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Organic maize grown with *Herbaspirillum seropedicae* and *Azospirillum brasilense* associated with green manures[1]

Milho orgânico cultivado com *Herbaspirillum seropedicae* e *Azospirillum brasilense* associados com adubos verdes

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HIGHLIGHTS:

Associative bacteria increase maize yield.
Green manure associated with diazotrophic bacteria is a viable alternative for organic maize cultivation.
Jack bean, sunn hemp, and velvet bean as green manure promote a good performance of the maize crop.

ABSTRACT: Organic maize cultivation is gaining relevance in the agricultural sector due to its rising profitability for producers and its contribution to reducing environmental impacts. This study aimed to evaluate the effects of green manure and inoculation with diazotrophic bacteria on maize yield under an organic cropping system. The experiment was conducted at Sítio Vale da Jaqueira, an organic-certified area located in the village of Estiva, municipality of Vitória da Conquista, Bahia, Brazil. A randomized block design in a 4 × 3 factorial scheme was used, with factors consisting of green manure (velvet bean, sunn hemp, jack bean, and weeds) with and without inoculation using *Herbaspirillum seropedicae* and *Azospirillum brasilense*, with four replications. The association between H. seropedicae and jack bean resulted in a 44.91% increase in grain yield compared to the control, providing a viable alternative for organic maize cultivation.

Key words: *Zea mays* L., biological fixation, sunn hemp, velvet bean, jack bean

RESUMO: O cultivo orgânico de milho vem ganhando maior relevância em todo o setor agrícola, com mercado em ascensão, mais rentável para os produtores além de contribuir com o meio ambiente, reduzindo impactos ambientais. O objetivo deste estudo foi avaliar o efeito da adubação verde e inoculação com bactérias diazotróficas na produtividade do milho, sob o sistema orgânico de cultivo. O experimento foi conduzido no Sítio Vale da Jaqueira (área com certificação orgânica), povoado da Estiva, município de Vitória da Conquista, Bahia. O delineamento foi em blocos casualizados, esquema fatorial 4 × 3. Os fatores foram: Adubação verde (mucuna preta, crotalária, feijão de porco, plantas espontâneas) com inoculação (*Herbaspirillum seropedicae*, *Azospirillum brasilense*) e sem inoculação, com quatro repetições. A associação entre Herbaspirillum seropedicae e feijão de porco proporcionou incremento de 44,91% na produtividade de grãos em relação a testemunha, sendo uma alternativa viável para o cultivo de milho em sistema orgânico.

Palavras-chave: *Zea mays* L., fixação biológica, crotalária, mucuna preta, feijão de porco

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Introduction

The agricultural sector has placed greater emphasis on organic maize cultivation due to its increasing profitability and market demand (Gazzola et al., 2019). Organic methods also promote reduced environmental impacts, lower risks for farmers, and healthier products for consumers.

As mineral nitrogen sources cannot be used in organic maize cultivation, biological nitrogen fixation (BNF) becomes a viable alternative for supplying this nutrient. Many authors have studied the contribution of associative bacteria in providing nitrogen for non-legume crops, including (Sabino et al., 2012; Moreno et al., 2019; Ventura et al., 2020; Ferreira et al., 2021; Marcolini et al., 2022; Matsuo et al., 2022). Dartora et al. (2013) studied the effects of *Azospirillum brasilense* and *Herbaspirillum seropedicae* inoculation on maize and observed that the associated inoculation of both strains increased grain yield compared to the control.

The use of cover crops is as effective as conventional fertilization (Leal et al., 2021; Oliveira et al., 2021), with leguminous plants as a source of fertilization associated with diazotrophic bacteria inoculation promoting positive performance for components of production and yield in maize crops. This study aimed to evaluate the effects of green manure and inoculation with diazotrophic bacteria on maize yield under an organic cropping system.

Material and Methods

The experiment took place from August 2021 to April 2022 at Sítio Vale da Jaqueira in Estiva village, Vitória da Conquista, Bahia, Brazil (14° 52' 6" S, 40° 44' 55" W, 917-m altitude). The region has an average annual temperature of 20.4 °C and receives an average annual rainfall of 733.9 mm (Figure 1), mostly from November to March (INPE, 2023).

