to the soil can be used as an exclusive source of N supply in the bean, contributing to lower costs and more sustainable production.

**Keywords:** *Phaseolus vulgaris* L.; *Rhizobium*; *Azospirillum*; sustainability; yield



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## 1. Introduction

Common bean (*Phaseolus vulgaris* L.) has great social and economic importance in several countries around the world as a major source of protein, especially for the poorest populations. However, its importance is not reflected in the technology that is normally employed by common bean producers, so the global average yield is low in relation to the crop yield potential [1]. Brazil is the largest producer of this species, with a production area in the 2022/2023 season of 2.7 million hectares and a production of 3.0 million tons, with an average yield of 1125 kg ha<sup>-1</sup> [2]. The main causes of the yield gap are water deficits and a lack of mineral nutrition. In the producing regions of Central Brazil, irrigation is essential for the success of crop bean, especially for winter crops (dry season) [3], due to the problems with high soil acidity, a predominant situation in the soils of the region.

Common bean genotypes developed with high production require large amounts of N to be supplied throughout their growth cycle to obtain high yields. The amount of N extracted by common bean is estimated at 140 kg ha<sup>-1</sup> [4], which makes N fertilization

a costly practice. In addition, as N is a mineral that requires the burning of fossil fuels to make the final product, its use causes negative environmental impacts, such as global warming and soil and water pollution, causing health and quality-of-life problems for the population [5].

Biological N fixation (BNF), a biochemical and natural process carried out by bacteria that make use of the enzyme nitrogenase, is an alternative that can meet the N demand of beans. These organisms are found in various environments in a free form, associated or in symbiosis with other living beings. BNF is performed by diazotrophic bacteria that live in symbiosis with the roots of host plants, thus positively affecting the formation of nodules, and can be influenced by abiotic and biotic factors [6,7]. N supply to bean via BNF represents a viable alternative in economic terms and is ecologically sustainable, which is essential for producers who aim to explore the demanding international markets today [8]. BNF is an important substitute for the use of N fertilizers to mitigate greenhouse gases, since N in the soil, through biochemical transformations, produces nitrous oxide (N<sub>2</sub>O), which has a global warming power about 300 times greater than that of CO<sub>2</sub> [9].

To improve Rhizobium performance and, consequently, BNF efficiency, the co-inoculation technique is beginning to be explored in some crops, especially soybean [10–13]. A recent review study published by [14] demonstrated that the co-inoculation of soybean with *Bradyrhizobium* spp. + *A. brasilense* increased root mass by 11%, number of nodules by 5.4%, nodule mass by 10.6%, shoot N concentration by 2.8%, and grain yield and grain N concentration by 3.2% and 3.6%, respectively, compared to conventional single inoculation with *Bradyrhizobium* spp. The authors also consider that co-inoculation can be important to mitigate the effects of water stress on plants, in addition to being an efficient technology that contributes to the sustainability of soybean production.

Co-inoculation is a management technique used to obtain benefits and increase the potential of BNF from the association between bacteria of the Rhizobium genus and plant-growth-promoting bacteria, such as *Azospirillum brasilense*. A group of associative bacteria represents this alternative, capable of promoting plant growth through physiological changes due to the release of hormones such as auxins and cytokinins that promote increased root growth [15,16].

Investigations involving the co-inoculation of *R. tropici* + *A. brasilense* in common bean crop are recent, such as the studies conducted by [17,18], whose results indicated that *Azospirillum* enhances the development of plants, especially roots, resulting in better conditions to supply the symbiosis with Rhizobium in N fixation, providing increases in grain yield, quality of seeds, and sustainability in common bean production systems [19,20]. Ribeiro et al. [21] conducted a study in “cerrado” conditions in Brazil involving the inoculation of seeds with *R. tropici*, associated with co-inoculation with *A. brasilense* plus the micronutrients molybdenum and cobalt with subsequent re-inoculation of the mixture under cover at the V4 stage, and concluded that the use of the technique has the potential to completely replace mineral nitrogen fertilization in bean plants. However, these studies need to be expanded in order to obtain more information about the efficiency of the technique in common bean.

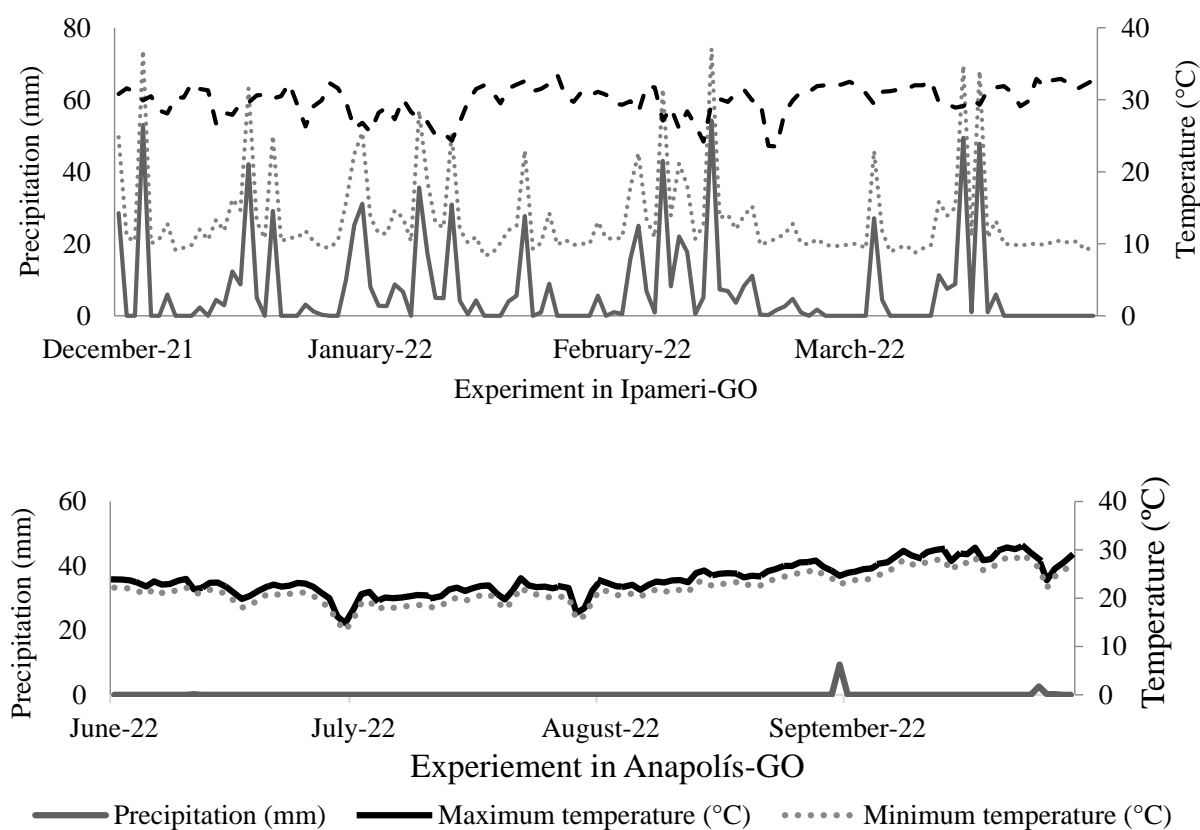
The objective of this study was to evaluate the growth and yield of bean cultivars subjected to inoculation with Rhizobium and co-inoculation with Rhizobium + *Azospirillum*, applied to seed and soil. The hypotheses tested in the study are the following: Hypothesis 1—The effectiveness of Rhizobium inoculation or co-inoculation with *Azospirillum* on the development and yield of bean plants depending on the form of application, with direct application to the soil being more effective than application to seeds; Hypothesis 2—Simultaneous inoculation with Rhizobium and *Azospirillum* gives better

results in terms of biological nitrogen fixation and yield of bean plants, compared to inoculation with *Rhizobium* alone, regardless of the application method.

## 2. Materials and Methods

### 2.1. General Information

The experiments were carried out in the spring–summer of 2021/2022 and winter of 2022, in an experimental area belonging to the State University of Goiás, Ipameri Unit (first season), and in the experimental area of Emater in an area associated with CET/UEG, in Anápolis (second season), located in the state of Goiás, Brazil, at the respective geographic coordinates of 17°43'27" S, 48°08'55" W and 16°20'12.13" S, 48°53'15.96" W, with average altitudes of 800 and 1058 m [22], and a distance of 208 km between the two municipalities. According to Köppen, the region's climate is type Aw (Rainy Tropical in spring–summer, with a dry season in winter) [23]. In Ipameri, the maximum and minimum air temperatures were 29.9 °C and 20 °C, respectively, with total precipitation of 863.6 mm. On the other hand, those averages were slightly lower in Anápolis (28.9 °C, 14.7 °C and 13.8 mm, respectively). The climatic conditions during the periods of the experiments are presented in Figure 1.



**Figure 1.** Climate data referring to precipitation (mm), maximum temperature (°C), and minimum temperature (°C) for the periods from 1 December 2021 to 31 March 2022, and 1 June to 30 September 2022.

In both experiments, the soil was classified as Oxisol and the texture was characterized as medium sandy, comprising the sandy loam textural class, with more than 520 g kg<sup>-1</sup> of sand [24]. Before the experiment, soil samples were collected from the 0–20 cm layer for physicochemical characterization (Table 1) at Solocria Laboratório Agropecuário Ltda (Goiânia, GO, Brazil) (Table 1).

**Table 1.** Results of the physicochemical analysis of the experimental areas of the Ipameri Unit and Emater of Anápolis, GO, Brazil.

Attributes	Common Bean Crop Seasons	
	2021/22 Spring-Summer	2022 Winter
pH (CaCl <sub>2</sub> )	5.7	5.1
P (mg dm <sup>-3</sup> )	31.6	18.1
K (mg dm <sup>-3</sup> )	317.8	140.7
Ca (cmol <sub>c</sub> dm <sup>-3</sup> )	5.1	2.9
Mg (cmol <sub>c</sub> dm <sup>-3</sup> )	1.5	1.0
Al (cmol <sub>c</sub> dm <sup>-3</sup> )	0.0	0.0
H + Al (cmol <sub>c</sub> dm <sup>-3</sup> )	1.4	3.8
V (%)	84.2	52.9
B (mg dm <sup>-3</sup> )	0.1	0.19
Cu (mg dm <sup>-3</sup> )	0.4	2.7
Fe (mg dm <sup>-3</sup> )	19.8	13.0
Mn (mg dm <sup>-3</sup> )	16.6	22.8
Zn (mg dm <sup>-3</sup> )	3.2	11.1
Organic matter (g dm <sup>-3</sup> )	22.0	31.0
Sand (g kg <sup>-1</sup> )	570.0	440.0
Silt (g kg <sup>-1</sup> )	90.0	110.0
Clay (g kg <sup>-1</sup> )	340.0	450.0

pH of (CaCl<sub>2</sub>) with a ratio of 1:1,0 and determined by potentiometry; phosphorus and potassium were extracted with Mehlich-1 solution and determined, respectively, by molybdate colorimetry and flame photometry; calcium, magnesium, and aluminum were extracted by a KCl<sup>-1</sup> 1N extractor and determined, respectively, by atomic absorption (Ca/Mg) and titration with NaOH solution; H + Al were obtained using a 0.5 mol L<sup>-1</sup> calcium acetate extractor at pH 7.0 and determined by potentiometry; the base saturation (V) was calculated from the obtained results; boron was extracted with hot water and determined colorimetrically with Azometina; copper, iron, manganese, and zinc were extracted with Mehlich-1 solution and determined by atomic absorption; organic matter was extracted with Na dichromate and determined by colorimetry; sand, silt, and clay were extracted with NaOH + Na Hexametaphosphate and analyzed by the pipette method.

