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Nutritional aspects and yield of corn intercropped with cover crops and inoculated with diazotrophic bacteria

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Intercropping corn with cover crops and inoculating with diazotrophic bacteria are sustainable low-cost practices that enhance nutrient cycling and uptake, leading to improved crop yield. Thus, this study evaluated the effects of monocropped corn versus intercropping with cover crops alongside foliar inoculation with *Azospirillum brasilense*. The experiment was conducted during the autumn-winter season in Dracena, within the Nova Alta Paulista region, Brazil, using a sandy-textured dystrophic Ultisol. The experiment was designed in a randomized blocks in a 4 × 2 factorial scheme, having four replicates. The treatments consisted of the cultivation of corn alone and intercropped with cover crops (*Crotalaria spectabilis*, *Urochloa ruziziensis* and *C. spectabilis* + *U. ruziziensis*) in the presence and absence inoculation with *Azospirillum brasilense* via foliar spray at crop growth stage V4. The inoculant contained strains AbV5 and AbV6 of *A. brasilense* (2×10^8 viable cells ml^{-1}) applied at 500 ml ha^{-1}. Intercropping corn with *U. ruziziensis* under inoculation, as well as corn with *C. spectabilis* + *U. ruziziensis*, regardless of inoculation, significantly increased grain yield in the second year. The corn intercropped with *C. spectabilis* irrespective of inoculation enhanced relative leaf chlorophyll content at the V4, V8, and R1 stages. In addition, intercropping corn with *U. ruziziensis* also enhanced leaf phosphorus (P) content while reducing sulfur (S) content. These findings suggest that farmers can optimize corn production by intercropping with *Urochloa ruziziensis* and *Crotalaria spectabilis* alongside diazotrophic bacterial inoculation to improve nutrient use efficiency and corn nutrition while reducing reliance on synthetic fertilizers and supporting low-carbon agriculture.

KEYWORDS

***Zea mays* L., *Crotalaria spectabilis*, *Urochloa ruziziensis*, biological nitrogen fixation, growth-promoting bacteria**

1 Introduction

Corn (*Zea mays* L.) holds an important role in global and Brazilian agricultural economies due to its prominent status among cultivated agricultural species (Môro and Fritsche-Neto, 2017). In addition to serve as a food for human consumption, corn is a primary component of feedstock for animals, particularly for poultry, cattle, and swine production. Therefore, advancing research to enhance grain yield remains critical to sustaining its agricultural and economic significance (Purwanto and Minardi, 2015). Intercropping is characterized as the innovative and sustainable alternative that allows cultivation of multiple plant species to enhance both yield and environmental benefits. Intercropping is considered part of the so-called fourth green revolution, due to its potential to render agricultural systems more ecologically sustainable (Martin-Guay et al., 2018).

Intercropping with cover crops increases soil organic matter, improving water retention and can provide greater resilience to extreme weather events, leading to higher yield and economic returns (Peng et al., 2024). In addition to yield improvements, cover crops improve soil health by enhancing physical, chemical, and biological properties, facilitating air and water movement, reducing compaction, enhancing recycling nutrients, and managing soil moisture to support biological nitrogen fixation (BNF) (Duchene et al., 2017; Peng et al., 2024). Intercropping cover crops contribute substantially to soil quality, particularly through increases in soil organic matter and carbon content, nutrient cycling, and the biological suppression of pests, pathogens, nematodes, and weeds (Martins et al., 2016; Schipanski et al., 2014).

Consequently, applied research aimed at improving the efficiency of BNF in tropical agriculture is essential and catalyzed by living organisms. The microorganisms responsible for nitrogen fixation, referred to as diazotrophs, are prokaryotes that utilize the enzyme nitrogenase to convert atmospheric nitrogen into ammonia through endergonic reaction (da Silveira and dos Santos Freitas, 2007). Among the bacteria species, *Azospirillum brasilense* has significant potential in enhancing nitrogen use efficiency and crop yield, particularly when co-applied with reduced nitrogen doses (Vogel et al., 2013; Galindo et al., 2021; Gato et al., 2024). The dose of 200 ml ha^{-1} applied via the sowing furrow of this inoculant provides an increase in productivity and a reduction in nitrogen application to the soil (Morais et al., 2016; Galindo et al., 2022). These bacteria associated with the corn root system can increase the capacity for root hair proliferation and greater soil exploration (Saikia et al., 2012; Fulchieri et al., 1993). This inoculant can enhance root architecture and nutrient uptake supports its use as a cost-effective technology via seed, furrow, or foliar application during early growth stages (Boleta et al., 2020; Silva et al., 2022; Hernández et al., 2024). The integrated use of diazotrophic bacteria with cover crops amplify soil organic matter, water retention, and nutrient cycling, reducing reliance on synthetic inputs while promoting ecological resilience (Duchene et al., 2017; Peng et al., 2024). The combination of foliar inoculation with *A. brasilense* and intercropping represents an innovative strategy to optimize nitrogen cycling and grain yield in sustainable tropical agriculture (Martin-Guay et al., 2018).

In addition, it can increase the contribution of organic matter, carbon, greater water retention, nutrient cycling and balance of the carbon/nitrogen (C/N) ratio in the soil–plant system. According to Lima Filho et al. (2023) and Vanolli et al. (2024), the *Crotalaria spectabilis* has C/N ratio of 18/1. However, due to the rapid decomposition and the annual cycle of *C. spectabilis*, a period of uncovered soil may occur until the next harvest. *C. spectabilis* has a high capacity to provide N via symbiotic BNF, between 60 and 120 kg ha^{-1} of N and a high potential for nutrient uptake, in average 222 kg ha^{-1} of N, 24 kg ha^{-1} of P and 220 kg ha^{-1} of K (Lima Filho et al., 2023; Vanolli et al., 2024). The *Urochloa ruziziensis* has a deep and vigorous root system, which helps in decompaction and formation of biopores in the soil. Furthermore, this this forage grass has a C/N ratio of 36/1, providing protection against the impacts of raindrops and reducing soil erosion processes, as well as a low capacity to provide N via symbiotic BNF between 30 and 40 kg ha^{-1} of N, and *U. ruziziensis* has high potential for nutrient cycling, extracting on average 200 kg ha^{-1} of N, 18 kg ha^{-1} of P and 372 kg ha^{-1} of K (Lima Filho et al., 2023; Vanolli et al., 2024).