The experimental design consisted of randomized blocks in a 4 × 3 factorial scheme with the following factors: green manure (weeds – control, velvet bean, sunn hemp, and jack bean) and inoculation (with *Herbaspirillum seropedicae* and *Azospirillum brasilense* strains and no inoculation), and four replicates.

The experiment was conducted in a family farming production unit that practices organic agriculture and has the participatory certification. The area has been undergoing continuous agroecological transition since 2016, with management practices conforming to the Organic Law. Farmers have already adopted practices such as the addition of straw, green manure, the use of organic compounds, and biological control for the organic cultivation of peppers, strawberries, and other vegetables.

The soil in the study area is classified as an Oxisol (United States, 2014), which corresponds to Latossolo Vermelho in the Brazilian Soil Classification System (EMBRAPA, 2018). Before legume cultivation, soil samples from the 0-20 cm depth layer had the following chemical characteristics: pH in H_2O = 6.0, P = 56 mg dm^{-3}, SB = 5.6 $cmol_c$ dm^{-3}, t = 5.8 $cmol_c$ dm^{-3}, T = 10.3 $cmol_c$ dm^{-3}, and V = 54%. Liming (broadcast distribution) was carried out using 2 Mg ha^{-1} of dolomitic limestone 75 days before the experiment. Subsequent soil analysis for maize cultivation revealed the following chemical characteristics in the 0-20 cm depth layer: pH in H_2O = 6.3, P = 54 mg dm^{-3}, SB = 6.0 $cmol_c$ dm^{-3}, t = 6.2 $cmol_c$ dm^{-3}, T = 11.9 $cmol_c$ dm^{-3}, and V = 54.5%.

The first stage of the experiment involved cultivating species used as green manure in the designated treatment plots, with a spacing of 0.50 m between rows, eight seeds per linear meter for jack bean and velvet bean, and 50 seeds per linear meter for sunn hemp. The control plots, which were left unplanted and unmanaged, were separated from the treatment plots. Only the naturally occurring plants within the delimited site were allowed to grow in the control plots.

Before sowing, legume seeds were inoculated with symbiotic diazotrophic bacteria of the species *Bradyrhizobium elkanii* strain BR2811 (SEMIA 6158), which is recommended by the Ministry of Agriculture, Livestock and Food Supply (MAPA). The inoculant was a peaty medium applied at a dose of 110 g per 20 kg of seeds, according to Ávila et al. (2020). Sowing was done manually in furrows opened with hoes, 120 days before maize sowing.

Before cutting the green manures and weeds, the plant material was characterized through the random and single release of a 0.50 × 0.50 m (0.25 m^2) quadrat on the plots, and the plants inside the frame were cut close to the soil. The collected material was weighed on a digital scale to measure fresh mass (FM) and subsequently taken to an air circulation oven at a temperature of 65 °C until reaching a constant weight