## 2.2. Experimental Design and Treatments

The experimental design used in both experiments was randomized blocks, in a 4 × 2 × 2 + 8 factorial scheme, with three replicates. Treatments consisted of four high-yielding common bean cultivars belonging to different groups recommended for cultivation in the Midwest region and most of the Brazilian states, BRS FC 402 (C1), BRS Estilo (C2), BRS Pitanga (C3) and BRS Esteio (C4), respectively. They were subjected to inoculation with *R. tropici* and co-inoculation with Azospirillum, applied to seed or soil, plus eight additional treatments corresponding to fertilization with mineral N source (N1) and its absence (N0) (control) for each cultivar studied. Additional treatments related to the cultivars were defined as follows: BRS FC 402 (C1N0 and C1N1), BRS Estilo (C2N0 and C2N1), BRS Pitanga (C3N0 and C3N1), and BRS Esteio (C4N0 and C4N1), respectively.

Seed treatment was not performed in order not to harm the population of bacteria inoculated before sowing. Seeds were initially inoculated at sowing with a liquid inoculant, containing *R. tropici* strains recommended for common bean (SEMIA 4088 and SEMIA 4077) (2 × 10<sup>9</sup> CFU mL<sup>-1</sup>), at a dose of 150 mL per 50 kg of seed, as suggested by the manufacturer for the use of the product. *A. brasilense* (3 × 10<sup>9</sup> CFU mL<sup>-1</sup>) was applied at the same time, at a dose corresponding to 100 mL per 50 kg of seed, recommended for common bean. Applications of biological inputs to the soil were carried out with a 20 L knapsack sprayer, using a fan-type nozzle with the jet directed at the soil. The dose of the inputs was doubled in the application to soil due to the greater interaction of microorganisms with the soil, using a spray volume of 200 L ha<sup>-1</sup> for both products [25]. Fertilization with mineral N was performed at sowing, along with basic topdressing fertilization only for the additional treatments.

### 2.3. Details on Experimental Plots, Setup, and Performance

In both experiments, the experimental plots were formed by four 5 m long rows, spaced 0.50 m apart. Liming was carried out only in the experimental area of Anápolis, GO, Brazil, by broadcasting 1.5 tons ha<sup>-1</sup> of limestone filler in the total area two months in advance. Initially, the areas were desiccated with Dimethylamine salt of (2,4-dichlorophenoxy) acetic acid, 2,4-D Nortox (Arapongas, PR, Brazil) at the dose of 1.0 L ha<sup>-1</sup>, and seven days after the application, conventional tillage was carried out, with one plowing and two harrowing operations.

All treatments received 200 kg ha<sup>-1</sup> of the fertilizer formulation 05-52-00 in the basal fertilization. It should be noted that this fertilizer contains a low amount of N, aiming at not interfering with the activities of the N-fixing bacteria in the inoculated and co-inoculated treatments. Sowing was carried out using 15 seeds per linear meter for all cultivars studied, and thinning was not necessary due to the uniform emergence of the plant population stands. At 25 days after emergence (DAE), N topdressing was performed only for the additional treatment with mineral N, using urea at a dose corresponding to 60 kg N ha<sup>-1</sup>, split and applied at 25 and 35 DAE. Potassium was applied as a topdressing at 25 DAE at the dose of 60 kg de K<sub>2</sub>O ha<sup>-1</sup> for all plots. The spring–summer season of 2021–2022 was carried out without irrigation due to the good water availability during the cultivation period. On the other hand, in the winter season of 2022, irrigation was used throughout the crop cycle, as it was a dry period, and 400 mm of water was used until harvest. The crops for the spring–summer season were planted on 5 December 2021 and harvested on 1 March 2022. The winter season crops were planted on 15 June 2022 and harvested on 23 September 2022.

Ants (*Atta* sp.) were controlled by distributing formifire granulated bait (RS)-5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile, Atratrex Fipronil® Bait (Campinas, SP, Brazil), dose of 10 g m<sup>-2</sup>, in the initial phase of the experiments. Weed control was carried out with the application of the post-emergent herbicide butyl (R)-2-[4-(5-trifluoromethyl-2-pyridyloxy) phenoxy]propionate, Fusilade® 250 EW (Huddersfield, West Yorkshire, United Kingdom), 1 L ha<sup>-1</sup>, Flex® Active 250 SL (Nantong City, Jiangsu, China), 1 L ha<sup>-1</sup>, at 20 DAE. The insecticides 3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine and (RS)- $\alpha$ -cyano-3-phenoxybenzyl (1RS,3RS; 1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate, Engeo Pleno™ S (Albert-Frank-Strasse 332, Trostberg, Germany), 30 mL 100 L<sup>-1</sup>, were used to control the cucurbit beetle (*Diabrotica speciosa*) and silverleaf whitefly (*Bemisia tabaci*). Fungal diseases such as anthracnose (*Colletotrichum lindemuthianum*) and angular leaf spot (*Phaeoisariopsis griseola*) were controlled by spraying the fungicides dimethyl 4,4'-(*o*-phenylene)bis(3-thioallophanate)A and 3-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)- $\alpha,\alpha,\alpha$ -trifluoro-2,6-dinitro-p-toluidine, Approve® 375 WG (São Paulo, SP, Brazil), 100 g c.p. 100 L<sup>-1</sup>.

### 2.4. Evaluated Characteristics

At the R<sub>5</sub> stage (full flowering plants), five plants with their root system were collected using a flat shovel from the usable area of the plot to evaluate the morphological characteristics (root length—RL; root dry mass—RDM; plant height—PH; shoot dry mass—SDM; leaf area index—LAI; and nitrogen content—NC).

The plants were collected, placed in plastic bags, and immediately taken to the laboratory. Next, the roots were sectioned, with subsequent washing. Main root length was measured with a graduated ruler as the distance between the collar and the final tip of the root (cap), and root dry mass was quantified after drying in an oven regulated at 72 °C until the material reached constant weight. Plant height was obtained by measuring the distance

between the plant collar and the apex of the main stem. Shoot dry mass was obtained by drying in an oven regulated at 72 °C. The leaf area index was determined with a leaf area meter CI—202<sup>®</sup> (CID-Bio-Science, Ave Camas, WA, EUA), and N content was evaluated according to the methodology proposed by [26], using the Kjeldahl method.

For the agronomic characteristics, 10 plants were harvested at stage R9 (mature plants) in the usable area of each plot to determine the production component's number of pods per plant (NPP), number of grains per pod (NGP), average hundred-grain weight (HGW), and grain yield (GY). HGW was expressed in grams, and GY was expressed in kg ha<sup>-1</sup> for 13% grain moisture.

### 2.5. Statistical Analysis

The data referring to each season were initially subjected to the Levene and Shapiro–Wilk tests to assess the homogeneity and normality of residuals. Then, an analysis of variance was performed; the means of the factorial were compared by the Tukey test, whereas the means of additional treatments and the interaction of factorial versus additional treatments were compared by the Dunnett test for each cultivar studied, both at a 5% probability level. R software, version Studio 2022 (Vienna, Austria) [27] was used for data analysis.

## 3. Results

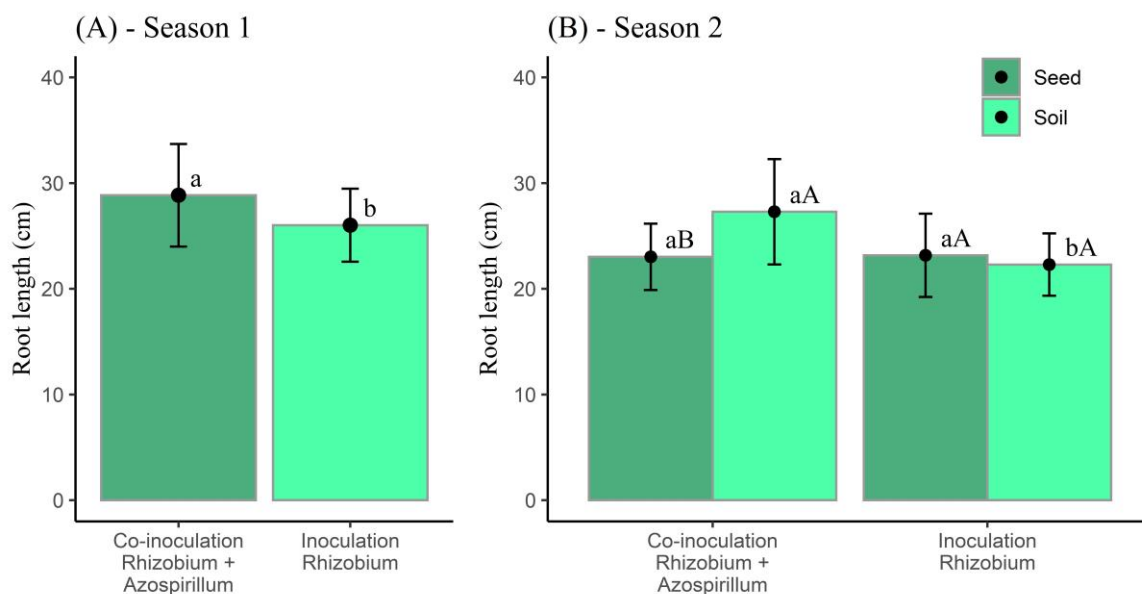
In the 2021/2022 spring–summer season, significant effects were found for the interactions between cultivars and inoculation (A × B), cultivars and application methods (A × C), and inoculation and application methods (B × C) on root length (RL), shoot dry mass (SDM), root dry mass (RDM), N content (NC), leaf area index (LAI), number of pods per plant (NPP), hundred-grain weight (HGW), and grain yield (GY). Plant height (PH) was not significantly influenced by the factors studied (Table S1 in Supplementary).

In the 2022 winter season, there were significant effects only for the interactions between cultivars and inoculation (A × B), cultivars and application methods (A × C), and inoculation and application methods (B × C). Root length (RL), shoot dry mass (SDM), root dry mass (RDM), N content (NC), number of pods per plant (NPP), hundred-grain weight (HGW), and grain yield (GY) were significantly influenced by the combinations of treatments (cultivar, inoculation, and application methods) or by the interaction of additional treatments versus factorial. Leaf area index (LAI) was only affected by the interaction of additional treatments versus factorial. Plant height (PH) was not significantly influenced by the factors studied.