Despite these advances, foliar inoculation of *A. brasilense* in corn, intercropped with cover plants remains underexplored. The hypothesis of this research was that the inoculation via foliar at the V4 stage of corn contributes positively to an increase in the plant nutrition and shoot dry mass yield of this cereal, both intercropped with the grass *U. ruziziensis* which demands more N, and intercropped with the legume *C. spectabilis*, which increases N in the agricultural system due to BNF, but it would not be enough to benefit the absorption of N by corn. In this context, this study introduces a novel approach of the foliar inoculation with *A. brasilense* in corn intercropped with cover crops. This approach represents a sustainable and low-cost strategy to enhance nutrient cycling, improve nitrogen fertilization efficiency, and boost nitrogen nutrition in corn, ultimately resulting in increased grain yield. Therefore, this study aims to evaluate the agronomic performance, nutritional composition and relative leaf chlorophyll content of intercropping systems involving corn, *Crotalaria spectabilis* and *Urochloa ruziziensis*, with and without foliar inoculation of *Azospirillum brasilense*. This research seeks to emphasize key physiological and nutritional indicators such as chlorophyll content, grain yield, and leaf nutrient concentrations to assess the effectiveness of such systems in enhancing yield and promoting sustainable soil management under the tropical conditions.

2 Materials and methods

2.1 Environmental characterization

The research was carried out during the autumn-winter of 2020 and 2021, in the municipality of Dracena, state of São Paulo, Brazil, located at 51°52' W longitude, 21° 29' S latitude, and an altitude of 420 m above the sea level, in a Ultisol, dystrophic, with sandy texture soil (Dos Santos et al., 2018).

The average annual values of precipitation, temperature and relative humidity are 1,261 mm, 24°C and 64%, respectively. According to the Köppen classification, the climate is Aw, humid tropical, with rainy summers and dry winters.

The climatic data on air temperature, rainfall, and relative humidity during the research were obtained at the Campbell Scientific CR10X meteorological station, installed at the Faculty of Agricultural and Technological Sciences (FCAT), belonging to the São Paulo State University (UNESP), located 200 m from the experimental area. The climatic data are presented in Figure 1, referring to the 2020 harvest (A) and 2021 harvest (B).

2.2 Experiment design

The experimental design was randomized blocks in a 4 × 2 factorial scheme, with four replicates. The treatments consisted of four intercropping systems and two inoculations (Figures 2, 3). The intercropping systems consisted of a single corn crop cultivation and three intercropping, corn with cover crops (*Crotalaria spectabilis, Urochloa ruziziensis* and *C. spectabilis* + *U. ruziziensis*) in association with and without foliar application of *Azospirillum brasilense* at the V4 phenological stage of the crop. The plot with corn alone and intercropped with cover crops (fabaceas and poaceas) consisted of four rows 5 m long spaced and 0.90 m apart, being sown at a depth of 0.05 m. All experimental plots consisted of an area of 18 m^2, considering the two central rows of corn as a useful area for evaluations, disregarding 0.5 m at each end. The size of the plot and the number of plants evaluated in each evaluation in the useful area of the plots are standard in most corn experiments.

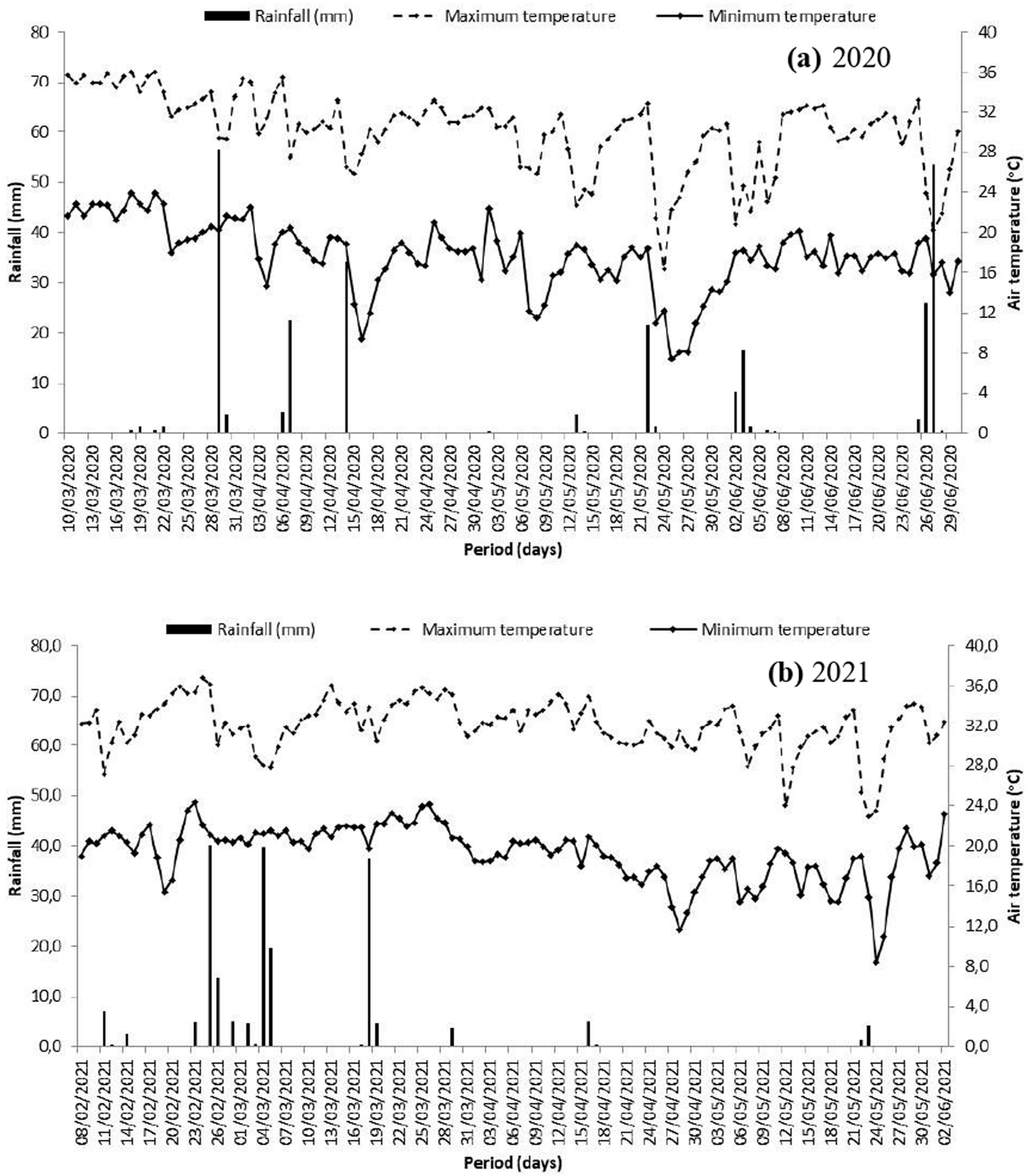

FIGURE 1
The behavior of rainfall (mm), maximum and minimum air temperature (°C), in Dracena, SP, Brazil, during the intercropping and inoculation systems experiment with *Azospirillum brasilense* in corn second harvest in 2020 **(a)** and 2021 **(b)**.