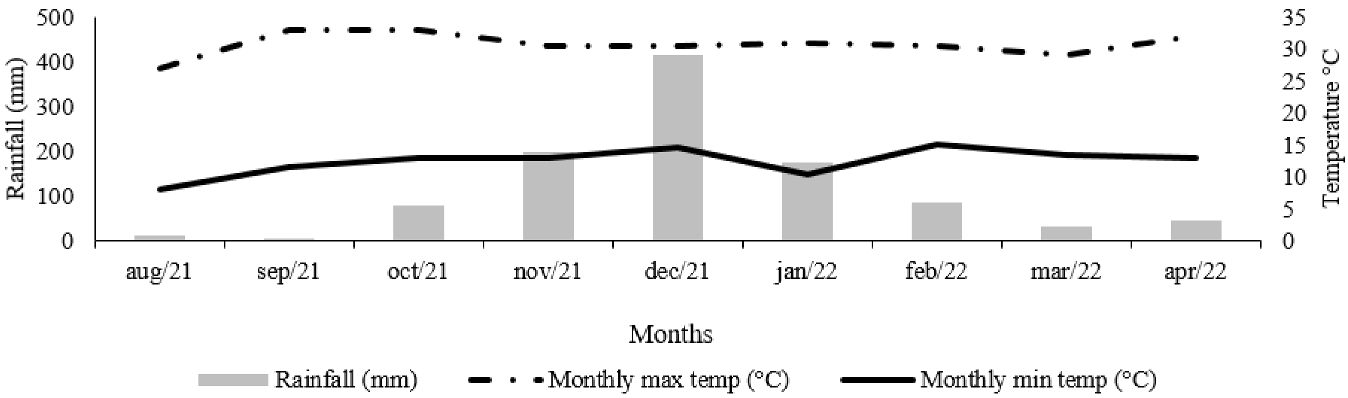

Figure 1. Monthly rainfall, maximum and minimum temperatures during the experimental period. Climatic data extracted from INPE website – National Institute for Space Research

to determine the dry mass (DM). The shoot fresh and dry mass results were extrapolated to Mg ha^{-1}.

Weeds and green manures were cut simultaneously using a manual brushcutter after the plant material had been collected. Since legumes have different growth cycles and their flowering stages do not coincide, the flowering of slower-growing species was taken into consideration. All plants were cut within their designated plots and screened to prevent mixing with nearby plots. The plant material was deposited onto the soil 15 days before maize sowing.

The maize genotype used was the AL Bandeirante variety. The experimental plots consisted of five rows, each 3.0 m in length, spaced 0.70 m apart, resulting in an area of 8.4 m^2 per plot. The observation area comprised the three central rows of each plot, with 0.50 m removed from each end. The distance between plots was 0.50 m, and the distance between blocks was 1.50 m.

The sowing of maize was done manually, with 25 seeds per row. The maize seeds were inoculated with diazotrophic bacteria of the *Herbaspirillum seropedicae* ZAE94 (BR 11417) and *Azospirillum brasilense* (AbV5 and AbV6), species, each with a population of 10^9 cells mL^{-1}. The inoculant was applied as a liquid medium at a rate of 100 mL ha^{-1}.

Thinning was performed 25 days after sowing to maintain 20 plants in each planting row. Cultural management and insect control were carried out according to the crop's requirements, following the norms established for organic cultivation. *Trichogramma* sp. micro-wasps were used to control fall armyworms.

The maize harvest was carried out in April 2022, and the ears were dried in the open air. The moisture level was approximately 20%, and the ears were left in place until the moisture level reached around 13%. Subsequently, production and grain yield components were evaluated.

Ten ears were randomly harvested from each experimental plot for evaluation. Ear diameter and length were measured using a digital caliper and a graduated ruler, respectively. Husked ear weight and cob weight were determined using a two-digit digital precision scale. The number of grains per row was obtained from the average of the grains in three rows in each ear.

The yield was determined for all the grains from the observation area's ears (including the cobs used for other evaluations), which were weighed on a two-digit digital precision scale. The values were extrapolated to Mg ha^{-1} after correcting for moisture to 13%.

The data were subjected to analysis for normality of errors using the Lilliefors test, and for homogeneity of variances using Bartlett's test, as recommended by Banzatto & Kronka (2011), followed by an analysis of variance. Mean values were compared using the Scott-Knott test at a probability level of 0.05. For analysis of the means and variances of legumes and spontaneous plants that preceded maize cultivation, the Tukey test was applied at a probability level of 0.05 using the software SISVAR 5.3 (Ferreira, 2019). Finally, multivariate analysis was performed by evaluating principal components using the XLSTAT software (Statistical Analysis Software).

Results and Discussion

Mass evaluations were conducted on the plants that preceded maize cultivation to characterize the amount of biomass deposited in the soil. This information is critical for interpreting the performance of the crop because the quantity of biomass can influence soil characteristics such as chemical composition, physical properties, and biological activity. A higher amount of biomass can enhance water retention, reduce soil temperature, and increase organic matter content, which creates favorable conditions for the development of soil microbiota that actively participate in nutrient-cycling processes. Therefore, mass characterization values are important indicators of soil quality and can help improve crop productivity.