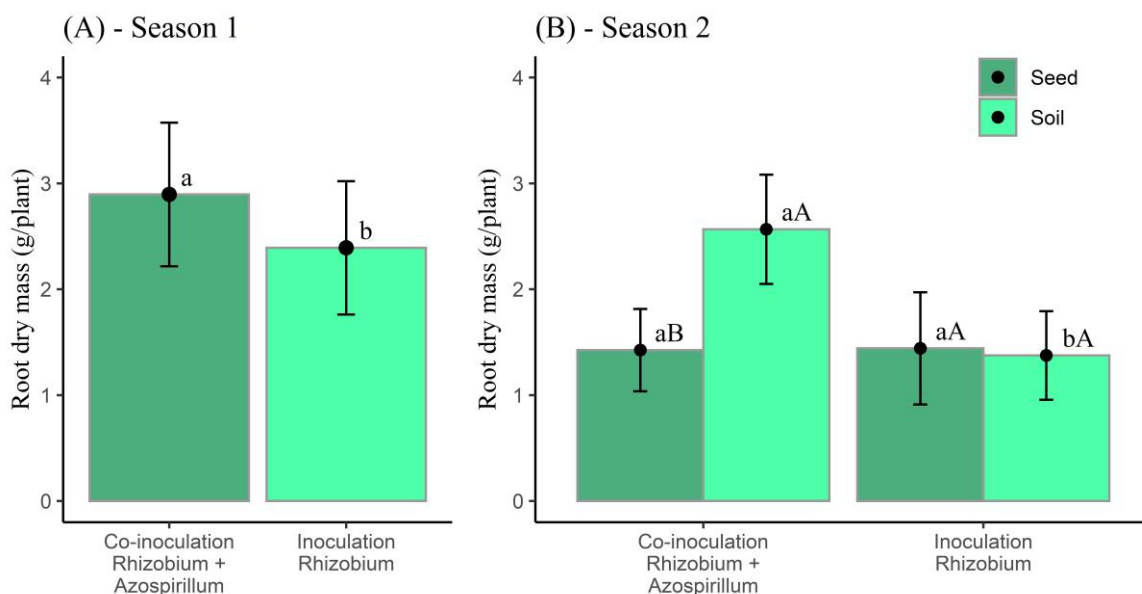
In season 1 (spring–summer), the highest root length (RL = 28.9 cm) was obtained under co-inoculation with Rhizobium + Azospirillum, being 2.9 cm higher than the value obtained under inoculation with Rhizobium (RL = 26 cm), representing an increase of 10.9% in RL regardless of the common bean cultivar analyzed (Figure 2A). In the plants from season 2 (Figure 2B), RL was affected by the interaction of inoculation and co-inoculation with the application methods; plants subjected to co-inoculation with Rhizobium + Azospirillum applied to the soil had higher RL (27.3 cm), and when Rhizobium + Azospirillum was applied to the seed, RL was equal to 22.3 cm, i.e., a difference of 5 cm compared to the co-inoculation carried out in the soil. When comparing the inoculation of Rhizobium (23 cm) and co-inoculation of Rhizobium + Azospirillum (27.3 cm) for soil application, a difference of 4.3 cm was observed in RL.

The root dry mass (RDM) obtained in season 1 (Figure 3A) showed the same behavior as RL with the highest value (2.9 g) obtained when the plants were subjected to co-inoculation with Rhizobium + Azospirillum, with a 0.5 g higher RDM when compared to plants inoculated with Rhizobium, which had an RDM equal to 2.4 g. For the RDM of

season 2, there was an interaction of inoculation and co-inoculation with the application methods, and plants subjected to co-inoculation with Rhizobium + Azospirillum, applied to soil, had the highest RDM (2.6 g), 1.2 g higher than the value obtained in plants inoculated via seed, equal to 1.4 g. On the other hand, RDM values obtained with the applications to seed and soil did not differ statistically from each other in the inoculation with Rhizobium (Figure 3B).

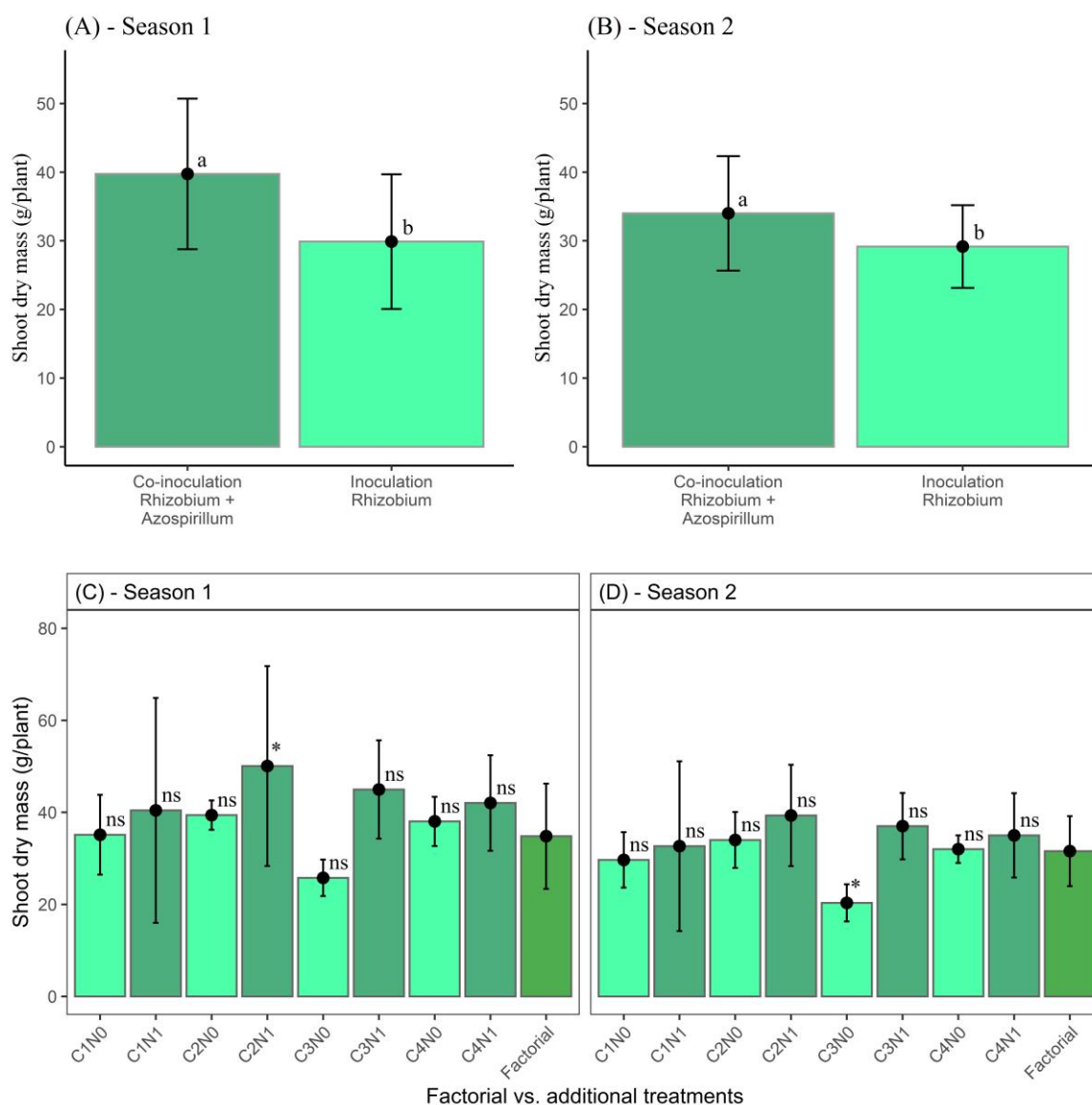


**Figure 2.** Root length of common bean plants under inoculation and co-inoculation (A) and seed and soil application methods as a function of inoculation and co-inoculation (B). Means followed by the same letter do not differ from each other by the Tukey test at 5% probability level. Means followed by the same letter, lowercase between co-inoculation and inoculation and uppercase between application methods (seed and soil), do not differ from each other by the F test ( $p \leq 0.05$ ).



**Figure 3.** Root dry mass of common bean plants under inoculation and co-inoculation (A) and seed and soil application methods as a function of inoculation and co-inoculation (B). Means followed by the same letter do not differ from each other by the Tukey test at 5% probability level. Means followed by the same letter, lowercase between co-inoculation and inoculation and uppercase between application methods (seed and soil), do not differ from each other by the F test ( $p \leq 0.05$ ).

Co-inoculation with Rhizobium + Azospirillum and inoculation with Rhizobium via seed did not differ statistically, while plants co-inoculated with Rhizobium + Azospirillum via soil obtained a higher RDM (2.6 g) than those inoculated with Rhizobium (1.5 g), a superiority of 1.1 g. For shoot dry mass (SDM) in season 1, the highest value (39.7 g per plant) was found in plants co-inoculated with Rhizobium + Azospirillum, showing a superiority of 9.8 g (32.8%) compared to plants inoculated with Rhizobium, whose value was 29.9 g per plant (Figure 4A). The same behavior was observed for SDM in season 2, as plants co-inoculated with Rhizobium + Azospirillum showed higher SDM accumulation (34 g per plant), while plants inoculated with Rhizobium had a value of 29.2 g per plant, i.e., plants co-inoculated with Rhizobium + Azospirillum were 14.2% superior to plants inoculated with Rhizobium (Figure 4B).