2.3 Experimental characterization

The soil at the research site has sand, silt, and clay values of 822 and 788 g kg^{-1}, 73 and 71 g kg^{-1}, and 105 and 140 g kg^{-1} for the depth of 0.00–0.20 and 0.21–0.40 m, respectively. The chemical attributes of the soil were determined before installing the experiment by collecting a composite sample, originating from 20 simple deformed soil samples, throughout the experimental area in the 0.00–0.20 m and 0.21–0.40 m depth, which presented the following results: 4 and 3 mg dm^{-3} of P (resin); 14 and 13 g dm^{-3} organic matter; pH 4.5 and 4.3 ($CaCl_2$); K^+, Ca^{2+}, Mg^{2+}, H^++Al^{3+}, sum of bases and cation exchange capacity = 2.6 and 1.6; 6 and 8; 5 and 3; 25 and 26; 12.7 and 12.4 and 37.7 and 38.4 $mmol_c$ dm^{-3}, respectively and 34 and 32% base saturation, respectively. Soil correction was carried out for the 0.00–0.40 m layer on 14th of January 2020, in the entire experimental area as recommended by Raij et al. (1997), 3,520 kg ha^{-1} of dolomitic limestone ($CaCO_3$) with total relative neutralizing power of 90%, using equipment with a broadcast distributor, to increase the base saturation to 70%.

The chemical analyses of the soil were carried out according to the methodology consolidated in Brazil by Raij et al. (2001). The soil pH of the soil suspensions was determined using 0.01 mol L^{-1} $CaCl_2$ solution at a ratio of 1:2.5. OM was determined by oxidation with $K_2Cr_2O_7$ in the presence of H_2SO_4 and titration of excess dichromate with a 0.4 mol L^{-1} solution of $Fe(NH_4)_2(SO_4)_2.6H_2O$. Exchangeable aluminum (Al^{+3}) was extracted with a 1.0 mol L^{-1} KCl solution and then titrated with 0.025 mol L^{-1} NaOH. Exchangeable calcium (Ca^{+2}) and magnesium (Mg^{+2}) were extracted by ion-exchange resin and quantified by atomic absorption spectrophotometry (AAS, Model Varian SpectrAA-55B, Varian, CA, USA). Exchangeable potassium (K^+) and phosphorus (P) were also extracted from the resin; K^+ was determined by flame photometry, and P was determined by colorimetry. The potential acidity (H^+ + Al^{+3}) was estimated by the SMP buffer pH method. Sulfur (S) was extracted from a solution of 0.01 mol L^{-1} $Ca(H_2PO_4)_2$, and turbidity was subsequently measured via the precipitation of sulfate by barium chloride via colorimetry. With these results, the sum of bases (SBs), cation exchange capacity (CEC) at pH 7, and base saturation (BS) were calculated. In the present study, a 10 cm^3 sample of prepared soil was used, which passed through a 2 mm sieve and a grinding process, to determine the concentration of chemical elements in the soil.

Before conventional soil preparation, desiccation was carried out with systemic herbicide in the total area on 15th of Janeiro and 13th of November 2020, with the herbicide's glyphosate (2,160 g ha^{-1} from active ingredient (a.i.)) + 2,4-D (670 g ha^{-1} from a.i.). The herbicides were applied with a tractor-mounted boom sprayer set at 240 L ha^{-1} of spray.

Conventional tillage operations were carried out between January and February 2020 and 2021 and consisted of a deep plowing operation with a disc plow and two operations with a light harrow. Conventional tillage is a traditional agricultural practice involving the mechanical preparation of soil for planting, according to methodologies used by Subbulakshmi et al. (2009), Schlütera et al. (2018), Suleymanov et al. (2022), Sangotayo et al. (2023), and Cakpo et al. (2025).

The desired plant population for corn was 60 thousand plants per hectare, consisting of seven seeds per linear meter. The sowing of single and intercropped corn with *U. ruziziensis, C. spectabilis* and occurred on the same date manually and mechanically.

A simple hybrid K9606 VIP3 of early corn cycle was used, adapted to local environmental conditions following the cultivar's technical recommendations for the low altitude tropical region (up to 500 m) as it presents high productive stability, being recommended for silage production. and grains. Regarding agronomic characteristics, the hybrid presents tolerance to the stunting complex, plant height of 2.25 to 2.40 m, 16 to 18 rows of grains, 1.25 to 1.35 m of ear insertion, semi-erect architecture, color of orange grain, semi-hard grain texture, 360 grams of one hundred grain mass and 730 grams of recommended hectoliter weight and adapted for the region in the second harvest.

Mechanical sowing of corn was carried out on the 10th of March 2020 and the 8th of February 2021 with a disc seeder-fertilizer. Soil

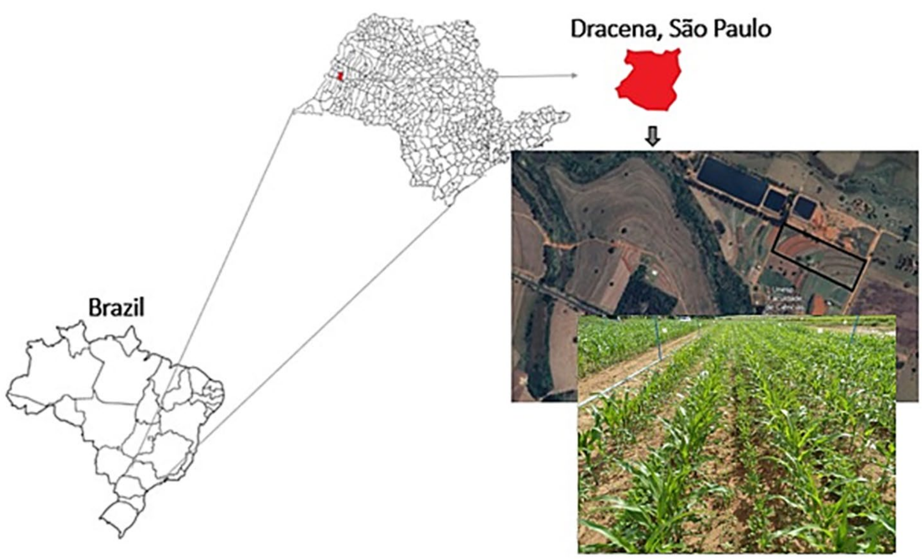

FIGURE 2
Image of the experimental area of the Faculty of Agricultural Sciences and Technology (FCAT), in Dracena, São Paulo state, Brazil, where the field experiment was carried. Image obtained from Google Earth. Map created using QGIS software (version 3.40.1) and Google Earth. QGIS Development por Rosas-Chavoya et al., 2022. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org. Image obtained from Google Earth.