Table 1 shows the results of the analysis of variance for shoot fresh and dry masses of legumes and spontaneous plants, including their corresponding coefficients of variation. The statistical analysis revealed significant differences in the evaluated characteristics between the treatments.

Sunn hemp had the highest dry mass among the legumes, followed by jack bean and velvet bean, while the weeds had the lowest dry mass, according to Table 2. This finding is consistent with a previous study by Nunes et al. (2022) that also reported sunn hemp having the highest dry mass among the plants studied.

Sunn hemp had the highest dry and fresh masses compared to the other plants evaluated (Table 2). This study showed higher results than those reported by Kappes et al. (2013), possibly due to the seed inoculation with a strain of diazotrophic bacteria. The symbiotic relationship between these microorganisms and legume plants improves performance since biological nitrogen fixation is well-established in this plant group.

Among the evaluated plants, jack bean and velvet bean had higher fresh mass values than spontaneous plants, while jack bean had the second-highest average for dry mass, followed by velvet bean and spontaneous plants (Table 2). Mass determination helps in understanding the results, as higher fresh mass values indicate greater potential for soil cover,

Table 1. Summary of the statistical analysis for shoot fresh and dry masses of legumes and weeds used as predecessor crops before planting AL Bandeirante maize under an organic system

SV	Mean squares		
	DF	FM	DM
Green manure	3	0.7611*	47.8266*
Block	3	1.1122^{ns}	0.2266^{ns}
CV (%)		2.70	4.77

*Significant (p≤0.05) by analysis of variance; ^{ns} non-significant (p ≤ 0.05) by analysis of variance

Table 2. Mean values of fresh and dry masses of green manure and spontaneous plants

Green manure	Fresh mass	Dry mass
	(Mg ha^{-1})	
Sunn hemp	43.6 a	12.6 a
Jack bean	35.6 b	8.0 b
Velvet bean	34.4 b	6.2 c
Weeds (Control)	15.8 c	4.6 d

Means followed by the same letter in the column do not differ from each other by Tukey's test at p > 0.05

and higher dry mass values indicate greater accumulation of nutrients in plant structures. These legumes show potential for use as green manure or cover crops.

Arf et al. (2018) studied maize intercropped with legumes for two consecutive years. After 46 and 49 days of sowing, they obtained lower dry masses than in this study for sunn hemp (4.36 and 4.97 Mg ha^{-1}) and velvet bean (3.34 and 4.28 Mg ha^{-1}) in the first and second year, respectively. This difference in results may be attributed to the cutting time of the legumes, which was shorter in the Arf et al. (2018) study, preventing their full development.

The cultivation period is an important factor to consider, as shown in the study by Teodoro et al. (2011) on legumes for green manuring. The authors reported higher dry mass production than the present study, with 13.9 Mg ha^{-1} for sunn hemp, 8.77 Mg ha^{-1} for jack bean, and 7.5 Mg ha^{-1} for velvet bean. The differences in results may be attributed to the sowing season, as the legumes studied by Teodoro et al. were grown in spring-summer, which is the optimal season for their development.

The control group (weeds) consisted mainly of *Physalis ixocarpa*, *Cyperus rotundus*, *Andropogon bicornis*, *Bidens pilosa*, *Cenchrus echinatus*, *Richardsonia grandiflora*, and *Desmodium tortuosum*. As these plants grew spontaneously without inoculation with diazotrophic bacteria, they had no symbiotic relationship with these bacteria as the legumes did. As a result, the shoot dry mass of the control group was the lowest, indicating a lower nitrogen supply.

The legumes studied in this research exhibit a potential for use as green manure due to their high fresh and dry mass content. However, these plants have diverse characteristics in terms of the growth cycle, habit, and carbon-to-nitrogen ratio (C/N), which need to be analyzed for their utilization in crop succession or intercropping. In a study by Pereira et al. (2017), cover crops were evaluated for nutrient cycling, and nitrogen accumulations of 817.33 kg ha^{-1} for sunn hemp, 737.17 kg ha^{-1} for jack bean and 219.50 kg ha^{-1} for velvet bean were observed, which fulfill the nitrogen demand of maize. These plants were assessed for their ability to provide nutrients as green manure in maize grown in rotation, which is an important factor in evaluating maize crop performance.