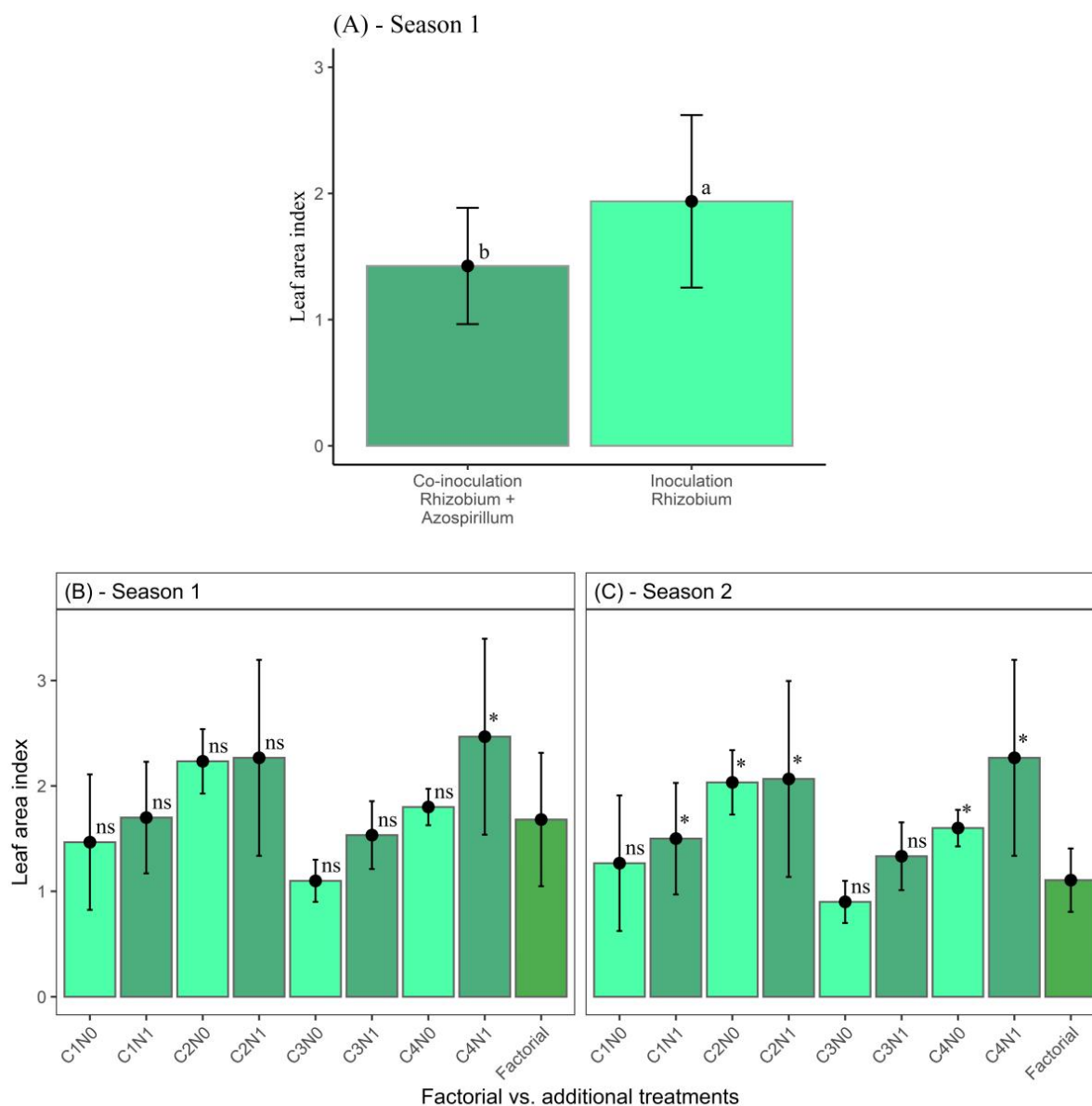


**Figure 4.** Shoot dry mass of common bean plants as a function of inoculation and co-inoculation in season 1 (A) and season 2 (B) and factorial vs. additional treatments in season 1 (C) and season 2 (D). Means followed by the same letter do not differ from each other by the Tukey test at 5% probability level. BRS FC 402 (C1), BRS Estilo (C2), BRS Pitanga (C3), and BRS Esteio (C4), in the presence of mineral nitrogen (N1) and in its absence (N0). ns = not significant; \* significant effect.

When the triple factorial was compared with the additional treatments (Figure 4C), a significant effect on SDM was observed only in the cultivar BRS Estilo (C2N1), with a

value of 50.1 g per plant, 15.3 g higher (43.8%) than the SDM obtained in the triple factorial (34.8 g per plant). The SDM obtained in season 2 showed a significant effect only for the cultivar BRS Pitanga (C3N0), with a value of 20.3 g per plant, being 11.3 g lower (35.7%) than the SDM obtained in the triple factorial (31.6 g per plant) (Figure 4D).

For the leaf area index (LAI) obtained in season 1, inoculation with *Rhizobium* increased LAI compared to co-inoculation with *Rhizobium* + *Azospirillum*, representing an increase of 35.9% (Figure 5A). On the other hand, in the LAI of the factorial vs. additional treatments, it was possible to only observe a significant effect for the cultivar BRS Esteio (C4N1), which had a maximum value of 2.5, representing a difference of 0.8 or 31.7% when compared to the value obtained in the triple factorial (1.7) (Figure 5B).



**Figure 5.** Leaf area index of common bean plants as a function of inoculation and co-inoculation (A), and factorial vs. additional treatments in season 1 (B) and season 2 (C). Means followed by the same letter do not differ from each other by the Tukey test at 5% probability level BRS FC 402 (C1), BRS Estilo (C2), BRS Pitanga (C3), and BRS Esteio (C4), in the presence of mineral nitrogen (N1) and in its absence (N0). ns = not significant; \* significant effect.

LAI in season 2 only showed a significant effect for the cultivars BRS Estilo (C2N0 and C2N1) and BRS Esteio (C4N0 and C4N1), in the absence and presence of N, with values of

2.0, 2.1, 1.6, and 2.3, respectively (Figure 5C). In the additional treatments, LAI was 45.8, 51.3, 31.3, and 46.6% higher than the value found in the factorial, equal to 1.1. The highest LAI values were obtained in C2N1 (2.1) and C4N1 (2.3), in the presence of N.

Regarding the N content (NC) of the cultivars for the two inoculants (Rhizobium + Azospirillum and Rhizobium) in season 1, the cultivar BRS Estilo showed higher NC in the shoots ( $31.8 \text{ g kg}^{-1}$ ) under co-inoculation with Rhizobium + Azospirillum, being superior to the cultivar BRS Esteio ( $28.8 \text{ g kg}^{-1}$ ), by  $2 \text{ g kg}^{-1}$  or 6.3% (Figure 6A). However, it did not differ statistically from the cultivars BRS FC 402 and BRS Pitanga. When the cultivars were inoculated with Rhizobium, the highest NC in the shoots was obtained in BRS FC 402 ( $28.5 \text{ g kg}^{-1}$ ), but it did not differ statistically from BRS Pitanga.

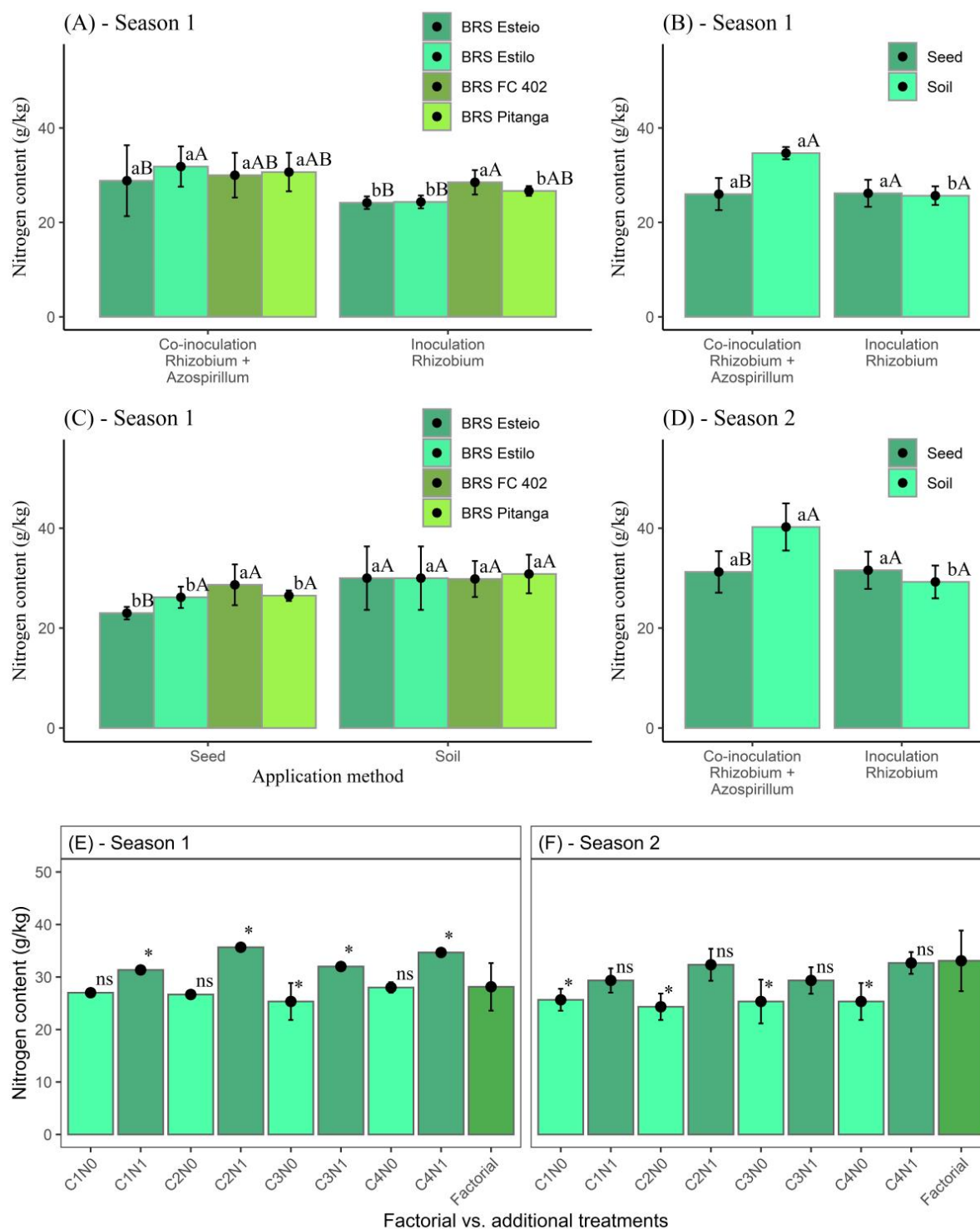
The other cultivars, BRS Estilo ( $24.3 \text{ g kg}^{-1}$ ) and BRS Esteio ( $24.2 \text{ g kg}^{-1}$ ), were inferior to BRS FC 402, with reductions in NC of 4.2 and  $4.3 \text{ g kg}^{-1}$ . When comparing the NC in the shoots of co-inoculated and inoculated cultivars, a higher value was observed in the co-inoculated ones, with increments of 7.5, 4.0, and  $4.7 \text{ g kg}^{-1}$ , or 23.6% for BRS Estilo, 13.1% for BRS Pitanga, and 16.2% for BRS Esteio, respectively. On the other hand, the cultivar BRS FC 402 did not show a statistical difference for the two inoculants (Rhizobium + Azospirillum and Rhizobium).

Joint application of Rhizobium + Azospirillum to soil promoted the highest NC in the shoots of common bean plants,  $34.7 \text{ g kg}^{-1}$ , while the application to seed led to an NC of  $25.7 \text{ g kg}^{-1}$ , i.e., a difference of  $9 \text{ g kg}^{-1}$ , or 25.9% compared to the soil application method (Figure 6B). In the inoculation with Rhizobium alone, there was no statistical difference between the application methods (seed and soil). Seed application also showed no statistical difference as a function of co-inoculation with Rhizobium + Azospirillum and inoculation with Rhizobium. Soil application in co-inoculation with Rhizobium + Azospirillum promoted higher NC ( $34.7 \text{ g kg}^{-1}$ ) compared to inoculation with Rhizobium ( $26 \text{ g kg}^{-1}$ ), representing a 24.9% increase in NC.

Based on the NC found in the shoots of the cultivars under seed and soil application, in season 1, it was possible to observe that, when subjected to seed application, BRS FC 402 stood out with  $28.7 \text{ g kg}^{-1}$ , a value that exceeded that of BRS Estilo ( $26.2 \text{ g kg}^{-1}$ ) by  $2.5 \text{ g kg}^{-1}$ , representing a difference of 8.9% (Figure 6C). The cultivar BRS FC 402 did not differ statistically from the other cultivars (BRS Pitanga and BRS Estilo). The cultivars subjected to soil application did not differ statistically from each other. When comparing each cultivar under the two application methods, it was observed that the NC was higher in BRS Estilo ( $30 \text{ g kg}^{-1}$ ), BRS Pitanga ( $30.83 \text{ g kg}^{-1}$ ), and BRS Esteio ( $30 \text{ g kg}^{-1}$ ) subjected to soil application, with increments of 3.8, 4.3, and  $7 \text{ g kg}^{-1}$ , or 12.8, 14.1, and 23.3%, respectively, except for the cultivar BRS FC 402, for which there was no statistical difference between the application methods.