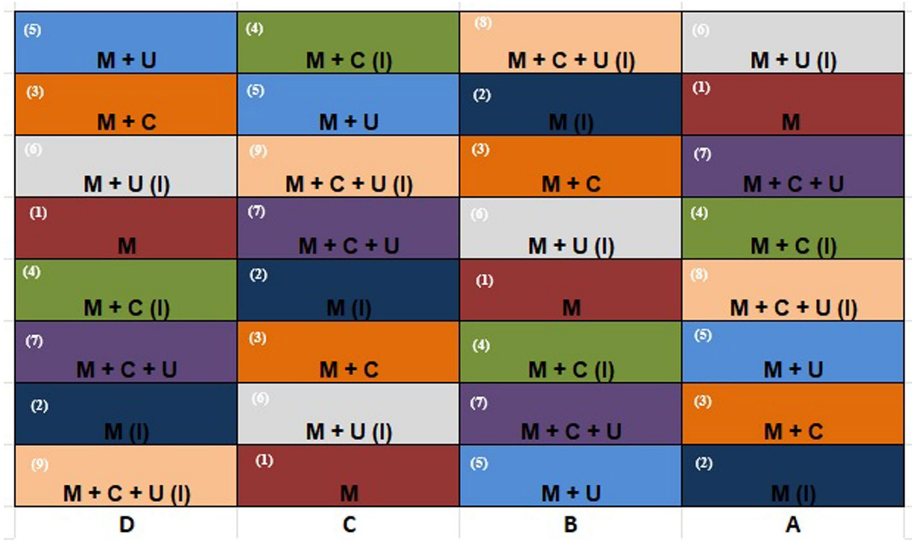

FIGURE 3
Experimental design of second harvest corn intercropped with cover crops and inoculated with *Azospirillum brasilense* via foliar, Dracena, SP, Brazil, 2020 and 2021. R: M: Single corn; C: *Crotalaria spcetabilis*; U: *Urochloa ruziziensis*; I: Foliar inoculation with *A. brasilense*. Treatments: (1) Single corn; (2) Single corn + Foliar inoculation with *A. brasilense*; (3) corn + *C. spcetabilis*; (4) corn + *C. spcetabilis* + Foliar inoculation with A. *A. brasilense*; (5) corn + U. ruziziensis; (6) corn + U. ruziziensis + Foliar inoculation with *A. brasilense*; (7) corn + *C. spcetabilis* + *U. ruziziensis*; (8) corn + *C. spcetabilis* + *U. ruziziensis* + Foliar inoculation with *A. brasilense*.

cover crops were sown manually with the aid of matraca equipment. The sowing of *U. ruziziensis* and *C. spectabilis* was regulated and distributed evenly in a single row in the middle of the corn rows. The quantity of seeds used for the species of *U. ruziziensis* was 2.5 kg ha^{-1} of pure viable seeds. The *U. ruziensis* seeds used in the research showed germination values of 80% and physical purity of 90%, consisting of a cultural value (VC) of 72%. The seed density used in sowing for *C. spectabilis* was 20.0 kg ha^{-1}. The *C. spectabilis* seeds used in the research showed germination values of 82% and physical purity of 99%. All ground cover species were sown at a depth of 0.03 m. Before sowing the corn, seeds were treated with pyraclostrobin + methyl thiophanate + fipronil (5 g + 45 g + 50 g a.i. per 100 kg of seeds, respectively). Seed treatment with two fungicides and one insecticide was carried out to control and protect plants in the initial stages of development against insects and fungi present in the soil that can compromise germination, emergence and initial plant stand.

Mineral fertilizer in the corn sowing furrows (N-P_2O_5-K_2O) in 2020 and 2021 was 365 and 475 kg ha^{-1} of formulation 08–28-16 and 04–30-10, respectively, calculated according to the chemical attributes from soil analysis and expected grain yield for the region, as recommended by Raij et al. (1997). The methodology of Raij et al. (1997) and Cantarella et al. (2022) is based on the calibration of N-P_2O_5-K_2O, following the recommended dose in the table for phosphorus and potassium based on the results of the soil chemistry analysis of the site and the expected grain yield. Mineral fertilization was not applied to the ground cover plants at the time of sowing.

Leaf inoculation with diazotrophic bacteria was carried out at phenological stage V4 (four expanded leaves). The application of inoculant was carried out on the 15th and 16th days after emergence (DAE) of the corn plants between 6 and 7 pm, aiming at mild temperature conditions, mainly to favor the penetration of the bacteria inside the stomata, lenticels, and natural openings of corn leaves. It is worth mentioning that this study did not perform seed inoculation in corn crops and in the intercropped cover crops (*Crotalaria spectabilis* and *Urochloa ruziziensis*).

In order to apply the inoculant, a knapsack sprayer was used at a constant pressure of 30 pounds in-2 (psi) pressurized by compressed CO_2, equipped with an XR 11002 "fan" flat jet nozzle tip, with an application rate proportional to 400 L ha^{-1}. The inoculant used contains the Ab-V5 (to fix nitrogen) and Ab-V6 (to promote plant growth through the synthesis of phytohormones) strains of *Azospirillum brasilense* and presents 2×10^{8} viable cells per gram of commercial product, using a dose of 500 ml ha^{-1} of the inoculant.

The application of nitrogen in top dressing was carried out in a single dose of 100 kg ha^{-1} in installments with half a dose (50 kg ha^{-1}) at 16 and 20 DAE of the plants when the corn plants had a phenological stage of five (V5) expanded leaves and the other half of the dose (50 kg ha^{-1}) at 29 and 33 DAE of the plants when the corn plants present the phenological stage of eight (V8) expanded leaves, using urea (46% of N) as a nitrogen source, in the 2 years of the research, respectively. In the second application of nitrogen (V8), 60 kg ha^{-1} of K_2O was applied, using potassium chloride (60% K_2O) as a source of potassium.