The results of the analysis of variance for maize traits are presented in Table 3, including ear diameter, ear length, number of grains per row, ear weight, cob weight, and grain yield, as well as their coefficients of variation. All of these characteristics showed a significant effect from the interaction of factors (green manure × inoculation).

Table 4 displays the follow-up of each green manure under different types of inoculation, highlighting that the control had the lowest means in all characteristics compared to the other green manures.

Legumes performed better than the control in all evaluated characteristics in this study when not inoculated, except for ear diameter. Sunn hemp had similar results to other legumes in this variable only when inoculated with *Herbaspirillum seropedicae* (Table 4). In a study by Ávila et al. (2020), a positive effect on maize was observed with the interaction between *Herbaspirillum seropedicae* and green manure.

Table 4 shows that sunn hemp, velvet bean, and jack bean had similar ear lengths when associated with *Azospirillum* or without inoculation. However, when *Herbaspirillum* was used, sunn hemp and jack bean showed greater efficiency.

Table 4. Follow-up of the interaction green manure × inoculation for ear diameter, ear length, number of grains per row, ear weight, cob weight, and grain yield in an organic maize cultivation

Green manure	Inoculation		
	Azospirillum	*Herbaspirillum*	No inoculation
	Ear diameter (mm)		
Sunn hemp	38.28 bC	43.62 aA	40.99 bB
Jack bean	43.44 aA	44.84 aA	43.62 aA
Velvet bean	44.00 aA	43.24 aA	43.17 aA
Control	39.30 bA	39.55 bA	37.28 cB
	Ear length (cm)		
Sunn hemp	14.82 aA	15.16 aA	14.63 aA
Jack bean	15.41 aA	15.82 aA	14.72 aB
Velvet bean	15.49 aA	14.44 bB	15.30 aA
Control	12.39 bA	12.45 cA	11.60 bB
	Grains per row		
Sunn hemp	31.71 aA	31.18 bA	31.54 aA
Jack bean	32.52 aA	33.13 aA	31.41 aA
Velvet bean	33.12 aA	29.60 bB	32.51 aA
Control	25.44 bA	25.63 cA	23.98 bA
	Ear weight (kg)		
Sunn hemp	0.1130 bA	0.0795 cB	0.1177 aA
Jack bean	0.1242 aB	0.1402 aA	0.1267 aB
Velvet bean	0.1335 aA	0.1195 bA	0.1265 aA
Control	0.0835 cA	0.0815 cA	0.0812 bA
	Cob weight (kg)		
Sunn hemp	0.0262 bA	0.0292 aA	0.0280 aA
Jack bean	0.0305 aA	0.0320 aA	0.0300 aA
Velvet bean	0.0305 aA	0.0287 aA	0.0297 aA
Control	0.0212 cA	0.0210 bA	0.0207 bA
	Yield (Mg ha^{-1})		
Sunn hemp	5.81 aA	5.96 aA	5.64 aA
Jack bean	6.01 aA	6.59 aA	5.75 aA
Velvet bean	6.42 aA	6.29 aA	5.76 aA
Control	3.68 bA	3.63 bA	3.58 bA

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other by the Scott-Knott test at 0.05 level of probability

Table 3. Summary of the analysis of variance for ear diameter (ED), ear length (EL), grains per row (GR), ear weight (EW), cob weight (CW), and yield of AL Bandeirante maize subjected to green manuring and inoculation with diazotrophic bacteria in organic cultivation