Regarding NC in the application methods (seed and soil) for the two inoculants (Rhizobium + Azospirillum and Rhizobium), in season 2, the behavior was similar to that observed in season 1, in which the joint application of Rhizobium + Azospirillum to soil led to the highest NC,  $40.3 \text{ g kg}^{-1}$ , while the application to seed led to an NC of  $29.3 \text{ g kg}^{-1}$ , a difference of  $11 \text{ g kg}^{-1}$ , or an increase of 27.3% in NC in the leaves of plants subjected to soil application of Rhizobium + Azospirillum (Figure 6D). In the inoculation with Rhizobium alone, the application methods (seed and soil) did not differ statistically.

Seed application also showed no statistical difference as a function of co-inoculation with Rhizobium + Azospirillum and inoculation with Rhizobium. Soil application in co-inoculation with Rhizobium + Azospirillum promoted higher NC ( $40.3 \text{ g kg}^{-1}$ ) compared to inoculation with Rhizobium ( $31.3 \text{ g kg}^{-1}$ ), representing an increase of 22.4% in NC in the shoots of common bean plants.



**Figure 6.** Nitrogen content of common bean cultivars as a function of inoculation and co-inoculation (A) and seed and soil application methods (C). Seed and soil application methods as a function of inoculation and co-inoculation in season 1 (B) and season 2 (D) and factorial vs. additional treatments in season 1 (E) and season 2 (F). Means followed by the same letter, lowercase between inoculation and co-inoculation uppercase between cultivars, do not differ from each other by the F test ( $p \leq 0.05$ ). Means followed by the same letter, lowercase between co-inoculation and inoculation and uppercase between application methods (seed and soil), did not differ from each other by the F test ( $p \leq 0.05$ ). Means followed by the same letter, lowercase between seed and soil application methods and uppercase between cultivars, did not differ from each other by the F test ( $p \leq 0.05$ ). BRS FC 402 (C1), BRS Estilo (C2), BRS Pitanga (C3), and BRS Esteio (C4), in the presence of mineral nitrogen (N1) and in its absence (N0). ns = non-significant; \* significant effect.

The NC for the triple factorial vs. additional treatments, in season 1, showed a significant effect only for the additional treatments C1N1, C2N1, C3N1, and C4N1 in the presence of N, with values of 31.3, 35.7, 32.0, and 34.7 g kg<sup>-1</sup>, respectively, which were 10.2, 21.2, 12.1, and 18.9% higher than the NC obtained in the factorial treatment (28.1 g kg<sup>-1</sup>) (Figure 6E). The additional treatment (C1N0) in the absence of N also had a significant effect, with a value of 25.6 g kg<sup>-1</sup>, 9.9% lower than the value found in the factorial treatment.

When comparing the additional treatments with the triple factorial in season 2, it was observed that the factorial had an NC of 33.1 g kg<sup>-1</sup>, being superior to the additional treatments C1N0 with 25.7 g kg<sup>-1</sup>, C2N0 with 24.3 g kg<sup>-1</sup>, and C3N0 and C4N0 with 25.3 g kg<sup>-1</sup>, which represent reductions of 22.4, 26.5, and 25.8% (Figure 6F).

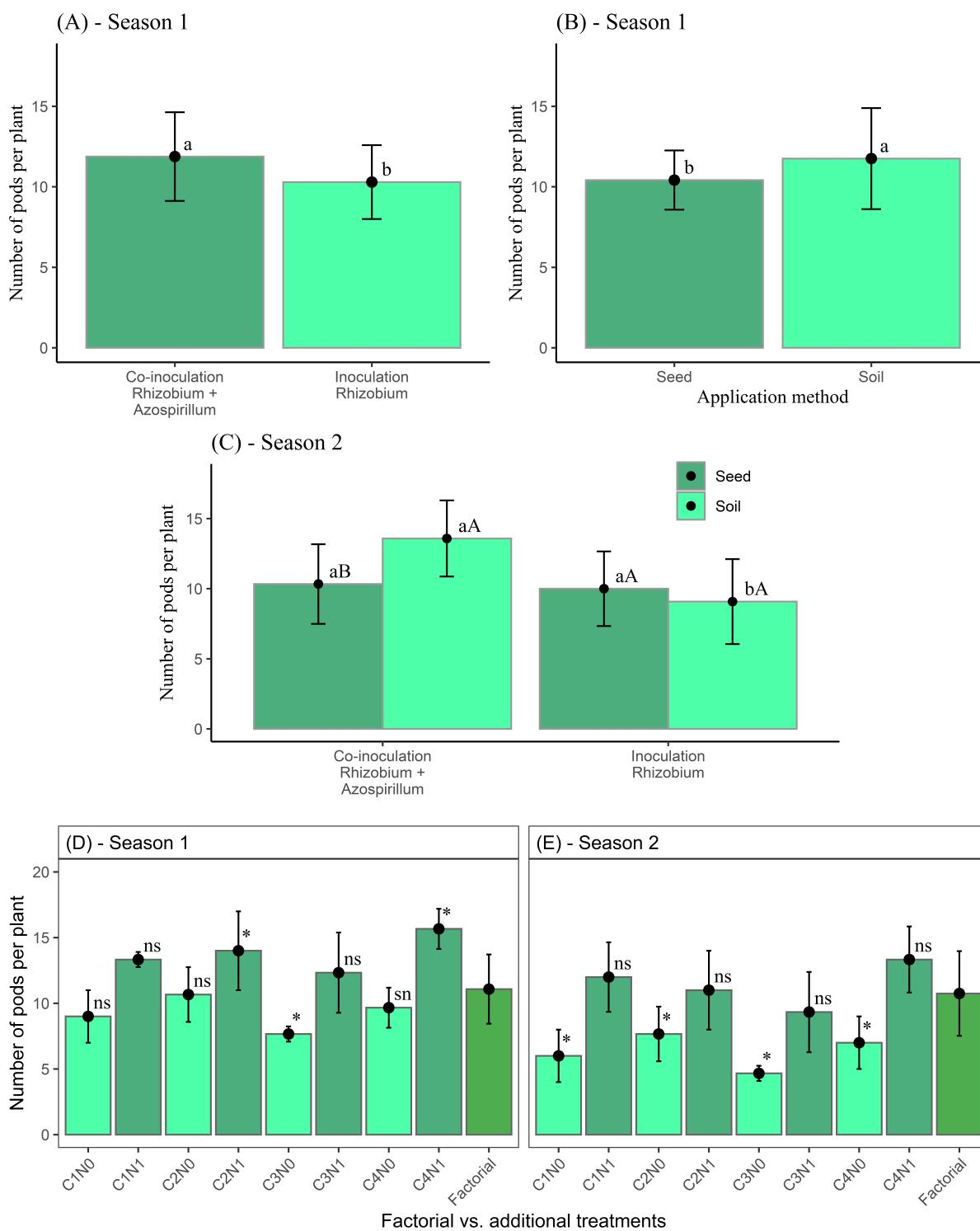
For the number of pods per plant (NPP), in season 1, plants subjected to co-inoculation with Rhizobium + Azospirillum had the highest NPP (11.9), representing a difference of 1.6 pods when compared to plants inoculated with Rhizobium, whose NPP was 10.3, i.e., co-inoculation was 15.1% superior to inoculation for NPP (Figure 7A). For NPP under the application methods, it was observed that soil application was the most effective (Figure 7B). In this form of application, there was an increase of 11.8 pods per plant compared to seed application, which resulted in 10.4 pods per plant. This difference of 1.3 pods represents an increase of 11.4% in pod production.

The NPP in season 2 showed the interaction of inoculation and co-inoculation with the seed and soil application methods (Figure 7C). Soil application associated with co-inoculation with Rhizobium + Azospirillum was superior in terms of pod production, and the plants under this condition had a total of 13.6 pods per plant. When the inoculant was applied to the seed, the NPP was reduced to 9.1, representing a decrease of 33.1% compared to the application carried out directly to the soil as a function of co-inoculation. There was no statistical difference between the soil and seed application methods as a function of Rhizobium inoculation.

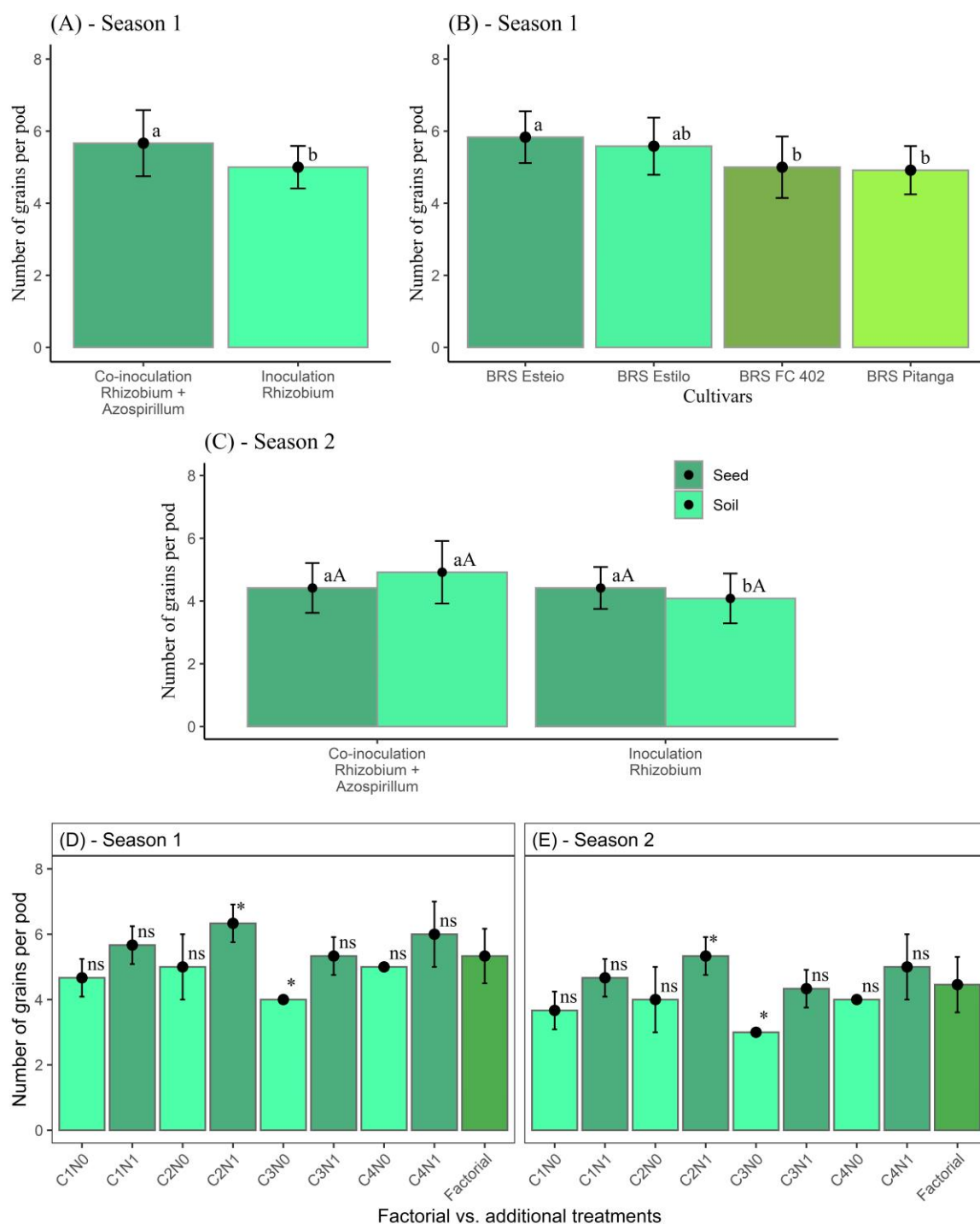
Seed application also did not differ statistically in the co-inoculation with Rhizobium + Azospirillum and inoculation with Rhizobium. On the other hand, co-inoculation with Rhizobium + Azospirillum led to higher NPP (13.6 pods per plant) than inoculation with Rhizobium (10.3 pods per plant), representing an increase of 3.3 pods per plant. When analyzing the NPP for the triple factorial with the additional treatments in season 1, a significant effect was observed only for the cultivars BRS Estilo (C2N1), BRS Pitanga (C3N0), and BRS Esteio (C4N1), with values of 14.0, 7.7, and 15.6, respectively (Figure 7D). The additional treatments in BRS Estilo (C2N1) and BRS Esteio (C4N1) in the presence of N showed superiority of 2.9 and 4.5 pods compared to the factorial, whose value was 11.1 pods per plant, representing increases of 20.7 and 28.8%, respectively. On the other hand, the cultivar BRS Pitanga (C3N0) produced 7.7 pods per plant, which is about 44.2% lower than that obtained in the triple factorial.

