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Research article

Impact of historical soil management on the interaction of plant-growth-promoting bacteria with maize (Zea mays L.)

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A R T I C L E I N F O

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A B S T R A C T

Edaphic factors can modulate the effects of microbial inoculants on crop yield promotion. Given the potential complexity of microbial inoculant responses to diverse soil management practices, we hypothesize that sustainable management of soil and water irrigation may improve soil quality and enhance the effects of plant growth-promoting bacteria (PGPB). Consequently, the primary objective was to assess the effectiveness of microbial inoculants formulated with *Herbaspirillum seropedicae* (Hs) and *Azospirillum brasilense* (Ab) on maize growth in soils impacted by different historical conservation management systems. We evaluated two soil management systems, two irrigation conditions, and four treatments: T0 - without bioinoculant and 100% doses of NPK fertilization; T1 - Hs + humic substances and 40% of NPK fertilization; T2 - Ab and 40% of NPK fertilization; T3 - co-inoculation (Hs + Ab) and 40% of NPK fertilization. Using a reduced fertilization dose (40% NPK) associated with microbial inoculants proved efficient in increasing maize shoot dry mass : on average, there was a 16% reduction compared to the treatment with 100% fertilization. In co-inoculation (Hs + Ab), the microbial inoculants showed a mutualistic effect on plant response, higher than isolate ones, especially increasing the nitrogen content in no-tillage systems irrigated by swine wastewater. Under lower nutrient availability and higher biological soil quality, the microbial bioinputs positively influenced root development, instantaneous water use efficiency, stomatal conductance, and nitrogen contents.

1. Introduction

Biological processes inserted in the productive development of agrosystems present significant potential for increasing the use efficiency of resources, reducing costs, and generating ecologically friendly products. The microbiota in the soil, rhizosphere,

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rhizoplane, and hosted in plants is a functional, active component that develops natural processes widely known and explored in biotechnological products. It actively contributes to plant development by harnessing its inherent bio-fertilization, bio-stimulation and bio-control capabilities, utilizing natural biological mechanisms [1,2].

The use of biological products derived from beneficial microorganisms has gained significant attention in agroecosystems. Regulatory oversight, as defined by Brazilian legislation, classifies them as bioinputs[82]. Within this category, inoculants based on plant growth-promoting bacteria (PGPB) have emerged as prominent contributors to enhanced plant production [3,4]. Scientific investigations on bioinputs provide substantiated evidence that aligns with the economic gains of sustainable agricultural practices [5]. However, the efficacy of plant growth-promoting rhizobacteria (PGPR) in soil applications encounters uncertainties owing to the complex interplay of various environmental factors that can influence their efficiency.

In order to enhance agricultural decision-making processes, further research endeavours should be dedicated to comprehending the intricate responses of microorganisms within the soil-plant system [6]. Generally, the PGPR efficiency varies with soil fertility, salt stress, temperatures, hydric stress, diseases, and abiotic factors [1,7,8]. Currently, persistent scientific investigations are imperative to address existing knowledge gaps on the efficacy of bio-inputs across diverse crop types, varieties, soil management techniques, edaphoclimatic conditions, as well as the intricate relationship between soil quality and the responses of plant growth-promoting rhizobacteria (PGPR) within the soil-plant ecosystem.

PGPBs are widely found in various soils, especially in the rhizosphere [9]. Their benefits are distinct, varying between species and strains of bacteria, with different mechanisms related to their effects on plant growth and development [10]. Several bacteria species have been evaluated as microbial products to boost plant growth and protection. Among them, *Herbaspirillum seropedicae* has been increasingly tested as a bioproduct, and *Azospirillum brasilense* formulation product has been placed as a best-seller inoculant bio-stimulation and bio-fortification effects in the crops [11–15]. *Azospirillum* is a facultative endophytic diazotrophic bacterium that colonizes the rhizosphere and roots [16]. *Herbaspirillum* seems to be an obligate endophyte, which has been isolated predominantly from the roots, stems, and leaves of Gramineae and have low survival in the soil system [13,17].

Azospirillum brasilense is widely used in Brazilian agriculture, especially maize crops [14]. The described mechanisms of action are linked to the modulation of phytohormones [18], increased nutrient and water uptake [19], the release of organic acids, which can solubilize calcium phosphates [20], and stimulation of plant resistance to abiotic stresses (Fukami et al., 2018; [21]). Similar underlined mechanisms were observed for *Herbaspirillum seropedicae* inoculated plants. Alves et al. [22] concluded that maize plants inoculated with *Herbaspirillum seropedicae* showed higher vegetative development under low N availability in the soil. These effects are related to biological nitrogen fixation (BNF) [23], modulation of enzymes in response to different types of stress [15,24], and/or bioprotection and mitigation of water stress [25–28] promoted by *Herbaspirillum seropedicae.*

The synergism and coexistence of bacteria in a microbiome depend on several factors, such as infection niches, endophytic competence, species functionality, and microorganism-soil-plant interactions [29]. Evaluating the potential for co-inoculation between *H. seropedicae* and *Azospirillum brasilense*, Ávila et al. [30] verified the strains's ability to increase production in organic corn culture compared to the control. Dartora et al. [31] concluded that co-inoculation can promote foliar P increment in corn plants, vegetative development, and increased crop productivity. To increase the efficiency of PGPBs, microbial consortia are being studied to verify bacterial strains' compatibility to enhance their co-inoculation benefits [32]. However, inconsistent results are reported [33], and such synergism may not be successful, mainly when co-inoculation is performed in stressful environments [34].