Weed management was carried out using herbicides applied by a tractor sprayer set at 270 L ha^{-1}. At 15 DAE of corn plants, the herbicide bentazon (600 g ha^{-1} a.i.) was used post-emergence. The other cultural and phytosanitary treatments were those normally recommended for the second corn crop in the region. The corn plots were harvested from the 30th of June 2020 to the 2th of June 2021.

In the management of irrigation water, crop coefficients (Kc) of the corn development phases were used: sowing up to 50% of germinated seedlings (S-VE) the value of 0.40; VE stage up to 50% of plants with eight fully expanded leaves (VE-V8) the value of 0.83; V8 stage up to 50% of plants with the presence of the tassel (V8-VT) the value of 1.13; VT stage up to 50% of plants with milky grain (VT-R2) the value of 0.89 and R2 stage up to 50% of plants at physiological maturity (R2-R6) the value of 0.58, according to the phenological scale of Ritchie et al. (1993) and the crop coefficient (kc) values proposed by Doorenbos and Kassam (1979).

Assessments carried out in the corn crop: relative chlorophyll content (SPAD Index): the SPAD reading was carried out at three phenological stages (V4, V8, and R1) of the corn hybrid in the field. For the SPAD chlorophyll reading, a digital chlorophyll meter (Clorofilog Falker CFL 1030) was used, which provides instantaneous readings. The readings taken by this equipment indicate proportional values of chlorophyll in the leaf and were calculated based on the amount of light transmitted by the leaf in two wavelengths with different chlorophyll absorbances. The readings to determine the chlorophyll content, SPAD Index, were carried out using the Clorofilog Falker CFL 1030, on five plants per plot. The leaf concentration of N, P, K, Ca, Mg and S was determined at the time of female flowering (ear emission), with the leaf blade removed, leaf below and opposite the ear, in five corn plants per plot. After drying in an oven with forced air circulation regulated at a temperature of 65°C for 72 h, they were ground in a Wiley mill and then subjected to digestion with sulfuric "acid" to determine the N concentration and digestion with nitro-perchloric acid to determine P, K, Ca, Mg and S concentrations. In terms of grain yield (kg ha^{-1}), the ears of two rows of three meters long plants were removed from the useful area of each plot. They were harvested and subjected to mechanical threshing. The grains obtained were weighed and the data converted to kg ha^{-1}, corrected to 13% wet basis.

2.4 Data analysis

The results were submitted to the F test of the analysis of variance and a significant interaction between the sources of variation was found. The results were broken down, comparing the means of the intercropping and foliar inoculation systems using the Tukey test, adopting the level of 5% significance, according to Pimentel-Gomes and Garcia (2002). For this statistical analysis, the SISVAR software developed by Ferreira (2019) was used.

3 Results

Corn plants emerged uniformly on the sixth day after sowing (DAS) in both experimental years. Female flowering (R1 stage) and plot harvest were carried out at 52 and 104 DAE in 2020, and at 50 and 108 DAE in 2021, respectively.

3.1 Relative chlorophyll content and yield grain

There was significant interaction for relative leaf chlorophyll content at the phenological stages of V8 in 2021 while the interactions for relative leaf chlorophyll content and grain yield were significant at R1 stage in both cropping seasons (Table 1). In addition, the isolated effect of the treatment indicated that relative leaf chlorophyll content was enhanced (58.9) with intercropping system between corn + *C. spcetabilis* + *U. ruziziensis* as compared to the other treatments in 2020 cropping season.

The interaction of the intercropping consortium with foliar inoculation for the relative leaf chlorophyll content in V4, V8, and R1 during 2021 cropping season showed variable response in the treatments with corn + *C. spcetabilis* as compared to corn monoculture (Table 2). However, all three intercropping systems showed substantial reductions in chlorophyll content as compared to monoculture (56.5 vs. 57.0), irrespective of inoculation. Further, the inoculated corn + *C. spectabilis* + *U. ruziziensis* consortium showed reduced chlorophyll content compared to inoculated monoculture corn.

The interaction between inoculation and cover crops consortium for grain yield showed that the consortia between corn + *U. ruziziensis* with the foliar inoculation of *Azospirillum brasilense* provided greater grain yield as compared to other treatments (Figure 4). The interaction of consortia within inoculation for corn grain yield indicated that the consortium between corn + *C. spectabilis* + *U. ruziziensis* in combination with and without foliar inoculation provided increases in grain yield (4,833 and 4,782 kg ha^{-1}), respectively, as compared to alone corn cultivation in the presence of inoculation (3,217 kg ha^{-1}) and the intercropping of corn + *U. ruziziensis* without inoculation (2,840 kg ha^{-1}) (Figure 4).

3.2 Leaf nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and sulfur (S) concentrations

Significant interactions were observed for leaf nitrogen and Ca concentration in 2021 Tables 3, 4). The interaction of consortia-inoculation demonstrated that the corn + *C. spectabilis* system increased leaf N and Ca levels, which were statistically not different from alone corn cultivation (Figures 5, 6). In addition, the corn + *U. ruziziensis* system consistently increased N (22 g kg^{-1}) and Ca (3.2 g kg^{-1}) concentrations regardless of inoculation as compared to alone corn cultivation.

When analyzing the isolated effects, the intercropping system of corn + *Urochloa ruziziensis* resulted in an increased leaf N concentration (2.3 g kg^{-1}), as well as an increase in leaf P concentration in the second cropping season. However, this treatment exhibited the opposite trend for S concentration, which was reduced to 0.9 g kg^{-1} as compared to the sole corn cultivation (control).

It is important to highlight that across both studied years (Tables 3, 4; Figures 5, 6), leaf N content remained below the recommended range of 25–35 g kg^{-1} in all treatments. In addition, the observed nutrients content like P (1.9–3.5 g kg^{-1}) and Ca (2.6–6.0 g kg^{-1}) concentrations in 2020, while K (17–30 g kg^{-1}), Mg (1.5–4.0 g kg^{-1}), and S (1.5–3.0 g kg^{-1}) concentrations in 2021, were observed in deficient across all treatments. This shows that the fertilizers management during both sowing and topdressing in future research and commercial areas needs to be reconsider. Nutrient management should be tailored to each production environment, corn genotype, and cropping year, especially when adopting intercropping systems with ground cover species aimed at enhancing corn development, according to Cantarella et al. (2022).