In season 2, the triple factorial had the highest NPP (10.8), with superiority of 44.2, 28.7 and 57.2% compared to the additional treatments in the absence of N, BRS FC 402 (C1N0) with 6.0, BRS Estilo (C2N0) with 7.7 and BRS Pitanga (C3N0) with 4.6 pods per plant, respectively (Figure 7E).

For the number of grains per pod (NGP) in season 1, co-inoculation with Rhizobium + Azospirillum led to an average of 5.6 grains per pod, showing an increase of 0.6 grains per pod compared to plants inoculated only with Rhizobium, which had 5 grains per pod, representing an increase of 11.6% in the number of grains (Figure 8A).



**Figure 7.** Number of pods per plant of common bean as a function of inoculation and co-inoculation (A) and seed and soil application methods (B), inoculation and co-inoculation in response to application, seed and soil (C), and factorial vs. additional treatments in season 1 (D) and season 2 (E). Means followed by the same letter do not differ from each other by the Tukey test at 5% probability level. Means followed by the same letter, lowercase between co-inoculation and inoculation and uppercase between application methods (seed and soil), did not differ from each other by the F test ( $p \leq 0.05$ ). BRS FC 402 (C1), BRS Estilo (C2), BRS Pitanga (C3), and BRS Esteio (C4), in the presence of mineral nitrogen (N1) and in its absence (N0). ns = not significant; \* significant effect.



**Figure 8.** Number of grains per pod of common bean plants as a function of inoculation and co-inoculation (A) and cultivar (B), seed and soil application methods as a function of inoculation and co-inoculation (C), and factorial vs. additional treatments in season 1 (D) and season 2 (E). Means followed by the same letter do not differ from each other by the Tukey test at 5% probability level. Means followed by the same letter, lowercase between seed and soil and uppercase between cultivars, do not differ from each other by the F test ( $p \leq 0.05$ ). BRS FC 402 (C1), BRS Estilo (C2), BRS Pitanga (C3), and BRS Esteio (C4), in the presence of mineral nitrogen (N1) and in its absence (N0). ns = not significant, \* significant effect.

When analyzing the NGP in the cultivars, it was found that BRS Esteio had the highest value, with an average of 5.8 grains per pod (Figure 8B). This value was 0.8 and 0.9 grains

per pod higher than those of the cultivars BRS FC 402 and BRS Pitanga, respectively, not differing statistically from that of BRS Estilo, with 5.5 grains per plant.

In season 2, the NGP of plants co-inoculated with Rhizobium + Azospirillum and inoculated with Rhizobium for both methods of application (seed and soil) did not differ statistically (Figure 8C). On the other hand, co-inoculation with Rhizobium + Azospirillum promoted higher NGP with soil application, with 4.9 grains per pod, being 18.5% higher than the number of grains observed in the treatment that received inoculation with Rhizobium.

When analyzing the NVG for the triple factorial versus additional treatments in season 1, a significant effect was found only for the additional treatments in BRS Estilo (C2N1) and BRS Pitanga (C3N0), with NVG of 6.3 and 4.0 grains per pod, respectively (Figure 8D). The additional treatment in BRS Estilo (C2N1) was superior to the factorial by 15.9% or 1.0 grain per pod, and BRS Pitanga (C3N0) was inferior to the factorial by 32.5% or 1.3 grains per pod.

As in season 1, the additional treatment in BRS Estilo (C2N1) in season 2 was superior (5.3 grains per pod) to the factorial treatment (4.5 grains per pod), with an increase of 0.8 grains per pod, representing an increase of 16.3% (Figure 8E). However, the triple factorial treatment was superior to the additional treatment in BRS Pitanga (C3N0), with 3 grains per pod, i.e., an increase of 1.5 grains per pod, or 32.7%.

For hundred-grain weight (HGW), in season 1, the cultivar BRS Esteio produced the highest HGW, 22.1 g, followed by BRS Estilo with 20.9 g, which are 9.9 and 4.8% higher than that of BRS FC 402 (19.9 g) and 13.1 and 8.1% higher than that of BRS Pitanga (Figure 9A). The highest HGW, in season 2, was observed in plants co-inoculated with Rhizobium + Azospirillum (19.4 g), with a difference of 2 g compared to the HGW of plants inoculated with Rhizobium, whose value was 17.4 g, i.e., an increase of 11.3% in HGW (Figure 9B).

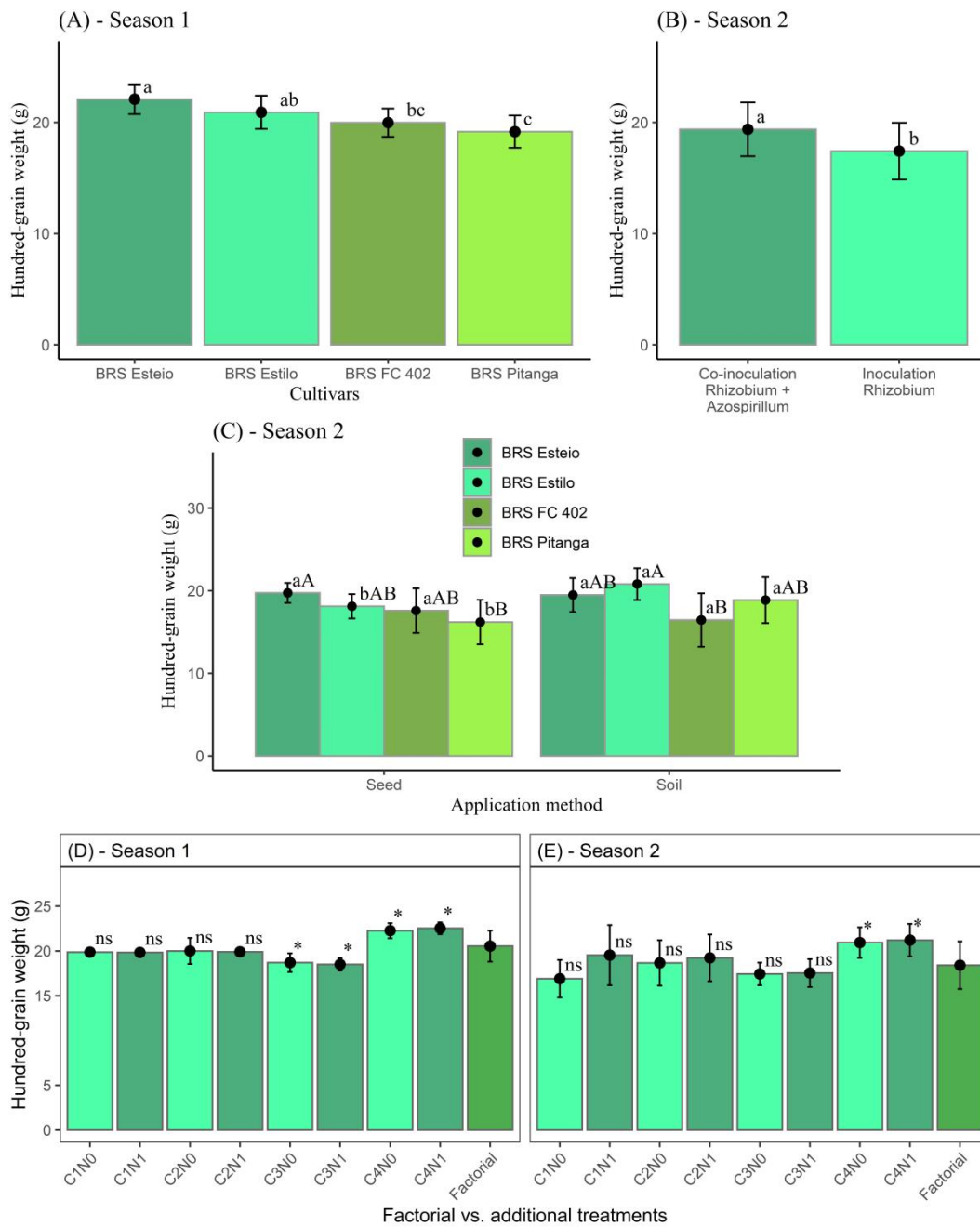
Interaction was observed between the cultivars and the seed and soil application methods. For soil inoculation, the highest HGW was obtained in the cultivar BRS Esteio, with 19.7 g, not statistically differing from the cultivars BRS Estilo and BRS FC 402. BRS Esteio was 17.8% superior to BRS Pitanga (Figure 9C). On the other hand, for the soil application, the highest HGW was obtained in the cultivar BRS Estilo, with 20.8 g, not statistically differing from the cultivars BRS Esteio and BRS Pitanga, with superiority of 20.7% in HGW compared to BRS FC 402.

When comparing the cultivars under the two application methods (seed and soil), it was observed that BRS Esteio and BRS FC 402 did not differ statistically from each other, regardless of the application method. The cultivars BRS Estilo and BRS Pitanga showed higher HGW when subjected to soil application, with values of 20.8 and 18.9 g, corresponding to differences of 2.7 and 2.6 g or 14.9 and 16.4%, respectively, compared to seed application.