SV	DF	Mean squares					
		ED	EL	GR	EW	CW	Yield
Green manure (M)	3	709.4572*	264.0995*	1422.0514*	0.0622*	0.0023*	17.4821*
Inoculation (I)	2	128.55*	10.265*	36.2725^{ns}	0.0035*	0.00002^{ns}	0.8002^{ns}
M × I	6	83.4003*	8.8686*	57.0613*	0.0062*	0.00004*	0.1852*
Block	3	132.3524*	42.6509*	223.6012*	0.0183*	0.0004*	1.743*
CV (%)		8.49	12.78	14.97	24.92	29.01	9.98

*Significant ($p \leq 0.05$) by analysis of variance; ns non-significant ($p \leq 0.05$) by analysis of variance; DF: degree of freedom; M × I: green manure × inoculation interaction

This strain's interaction with sunn hemp and jack bean plants improved maize performance, resulting in increased production components.

In terms of grains per row, the results were similar for the association with *Azospirillum* and without inoculation, while the use of *Herbaspirillum* with jack bean showed a significant increase compared to the control (29.26%), velvet bean (11.92%), and sunn hemp (6.25%) (Table 4). These strains can produce indole acetic acid - IAA (Sabino et al., 2012; Zhao et al. 2018; Mondal et al. 2019), which promotes root growth and enhances the plant's capacity to absorb water and nutrients.

Cob and ear weights showed similar responses to *Azospirillum* inoculation or absence thereof, with green manures outperforming the control. Jack bean was the most productive legume, showing a 72% increase in ear weight when *Herbaspirillum* was also present. Green manures had higher cob weight values than weeds, with growth ranging from 36.66 to 52.38% (Table 4).

Alves et al. (2020) found that inoculating maize with diazotrophic bacteria, specifically the *Herbaspirillum seropedicae* strain, increased grain yield even in the absence of nitrogen fertilizer and at the lowest dose tested. Bacteria have the potential to enhance crop performance by providing nitrogen and promoting plant growth, particularly when conditions for their growth are favorable.

Treatments with all the studied legumes promoted a positive effect on grain yield, demonstrating the efficiency of green manuring for organic maize cultivation. For instance, Saldanha et al. (2017) reported that including jack bean as a green manure significantly increased maize development. Rani et al. (2022) also found that growing legumes in succession with maize releases nutrients and makes them available for the following crops, resulting in lower production costs and more sustainable crops without yield losses. Compared to the average maize yield in Brazil (5.24 Mg ha^{-1}) and Bahia (3.0 Mg ha^{-1}) under conventional cultivation systems (CONAB, 2022), yields achieved with green manure in this study (Table 4) were higher. Therefore, the use of green manure has the potential to improve maize yield and sustainability.

Regarding the interaction between inoculation and green manure source, the control treatment (spontaneous plants) showed a significant difference. Inoculated plants with the strains had larger ear diameters and lengths than non-inoculated plants (Table 4), which is consistent with the findings of Matsuo et al. (2022) who reported that inoculating maize with diazotrophic bacteria promotes better crop performance.

The statistical analysis of the inoculation interaction within each green manure source revealed no significant difference in grain yield between the inoculation factors. However, when comparing jack bean treatments, the use of the *Herbaspirillum seropedicae* strain resulted in a 14-bag per hectare increase in grain yield compared to the treatment without inoculation (Table 4). This suggests that associative bacteria have the potential to promote root system expansion and nutrient absorption, leading to improved crop productivity.

The grain yield of treatments without legumes (control) reached 3.68 Mg ha^{-1} when inoculated with *Azospirillum brasilense* and 3.63 Mg ha^{-1} with *Herbaspirillum seropedicae*, which are higher than the average yield of 36 maize varieties in an organic cropping system reported by Valentine et al. (2021). Additionally, the findings in this study are consistent with Alves et al. (2020), who observed that inoculation with *Herbaspirillum seropedicae* and *Azospirillum brasilense* strains in the absence of nitrogen fertilization in maize increased grain yield.