4 Discussion

The integration of cover crops with inoculation of *Azospirillum brasilense* has emerged as a transformative strategy for sustainable agriculture, enhancing soil health while reducing reliance on synthetic inputs. Cover crops contribute to soil organic matter accumulation (Schipanski et al., 2014), nutrient cycling (Pacheco et al., 2013), and suppression of pests, pathogens, and weeds (Martins et al., 2016;

TABLE 1 Average values of relative chlorophyll content (Clorofilog Falker CFL 1030) in leaves at phenological stages V4, V8, R1 and grain yield of second harvest corn intercropped with cover crops and inoculated with *Azospirillum brasilense* via foliar, Dracena, SP, Brazil, 2020 and 2021.

	Relative leaf chlorophyll content (phenological stages)						Grain yield	
	V4		V8		R1		(kg ha^{-1})	
	2020	2021	2020	2021	2020	2021	2020	2021
Inoculation with *Azospirillum brasilense* via foliar (I)								
Without	52.1	45.7	56.4	45.1	59.2	49.3	4.038	3.934
With	52.4	44.0	57.1	45.3	59.6	49.2	4.145	4.014
Consortium (C)								
M	52.4	45.5	57.6 ab	45.9	59.0	59.0	3.720	3.635
M + C	53,3	45.0	55.7 b	45.1	59.1	59.1	3.779	4.009
M + U	52.2	44.9	54.8 b	44.6	60.2	60.2	4.294	3.443
M + C + U	51.2	44.1	58.9 a	45.2	59.0	59.0	4.574	4.897
F values								
I	0.157^{ns}	13.122*	0.000^{ns}	0.215^{ns}	0.261^{ns}	0.041^{ns}	1.44^{ns}	0.116^{ns}
C	1.187^{ns}	1.518^{ns}	5.106*	2.269^{ns}	0.611^{ns}	148.039*	21.41*	6.636*
I x C	1.507^{ns}	5.897*	2.274^{ns}	5.835*	3.391*	12.696*	7.089*	3.247*
Minimum significant difference (5%)								
I	--	--	--	--	--	--	--	--
C	--	--	3.0221	--	--	--	--	--
CV(%)	9.38	6.56	9.16	4.94	8.11	5.32	6.15	16.67

M: Single corn; C: *Crotalaria spcetabilis*; U: *Urochloa ruziziensis*; *significant at 5% significance; ns—not significant by the F test. Means followed by the same letter, within Inoculation and Consortium do not differ statistically at 5% significance.

Schipanski et al., 2014). The intercropping consortium (corn + *Cajanus cajan* or *Crotalaria spectabilis*) combines economic viability and ecological benefits, aligning with the principles of the fourth green revolution (Martin-Guay et al., 2018).

Tropical forage species like *Urochloa* spp. are particularly advantageous due to their high biomass production and high carbon/nitrogen (C/N) ratio, which prolongs soil cover and improves soil structure (Mingotte et al., 2014; Silveira et al., 2011). In this study, the corn + *U. ruziziensis* consortium, especially when inoculated with *A. brasilense* was observed with higher grain yield (~4,800 kg ha^{-1}), as compared to monoculture systems (3,217 kg ha^{-1}) (Table 1; Figure 4). This aligns with findings by Seidel et al. (2022), who reported that intercropping increased corn grain yield as compared to monoculture due to the enhanced soil aggregation and stability in diversified systems. The synergistic effects of *U. ruziziensis* and *A. brasilense* contribute to higher yield gains, demonstrating the potential of strategic consortia to mitigate interspecific competition for nitrogen fixation and root growth promotion (Oliveira et al., 2010). It is reported in the literature that the species *U. ruziziensis* in monoculture has a deep and vigorous root system, which helps in decompaction and forming biopores in the soil profile. Furthermore, it presents high dry mass yield of around 10,000 to 14,000 kg ha^{-1} and a high C/N ratio of 36/1, providing protection against the impacts of raindrops and reducing soil erosion processes, as well as a low capacity to provide N via symbiotic BNF between 30 and 40 kg ha^{-1} of N with interaction with bacteria of the genus *Rhizobium* present or native to the soil, and high potential for nutrient cycling, extracting on average 200 kg ha^{-1} of N, 18 kg ha^{-1} of P and 372 kg ha^{-1} of K, according to Vanolli et al. (2024). Therefore, we reinforce in this research the beneficial effects of the *U. ruziziensis* species in the soil–plant system verified in the results between the corn + *U. ruziziensis* consortium, especially when inoculated with *A. brasilense*, was observed with greater grain yield (~4,800 kg ha^{-1}), compared to monoculture systems (3,217 kg ha^{-1}).

The use of legumes such as *C. spectabilis* further enhance system sustainability by supplying up to 160 kg ha^{-1} of N via biological nitrogen fixation (BNF) (Calegari and Carlos, 2014), reducing dependence on synthetic fertilizers. However, our results revealed persistent nitrogen deficiencies across treatments (<25 g kg^{-1}), suggesting that while legumes contribute significantly, supplementary nitrogen management remains critical. Additionally, *C. spectabilis* helps in nematode suppression (Wang et al., 2002) and diversifies residue quality for no-till systems (Kappes and Zancanaro, 2015), though its lower C/N ratio accelerates decomposition, necessitating careful residue management to sustain soil cover. Besides this, sunn hemp is classified as green manures due to their ability to associate with nitrogen-fixing microorganisms. These interactions facilitate nitrogen release into the soil either through root exudates (Murrell et al., 2017) or residue decomposition, effectively enriching soil nitrogen stocks (Marcelo et al., 2012). It is worth noting that *C. spectabilis* in monoculture has high dry mass yield of around 4,000 to 7,000 kg ha^{-1} and a low C/N ratio of 18/1. However, due to rapid decomposition and the annual cycle, a period of uncovered soil may occur until the next harvest. *C. spectabilis* has a high capacity to provide N via native symbiotic BNF between 60 and 120 kg ha^{-1} of N, and a high potential for nutrient uptake, extracting on average 222 kg ha^{-1} of N, 24 kg ha^{-1} of P, and 220 kg ha^{-1} of K (Lima Filho et al., 2023; Vanolli et al., 2024).