When comparing the triple factorial with the additional treatments, in season 1, it was possible to observe that only the cultivars BRS Pitanga (C3N0 and C3N1) and BRS Esteio (C4N0 and C4N1) differed statistically from each other, with the highest HGW found in the additional treatments (C4N0 and C4N1), with 22.3 and 22.5 g, respectively, values that are 7.7 and 8.8% higher than that observed in the factorial treatment, 20.5 g (Figure 9D). On the other hand, the factorial treatment showed increases of 9.9 and 9% in HGW when compared with the cultivar BRS Pitanga and its additional treatments (C3N0 and C3N1), with values of 18.5 and 18.7 g, respectively.

When comparing the additional treatments with the triple factorial in season 2, an increase in HGW was observed in the cultivar BRS Esteio (C4N1) in the presence of N, with

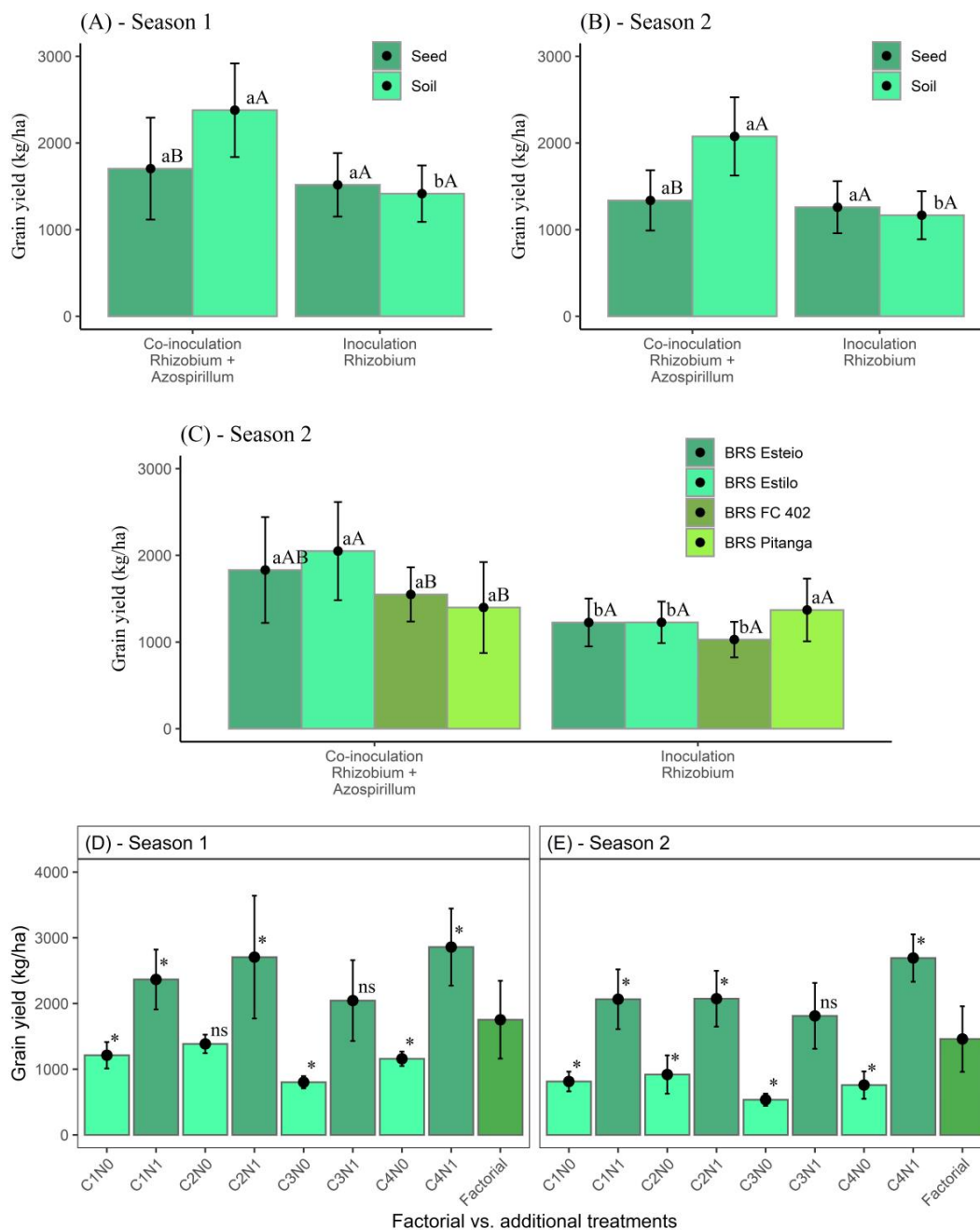
a value of 20.9 g, showing a higher HGW when compared to the triple factorial (18.4 g), i.e., a difference of 2.5 g, which represents an increase of 12.1% (Figure 9E).



**Figure 9.** Hundred-grain weight for common bean cultivars (A) as a function of inoculation and co-inoculation (B), and for cultivars as a function of seed and soil application methods (C) and factorial vs additional treatments in season 1 (D) and season 2 (E). Means followed by the same letter do not differ from each other by the Tukey test at 5% probability level. Means followed by the same letter, lowercase between seed and soil application methods and uppercase between cultivars, do not differ from each other by the F test ( $p \leq 0.05$ ). BRS FC 402 (C1), BRS Estilo (C2), BRS Pitanga (C3), and BRS Esteio (C4), in the presence of mineral nitrogen (N1) and in its absence (N0). ns = not significant; \* significant effect.

For grain yield (GY) in season 1, the joint application of Rhizobium + Azospirillum to the soil resulted in the value of 2378.9 kg ha<sup>-1</sup>, which is 963.8 kg ha<sup>-1</sup> or 40.51% higher than that obtained with seed application, which led to yield of 1415.1 kg ha<sup>-1</sup> (Figure 10A). No statistical difference was detected between the soil and seed application methods as a

function of *Rhizobium* inoculation. Seed application also did not differ statistically in the co-inoculation with *Rhizobium* + *Azospirillum* and in the inoculation with *Rhizobium*. When compared to the inoculation with *Rhizobium* alone via soil application (1704.1 kg ha<sup>-1</sup>), the co-inoculation of *Rhizobium* + *Azospirillum* (2378.9 kg ha<sup>-1</sup>) was also superior by 674.8 kg ha<sup>-1</sup>, representing an increase of 28.4% in grain yield.



**Figure 10.** Grain yield of common bean plants as a function of inoculation and co-inoculation carried out in seed and soil, in season 1 (A) and season 2 (B), and of cultivars as a function of inoculation and co-inoculation (C) and factorial vs. additional treatments in season 1 (D) and season 2 (E). Means followed by the same letter, lowercase between co-inoculation and inoculation and uppercase between application methods (seed and soil), did not differ from each other by the F test ( $p \leq 0.05$ ). Means followed by the same letter, lowercase between co-inoculation and inoculation and uppercase between cultivars, did not differ from each other by the F test ( $p \leq 0.05$ ). BRS FC 402 (C1), BRS Estilo (C2), BRS Pitanga (C3), and BRS Esteio (C4), in the presence of mineral nitrogen (N1) and in its absence (N0). ns = not significant; \* significant effect.

The GY obtained in season 2 showed the same behavior as in season 1, when co-inoculation with *Rhizobium* + *Azospirillum* applied to the soil allowed a yield of 2076.1 kg ha<sup>-1</sup> to be obtained, while application to seed resulted in a yield of 1165.9 kg ha<sup>-1</sup>, equivalent to a difference of 910.2 kg ha<sup>-1</sup> or 43.9% when comparing the two methods of application. The seed and soil application methods did not show statistical differences as a function of *Rhizobium* inoculation (Figure 10B).

Seed application also showed no statistical difference in the co-inoculation with *Rhizobium* + *Azospirillum* and in relation to the inoculation with *Rhizobium*. In addition, when comparing the GY of plants under soil application for the two types of inoculants, *Rhizobium* + *Azospirillum* (2076.1 kg ha<sup>-1</sup>) and *Rhizobium* alone (1337.3 kg ha<sup>-1</sup>), a superiority of 738.9 kg ha<sup>-1</sup> was observed in the co-inoculation of with *Rhizobium* + *Azospirillum*, representing an increase of 35.6%.

In the interaction of the cultivars with the inoculation and co-inoculation for season 2, it was found that for co-inoculation, the highest GY was obtained in the cultivar BRS Estilo, 2049.3 kg ha<sup>-1</sup>, not differing statistically from BRS Esteio, showing superiority in the GY of 500.4 kg ha<sup>-1</sup> and 651.4 kg ha<sup>-1</sup>, or 24.4 and 31.8%, compared to BRS FC 402, with 1548.9 kg ha<sup>-1</sup>, and BRS Pitanga, with 1397.9 kg ha<sup>-1</sup> (Figure 10C). On the other hand, the GY values of the cultivars subjected to inoculation only did not differ statistically from each other.

When comparing the cultivars considering the two inoculant application procedures, it was possible to observe that BRS Estilo (2049.3 kg ha<sup>-1</sup>), BRS Esteio (1830.7 kg ha<sup>-1</sup>), and BRS FC 402 (1548.9 kg ha<sup>-1</sup>) subjected to co-inoculation showed a greater increase in GY than when subjected to inoculation with *Rhizobium*, with differences of 822.2, 605.3, and 520.9 kg ha<sup>-1</sup>, representing increments of 40.1, 33.1, and 33.6%, respectively, in GY for these cultivars.

Analysis of the additional treatments with the triple factorial showed that the triple factorial (1753.5 kg ha<sup>-1</sup>) was inferior to the additional treatments in the presence of N, except for the cultivar BRS Pitanga, which did not differ statistically (Figure 10D). The additional treatments showed increments in GY of 25.9% for BRS FC 402 (C1N1), with 2365.8 kg ha<sup>-1</sup>; 35.2% for BRS Estilo (C2N1), with 2706.2 kg ha<sup>-1</sup>; and 38.7% for BRS Esteio (C4N1), with 2859.2 kg ha<sup>-1</sup>. However, the triple factorial was superior to the additional treatments in the absence of N, except for BRS Estilo, since it did not differ statistically. The additional treatments under this condition showed inferiority of 30.8% for the cultivar BRS FC 402 (C1N0), with 1213.4 kg ha<sup>-1</sup>; 54.2% for BRS Pitanga (C3N0), with 802.6 kg ha<sup>-1</sup>; and 33.9% for BRS Esteio (C4N0), with 1158.9 kg ha<sup>-1</sup>.

When comparing the additional treatments with the triple factorial in season 2, the factorial showed a GY of 1459.6 kg ha<sup>-1</sup>, which is, respectively, 29.3, 29.9, and 45.8% lower than those obtained by the additional treatments in the presence of N, which had values of 2065.8 kg ha<sup>-1</sup> for BRS FC 402 (C1N1), 2072.8 kg ha<sup>-1</sup> for BRS Estilo (C2N1), and 2692.6 kg ha<sup>-1</sup> for BRS Esteio (C4N1) (Figure 10E). The additional treatment in BRS Pitanga (C3N1) did not differ statistically from the factorial treatment. On the other hand, in the additional treatments in the absence of N, the triple factorial had a higher GY than the additional treatments, which showed an inferiority of 44.3% for the cultivar BRS FC 402 (C1N0), with 813.4 kg ha<sup>-1</sup>; 37% for BRS Estilo (C2N0), with 919.4 kg ha<sup>-1</sup>; 63.3% for BRS Pitanga (C3N0), with 535.9 kg ha<sup>-1</sup>; and 48% for BRS Esteio (C4N0), with 758.9 kg ha<sup>-1</sup>.