The association of inoculation and green manure significantly increased grain yield compared to the control treatment, demonstrating the positive effect of this association. Specifically, the combination of *Azospirillum* inoculation and velvet bean increased yield by 2.64 Mg ha^{-1}, while the combination of no inoculation and velvet bean increased from 2.18 Mg ha^{-1} (Table 4). The inoculation of *H. seropedicae* associated with jack bean led to a significant increase in grain yield (2.96 Mg ha^{-1}), which was 44.91% higher than the control treatment (Table 4). Therefore, the interaction between green manure and associative bacteria improved the agronomic performance of maize in organic cultivation. This may be attributed to the benefits provided by organic matter, such as soil cover, temperature regulation, and adequate moisture for microorganism proliferation. Despite having a lower fresh mass volume than sunn hemp, jack bean showed high efficiency in nitrogen supply, as already demonstrated by Pereira et al. (2017).

The positive response observed from the association between diazotrophic bacteria and green manure in organic maize cultivation is significant for crop management. Nitrogen is one of the key limiting factors that affect maize production, and the use of synthetic fertilizers is not allowed in organic farming systems. Therefore, alternative methods that can enhance soil fertility and increase nutrient availability are crucial for sustainable agriculture.

The inoculation of sunn hemp with *Herbaspirillum* was superior only for ear diameter. On the other hand, there was no significant difference between the types of inoculation for ear length, grains per row, cob weight, and productivity. For optimal bacterial performance, initial nitrogen availability is necessary. Therefore, sunn hemp, due to its more lignified structures, may have hindered the decomposition process for cycling.

In the control treatment (spontaneous plants), inoculations with *Herbaspirillum* and *Azospirillum* were superior for ear diameter and length and promoted an increase of 100 and 50 kg ha^{-1} in grain yield, respectively.

Figures 2A and 2B depict the multivariate analysis of principal components for the association between diazotrophic bacteria inoculants and green manure in organic maize cultivation. The analysis explains 94.38% of the components, with the x-axis explaining 88.54% of the interactions.

In Figure 2A, the treatment without inoculation differed significantly from the others, showing no notable influence on the development of the studied characteristics. Yield and cob weight were the characteristics closest to the x-axis and strongly correlated with the use of *Herbaspirillum*.

Figure 2B shows that weeds are isolated from the interaction of organic maize growth factors, while yield and other characteristics are strongly influenced by the action of jack bean and velvet bean.