TABLE 2 Interaction of the analysis of variance regarding the relative content of leaf chlorophyll (Clorofilog Falker CFL 1030) in the phenological stages V4, V8, R1 of second harvest corn intercropped with cover crops and inoculated with *Azospirillum brasilense* via foliar, Dracena, SP, Brazil, 2020 and 2021.

Consortium (C)				
	Relative leaf chlorophyll content V4–2021			
Inoculation (I)	M	M + C	M + U	M + C + U
Without	46.1 AB	47.4 a A	45.3 AB	44.1 B
With	44.9 A	42.5 b B	44.6 AB	44.2 AB
LSD (5%)	I in C (1.8415) and C in I (2.4222)			
	Relative leaf chlorophyll content V8–2021			
Inoculation (I)	M	M + C	M + U	M + C + U
Without	45.4	44.0 b	45.2	45.9
With	46.3 A	46.3 a A	44.0 B	44.6 AB
LSD (5%)	I in C (1.3965) and C in I (1.8369)			
	Relative leaf chlorophyll content R1–2020			
Inoculation (I)	M	M + C	M + U	M + C + U
Without	57.4 b	60.3	59.0	59.9
With	60.6 a	58.0	61.5	58.2
LSD (5%)	I in C (3.0072) and C in I (3.9554)			
	Relative leaf chlorophyll content R1–2021			
Inoculation (I)	M	M + C	M + U	M + C + U
Without	57.0 A	46.5 b BC	48.5 a B	45.1 b C
With	56.5 A	48.4 a B	44.4 b C	47.5 a B
LSD (5%)	I in C (1.6375) and C in I (2.1539)			

M: Single corn; C: *Crotalaria spectabilis*; U: *Urochloa ruziziensis*; means followed by the same letter, lowercase in the columns and uppercase in the lines, do not differ statistically from each other using the Tukey test at 5% significance.

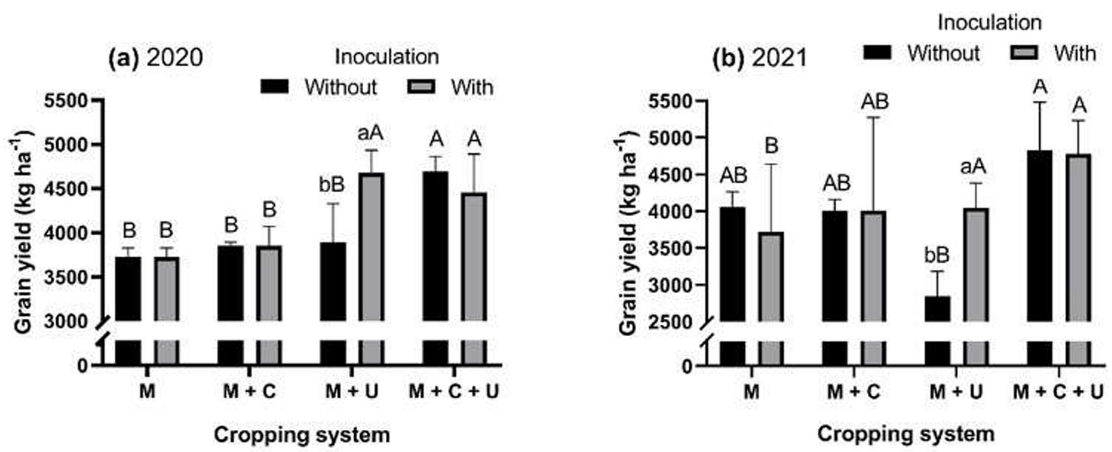

FIGURE 4
Corn grain yield influenced by corn intercropped with cover crops and inoculation with *Azospirillum brasilense* via foliar, Dracena, SP, Brazil, **(a)** 2020 and **(b)** 2021. M: Single corn; C: *Crotalaria spectabilis*; U: *Urochloa ruziziensis*. Bars followed by the same letter, lowercase without and with inoculation and uppercase in the cropping system, do not differ statistically according to the Tukey test at 5% significance.

Chlorophyll content act as a proxy for nitrogen status varied among the treatments with intercropping systems showing reductions in 2021 as compared to monoculture, likely due to resource competition during reproductive stages. This emphasized the need for optimized planting schedules to balance competition and facilitation. Furthermore, deficiencies in phosphorus, potassium, and sulfur across both seasons highlighted the importance of integrated soil fertility management, combining organic inputs with targeted mineral supplementation (Vanlauwe et al., 2010).

Moreover, cover cropping contributes to the build-up of soil organic matter, which in turn improves the soil's water-holding capacity, making agricultural land more resilient to extreme

TABLE 3 Average values of leaf concentration of nitrogen (N), phosphorus (P), and potassium (K) at the R1 phenological stage of second-crop corn intercropped with cover crops and inoculated with *Azospirillum brasilense* via foliar, Dracena, SP, Brazil, 2020 and 2021.

	N		P		K	
	g kg^{-1}					
	2020	2021	2020	2021	2020	2021
Inoculation with *Azospirillum brasilense* via foliar (I)						
Without	22	20	1.8	2.1	18	17
With	22	20	1.7	2.1	17	16
Consortium (C)						
M	22	21	1.78	1.9 b	17	16
M + C	23	19	1.74	2.1 ab	17	16
M + U	22	21	1.76	2.3 a	18	17
M + C + U	23	19	1.75	2.1 ab	18	16
F values						
I	0.006^{ns}	4.624*	0.392^{ns}	1.209^{ns}	2.058^{ns}	3.133^{ns}
C	0.620^{ns}	9.839*	0.041^{ns}	4.594*	0.580^{ns}	2.320^{ns}
I x C	0.410^{ns}	17.305*	0.376^{ns}	0.436^{ns}	0.898^{ns}	1.843^{ns}
Minimum significant difference (5%)						
I	--	--	--	--	--	--
C	--	--	--	0.2465	--	--
CV (%)	9.30	4.85	12.86	8.46	10.69	5.80

M: Single corn; C: *Crotalaria spcetabilis*; U: *Urochloa ruziziensis*; *significant at 5% significance; ns—not significant by the F test. Means followed by the same letter, within Inoculation and Consortium do not differ statistically at 5% significance.