#### 4. Discussion

In season 1, the treatments with co-inoculation with *R. tropici* and *A. brasilense* stood out, being superior to the others in root length (Figure 2A), root dry mass (Figure 3A), and shoot dry mass (Figure 4A,C). The treatments that received this combination showed

greater root growth, increasing root and shoot dry mass. This can be attributed to the fact that these plants had a more accentuated root growth, which increased the surface of contact between the roots and the soil. As a result, there was a greater absorption of nutrients and water, increasing both root and shoot dry mass [28].

It should be noted that the results obtained may be influenced by soil conditions. Table 1 shows that in season 1 there was higher soil fertility, with a V% value of 84%, indicating optimal conditions for plant development [29,30]. These favorable soil conditions may have contributed to intensifying the positive effects of co-inoculation with *R. tropici* and *A. brasilense* under this condition.

For the leaf area index, in season 1, the behavior differed from the other morphological traits, with the highest value in plants inoculated only with Rhizobium (Figure 5A–C). This can be attributed to the fact that co-inoculation, despite promoting root growth, may require higher energy expenditure of the plants. As a result, co-inoculated plants may direct more resources to root growth at the expense of leaf development. This phenomenon of prioritizing root growth to the detriment of shoots was also observed by [31], in a study with *R. tropici* in seed treatments with fungicides, insecticides, and polymers in common bean plants. The results obtained in the additional treatment with the triple factorial in seasons 1 and 2 can be explained by the action of N on the leaf structure, which is more viable when compared to inoculation and co-inoculation because it has its costs, so the use of mineral N may have reduced the defoliation of common bean [32].

For root length, root dry mass and shoot dry mass, in season 2, the behavior was similar to that of season 1, but with lower mean values (Figures 2B, 3B, 4B, and 4D, respectively). This may be related to the fertility of the soil in season 2 (Table 1), with a V% value of 53%, considered below the recommended level for common bean cultivation, which is 60%. In this context, treatments with co-inoculation with *R. tropici* and *A. brasilense* showed superior results in terms of root growth (RL), root dry mass (RDM), and shoot dry mass (SDM). These results can be attributed to the fact that plants that received this combination of inoculants showed higher root growth, which increased the surface area of contact between the roots and the soil. This larger contact surface allowed for greater absorption of nutrients and water by the plants, resulting in better development and higher biomass production, leading to greater dry mass in both roots and shoots, as described by [33], demonstrating that this result is due to the presence of an amount of phytohormone in the plant promoted by these bacteria. The result obtained in the additional treatment with the factorial is possibly associated with the use of fertilizer, which can help reduce the defoliation of common beans, as stated by Pratişoli et al. (2012) [32].

The results for nitrogen content (Figure 6A–C) and hundred-grain weight (Figure 8A) were similar to those found by [34] in studies carried out with maize. When *A. brasilense* was added at a dose of 200 mL ha<sup>-1</sup>, an increase in the physiological response of the crop was observed. On the other hand, in the study by Bárbaro-Torneli et al. (2018) [35], in soybean crop, when *A. brasilense* was applied in the furrow at a dose of 450 mL ha<sup>-1</sup>, an increase in agronomic parameters was observed. These studies highlight the benefits of co-inoculation with *A. brasilense* in different crops, in terms of both physiological responses and agronomic performance, corroborating the results obtained with the cultivars studied.

For nitrogen content in season 2 (Figure 6E,F), it was observed that the cultivars BRS Estilo (C2N1) and BRS Esteio (CAN1), in the presence and absence of mineral N, showed superiority in the values, thus showing that these cultivars in both seasons responded well to the dose of 70 kg of N ha<sup>-1</sup>, corroborating the yield potentials described by Oliveira et al. (2024) [17] and Reis et al. (2023) [18], as these cultivars show high yield when cultivated with mineral N.

The results observed for the agronomic variables of number of pods per plant (Figure 7A,B) and number of grains per pod (Figure 8A), in season 1, may be associated with the more efficient use of N available to the plants due to greater precipitation during the common bean flowering period, corroborating the research results obtained by [36]. In addition, it is important to highlight that both NGP and HGW are agronomic traits with high heritability, meaning that they are strongly influenced by plant genetics [6]. Unless the plants are adequately nourished, these traits are less susceptible to modification through management. An adequate supply of nutrients can result in a greater development of plants and an increase in the number of productive branches, and consequently the number of pods per plant. This more vigorous development and adequate availability of resources are usually reflected in a higher number of pods and grains produced [8,37], as found in this research.

The increase in agronomic variables due to *A. brasilense* occurs because these bacteria produce phytohormones, such as indoleacetic acid, which acts as a root and shoot growth promoter. This results in a greater absorption of water and nutrients by plants, which in turn leads to increased production of photoassimilates (organic substances produced by the plant through photosynthesis) and resistance to biotic and abiotic stresses [38,39].

The variables NC, NPP, NGP, and HGW, in season 2, as observed in season 1, showed a similar behavior in promoting significant increases when subjected to co-inoculation with the bacteria *R. tropici* and *A. brasilense*, through soil application, and these increases can be associated with the behavior of the diazotrophic bacteria, which promoted the supply and improved the use of N available to the plants [36,38]. However, when these parameters are evaluated considering the lower fertility in season 2, a reduction in the means found can be observed, since the availability of nutrients limits common bean production, especially in soils such as those found in the Brazilian “cerrado” biome [40], even after correction.

Co-inoculation with *R. tropici* and *A. brasilense* may contribute to better utilization of photoassimilates by plants, and this is related to the ability of *A. brasilense* to secrete indole-3-acetic acid (IAA), a plant hormone known to trigger cellular responses, such as increased cell elongation, in addition to influencing slower processes, such as cell division and differentiation [38]. These responses can promote more efficient plant growth and development. Such improvement in plant performance with the co-inoculation of *R. tropici* and *A. brasilense* is consistent with the results found by [41], who observed that inoculation carried out via seed treatment and the association between the bacteria *Bradyrhizobium japonicum* and *Azospirillum brasilense*, applied in the sowing furrow, resulted in improvements in the physiological and morphological aspects of the soybean crop, when compared to non-inoculated plants (control).

Grain yield showed significant interaction in season 1 for cultivars subjected to co-inoculation of *R. tropici* and *A. brasilense* applied to the soil, with a value of 2378.9 kg ha<sup>-1</sup>, regardless of the cultivar studied (Figure 10A). However, treatments with mineral N were superior to the triple factorial. It is worth mentioning that this yield is more than double when compared to Brazil’s average yield for the common bean crop. In addition, this yield value obtained is in accordance with the result reported by [6], who found that the combination of seed inoculation and supplementary topdressing inoculation (re-inoculation) resulted in a significant increase in the grain yield of the cultivar BRS Valente, specifically, an increase of 2827.0 kg ha<sup>-1</sup> compared to the application of N fertilization of 20 kg ha<sup>-1</sup> as basal and 40 kg ha<sup>-1</sup> of mineral N as topdressing.

As in season 1, an interaction of co-inoculation via soil was observed in season 2; however, possibly due to the lower natural fertility of the soil, the mean values of yield were not equal, even after liming at the dose of 1.5 ton ha<sup>-1</sup>. The increments in GY in both crop seasons, promoted by the application of N<sub>2</sub>-fixing microorganisms associated

with phytohormone promoters, corroborate the study carried out by [29], who demonstrated that the highest grain yields of common bean in the Carioca group were obtained with the association of *Rhizobium tropici* + *Azospirillum brasilense*, which led to a value of 2448.4 kg ha<sup>-1</sup>. Also in season 2, it was observed that the cultivars BRS Esteio and BRS Estilo showed higher GY under co-inoculation, demonstrating that these genetic materials respond well to bacteria inoculated with this technique, because BNF varies according to the species and/or varieties/cultivars used, in addition to the fact that common bean is susceptible to colonization with these bacteria, which in turn act by promoting an increase in plant nutrition.

In relation to the triple factorial treatments with the additional treatments, it can be observed that the cultivars BRS FC 402 (C1N0), BRS Estilo (C2N1), and BRS Esteio (C3N0) in the presence of mineral N in seasons 1 and 2 were superior to treatments subjected to inoculation and co-inoculation, regardless of the method of application of the products (Figure 10D,E). This is related to the addition of N, which promotes greater conditioning for production; however, its use promotes negative impacts on the environment and increases production costs [5]. Therefore, the adoption of sustainable alternatives and efficient management is essential to guarantee the economic viability of agriculture and environmental preservation.

This study is innovative in exploring the combination of inoculation of *R. tropici* and co-inoculation of *R. tropici* and *A. brasilense*, applied to seed or soil in four high-yielding cultivars, and it was possible to observe that the soil co-inoculation technique, an uncommon practice for common bean, has promising results for use by farmers who cultivate common bean. This statement is true when comparing data from [2] referring to Brazil, with an average yield of the common bean crop obtained in the last season (2021/2022) with a value of only 1036.0 kg ha<sup>-1</sup>, to the results obtained in the present study, which demonstrated that the effects of co-inoculation carried out via soil were positive, resulting in GY of 2378.9 kg ha<sup>-1</sup>, significantly surpassing the national average.

## 5. Conclusions

The co-inoculation technique, applied to the soil, favors the common bean's morphological and agronomic aspects, regardless of the crop season.

Co-inoculation with *R. tropici* + *A. brasilense* promotes the highest yields of the common bean cultivars BRS Estilo and BRS Esteio, with respective values of 2049.3 and 1830.7 kg ha<sup>-1</sup>.

Co-inoculation with *R. tropici* + *A. brasilense* applied directly to the soil can be an alternative to nitrogen fertilization in common bean crops.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15020377/s1>, Table S1: Analysis of variance (Medium Square) for morphophysiological and agronomic variables of bean cultivars (A), subjected to inoculation with rhizobia and co-inoculation with rhizobia + *Azospirillum* (B), applied via seed and soil (C), in the spring-summer 2021/2022 season and winter 2022 season conducted respectively in Ipameri-GO and Anápolis-GO, Brazil.

**Author Contributions:** Conceptualization, G.R.S. and I.R.T.; methodology, I.R.T.; software, E.C.R.; validation, G.R.S. and N.M.d.B.C.; formal analysis, G.R.S.; investigation, G.R.S. and I.R.T.; resources, I.R.T.; data curation, G.R.S., A.B.F. and M.R.B.N.; writing—original draft preparation, G.R.S. and I.R.T.; writing—review and editing, G.C.d.S., F.L.S.S. and D.C.; visualization, G.R.S.; supervision, I.R.T.; project administration, I.R.T.; funding acquisition, I.R.T. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The data that support the findings of this study are available within the article and in the Supporting Information of this article.

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