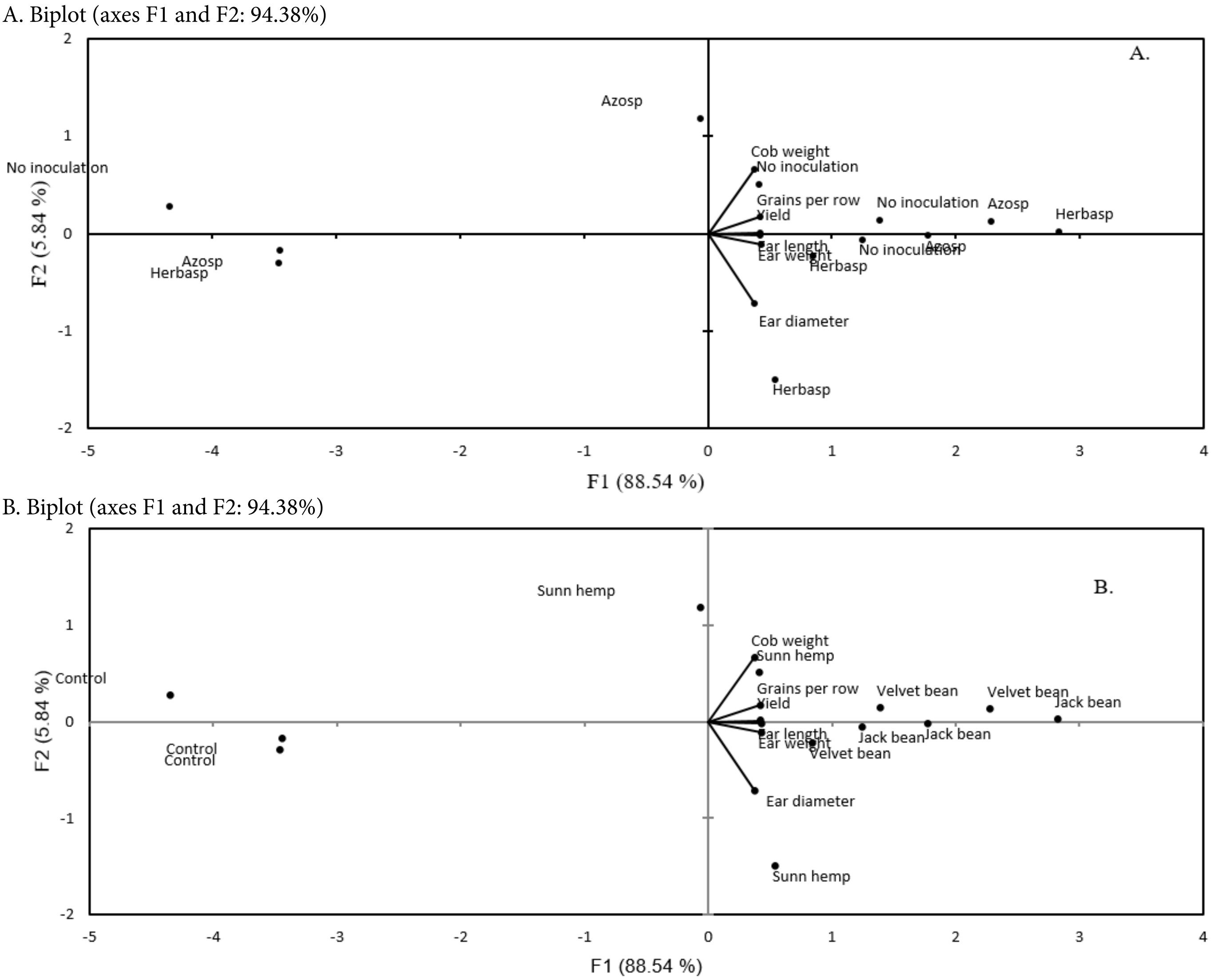

Figure 2. Principal components for the association between diazotrophic bacteria inoculants and green manure in organic maize cultivation

The multivariate analysis provided further evidence of the positive influence of *Herbaspirillum* in association with velvet bean and jack bean on maize production. Although sunn hemp produced more dry mass than the other legumes (Table 2), its slower decomposition rate and higher C/N ratio may have prolonged its stay in the soil. Pereira et al. (2017) reported that sunn hemp and jack bean did not differ significantly in terms of their nitrogen contribution.

Chieza et al. (2017) evaluated the production and economic aspects of maize intercropped with sunn hemp at different sowing intervals under organic management. Their findings showed that sunn hemp has the potential to serve as an alternative nitrogen source to mineral fertilization when grown in spring-summer.

The use of cover crops is an alternative that can improve soil quality, benefit commercial crops in succession, and contribute to the intensification of sustainable agriculture (Silva et al., 2021). In the present study, treatments with legumes produced positive results due to the contribution of nitrogen and other nutrients returned in the cycling process, as well as the protection provided by legumes without incorporating the plant material. This allowed for greater soil coverage, less soil disturbance, and the maintenance of adequate conditions for the activity and proliferation of soil microbiota.

Bacteria associations are crucial for achieving high yields with lower environmental impacts. Besides assisting in biological nitrogen fixation, some strains, such as those of the genus *Azospirillum*, can produce auxins that improve crop performance. Matsuo et al. (2022) investigated the initial growth of hybrid maize co-inoculated with *Azospirillum brasilense* and found higher root development and shoot phytomass in plants receiving these microorganisms.

This study found that the use of diazotrophic bacteria and green manure in organic maize cultivation significantly increased crop yield. This approach can improve soil health, reduce the need for synthetic fertilizers, and ultimately provide a sustainable alternative for organic farmers.

Conclusions

1. Inoculating corn with bacteria associated with green manures resulted in higher corn grain yields than the average maize yield in Brazil during the 2021/2022 crop year.

2. The association between *Herbaspirillum seropedicae* and jack bean provided the highest ear weights.

3. Using legumes as green manures resulted in higher yield increases from inoculation.

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