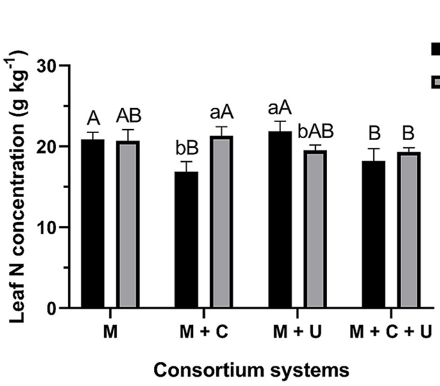

FIGURE 5
Leaf nitrogen (N) content in the R1 phenological stage of second-crop corn intercropped with cover crops and inoculated with *Azospirillum brasilense* via foliar, Dracena, SP, Brazil, 2021. M: Single corn; C: *Crotalaria spectabilis*; U: *Urochloa ruziziensis*. Bars followed by the same letter, lowercase without and with inoculation and uppercase in the cropping system, do not differ statistically according to the Tukey test at 5% significance.

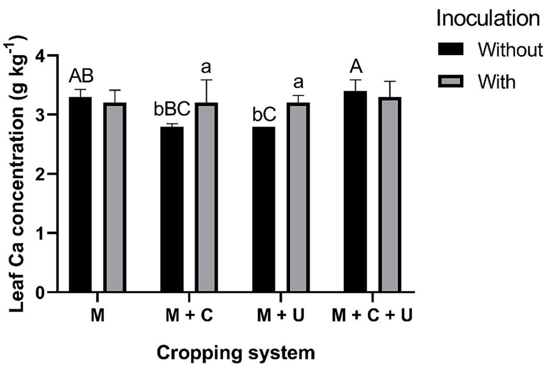

FIGURE 6
Leaf Calcium (Ca) content in the R1 phenological stage of second-crop corn intercropped with cover crops and inoculated with *Azospirillum brasilense* via foliar, Dracena, SP, Brazil, 2021. M: Single corn; C: *Crotalaria spectabilis*; U: *Urochloa ruziziensis*. Bars followed by the same letter, lowercase without and with inoculation and uppercase in the cropping system, do not differ statistically according to the Tukey test at 5% significance.

weather conditions. Practices that enhance soil health, such as cover cropping, result in higher soil organic matter content and increased yield potential, particularly for corn (Duchene et al., 2017). The present study supports these findings, demonstrating not only agronomic benefits but also a favorable return on investment, representing genuine economic growth (Peng et al., 2024). Beyond yield gains, cover crops contribute holistically to soil health by improving physical, chemical, and biological soil properties. They enhance nutrient cycling, increase soil porosity and infiltration, reduce compaction, optimize water dynamics, and promote biological nitrogen fixation (Duchene et al., 2017; Peng et al., 2024).

TABLE 4 Average values of leaf concentration of calcium (Ca), magnesium (Mg) and sulfur (S) in the R1 phenological stage of second-crop corn intercropped with cover crops and inoculated with *Azospirillum brasilense* via foliar, Dracena, SP, Brazil, 2020 and 2021.

	Mg		Ca		S	
	g kg^{-1}					
	2020	2021	2020	2021	2020	2021
Inoculation with *Azospirillum brasilense* via foliar (I)						
Without	3.5	1.3	1.3	3.1	1.5	1.0 a
With	3.3	1.2	1.2	3.2	1.6	0.9 b
Consortium (C)						
M	3.4	1.2	1.2	3.2	1.5	1.1 a
M + C	3.3	1.2	1.2	3.0	1.5	1.0 ab
M + U	3.4	1.3	1.2	3.0	1.4	0.9 b
M + C + U	3.6	1.4	1.3	3.3	1.6	1.0 ab
***F* values**						
I	2.827^{ns}	1.899^{ns}	5.521^{ns}	2.377^{ns}	2.965^{ns}	5.609*
C	1.282^{ns}	2.884^{ns}	0.382^{ns}	5.204*	1.784^{ns}	4.868*
I x C	1.042^{ns}	2.884^{ns}	2.462^{ns}	3.132*	0.384^{ns}	1.263^{ns}
Minimum significant difference (5%)						
I	--	--	--	--	--	0.06
C	--	--	--	--	--	0.12
CV (%)	10.81	12.44	11.70	6.95	8.11	8.45

M: Single corn; C: *Crotalaria spcetabilis*; U: *Urochloa ruziziensis*; *significant at 5% significance; ns—not significant by the F test. Means followed by the same letter, within Inoculation and Consortium do not differ statistically at 5% significance.

5 Conclusion

The relative chlorophyll and leaf nitrogen, phosphorus, potassium, magnesium and calcium concentrations in corn plants remained unaffected by the foliar application of *Azospirillum brasilense*. However, intercropping systems of corn + *Urochloa ruziziensis* with inoculation and corn + *Crotalaria spectabilis* + *U. ruziziensis*, regardless of inoculation increased corn grain yield. Furthermore, intercropping corn with *C. spectabilis*, irrespective of inoculation enhanced the relative chlorophyll content at the V4, V8, and R1 stages. The consortium between corn + *U. ruziziensis* increased leaf P concentration while reducing the leaf S concentration of corn.

A key limitation of this study was the absence of data on gas exchange and broader physiological traits in corn. Future research should focus on the investigation of early phenological stages (V1–V4), optimizing foliar inoculant doses, and refining application parameters such as spray volume, nozzle type, and pressure. Exploring novel cover crop combinations intercropped with corn could further advance sustainable soil management and low-carbon agriculture. Additionally, elucidating the specific physiological and molecular mechanisms by which *A. brasilense* and cover crops interact with corn may provide insights to further improve resource use efficiency and sustainability in corn-based cropping systems.

For practical implementation, farmers should consider intercropping corn with *Urochloa ruziziensis* and *Crotalaria spectabilis* alongside diazotrophic bacterial inoculation. This strategy can enhance nutrient uptake, reduce dependence on synthetic fertilizers, and support eco-friendly agricultural practices aligned with low-carbon goals.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

VN: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing. SF: Investigation, Methodology, Validation, Writing – review & editing. RL: Investigation, Methodology, Software, Writing – review & editing. CB: Formal analysis, Investigation, Methodology, Writing – review & editing. EP: Investigation, Methodology, Software, Writing – review & editing. FG: Data curation, Investigation, Methodology, Writing – review & editing. JD: Investigation, Methodology, Software, Writing – review & editing. OA: Investigation, Resources, Supervision, Validation, Writing – review & editing. LS: Investigation, Methodology, Validation, Writing – review & editing. MM-R: Investigation, Methodology, Validation, Writing – review & editing. BV-M: Investigation, Methodology, Validation, Writing – review & editing. SK: Data curation, Formal analysis, Software, Validation, Writing – review & editing. EA: Data curation, Methodology, Software, Writing – review & editing. AJ: Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. MT: Conceptualization, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

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