A comprehensive understanding of the complex interconnections among soil, plants, and microorganisms is crucial across diverse management conditions. However, despite several studies evaluating the effects of soil fertility and water availability on the efficiency of PGPB, few studies have assessed the effects of historical soil management practices, such as the simultaneous adoption of no-tillage systems and swine wastewater irrigation, which is strongly emerging in Brazil. Therefore, the objective of this study was to assess, within a controlled greenhouse setting, microbial inoculants formulated with *H. seropedicae* (strain HRC54) and *Azospirillum brasilense* Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084), applied as single inoculum or co-inoculated and understand its action on the initial growth of maize (creole corn, Fortaleza variety) in different edaphic conditions. These conditions represent a history of soil use and management characterized by irrigation with swine wastewater and raw water and the implementation of two tillage systems (conventional vs no-tillage). This study aims to contribute scientific insights into the interactions among soil, plants, and microorganisms, as well as propose specific soil management strategies for future implementation.

2. Material and methods

2.1. Historical management and soil characterization

The soil was collected in an experimental permanent plot to evaluate the dynamics of organic matter and nutrients in the long term under the effect of two sustainable management models: no-tillage and the use of swine wastewater. The permanent plots were implemented in early 2017. Since then, five consecutive cycles of corn (*Zea mays* L.) were conducted in two soil management systems: a no-tillage system with five years of implementation (NT) and conventional tillage (CT); associated with the use of swine wastewater (SW) in the irrigation of the corn crop, compared with raw water (RW) from the river (supplying 100% of the crop's water demand). In the NT, during the fallow period of the soil, was planted the velvet bean (*Stizolobium aterrimum*). In the CT, tillage with moldboard plow and disking was carried out before the corn crop cycle. Regarding accumulated water in SW, approximately 5169.5 m^3 ha^{-1} was applied during the four corn crop cycles.

The present study was carried out on representative samples of the effect of the 5-year historical use representative of four soil managements: NT irrigated with SW; CT irrigated with SW; NT irrigated with RW and; CT irrigated with RW. Composite samples (10 simple samples at a depth of 0–20 cm without surface plant residues) representative of the soil of each management were collected to experiment in a greenhouse. The soil was previously sieved (4.0 mm) and incubated for 30 days to acidity correction objectivity raising to 60% of base saturation [35].

Soil aliquots of 200 g from composite soil sample were stored in a cold chamber ±4 °C for enzymatic characterization: β-glucosidase (μg *p*-nitrophenol h^{-1} g^{-1}) (β-G) [36], dehydrogenase (μg TPF g^{-1} soil) (DGS) [37], microbial biomass nitrogen (MBN), microbial biomass carbon (MBC) [38–40]. No-cold samples were used to total organic carbon (TOC) and potassium sulfate-extracted carbon (C-soluble) [41,42] (Table 1). The chemical attributes (no-cold samples) evaluated were: pH in water; Exchangeable $Al^{3}+$, $^{Ca2+}$, Mg^{2+} and Na^{+}; K and P available; and H + Al, using the methods recommended by Teixeira et al. [43] (Table 1). Total organic carbon (TOC) was evaluated by wet oxidation and dichromatometry with external heating [44] (Table 1). The granulometry of the soil [43] used in work was composed of 640 g kg^{-1} of sand, 100 g kg^{-1} of silt and 260 g kg^{-1} of clay, being a soil of medium texture.

2.2. Experimental design

The experiment was conducted in a greenhouse at latitude 20° 45′ 01″ South, longitude 41° 29′ 18″ West, with an altitude of 112 m (Southeast region, Brazil). The greenhouse's maximum air temperatures varied between 35 °C to 25 °C and minimum air temperatures varied between 20 °C to 12 °C. The study was implemented in a 2 × 2 x 4 factorial scheme with four replications. The first factor evaluated the effect of historical management in the soil: no-tillage system (HNT) and conventional tillage system (HCT). The second factor evaluated the historical effect of successive irrigations with swine wastewater (HSW) and raw water (HRW), equivalent to 100% of the actual evapotranspiration (ETC) of the corn crop. The third factor was outlined in four treatments: T0 - without microbial inoculant and 100% of fertilization doses of NPK; T1 – *Herbaspirillum seropedicae* (strain HRC54) + Humic Substances as a vehicle to foliar spray (HS) with 40% of fertilization doses of NPK; T2 – *Azospirillum brasilense* (seed inoculation with Ab-V5=CNPSo 2083 and Ab-V6=CNPSo 2084) with 40% of fertilization doses of NPK; and T3 - Co-inoculation of *Herbaspirillum seropedicae* (strain HRC54) (T1 treatment) + *Azospirillum brasilense* (strains CNPSo 2083 and CNPSo 2084) (T2 treatment) and 40% of fertilization doses of NPK. The *Herbaspirillum seropedicae* (strain HRC54) was inoculated via foliar spray and *Azospirillum brasilense* (CNPSo 2083 and CNPSo 2084) was inoculated in seeds in differents periods.

The doses of mineral fertilizers (N, P_2O_5 and K_2O - NPK) were based on the recommendations of Novais et al. [45].

2.3. Implementation and conduction of the experiment

We used disinfected pots of 5 dm^3 [46], and the Fortaleza creole variety corn seeds came from small regional producers. Before planting, the seeds were also disinfested [11].

Fertilization followed the recommendations of Novais et al. [45] for studies in a controlled environment (100 mg dm^{-3} of N, 300 mg dm^{-3} of P, and 150 mg dm^{-3} of K). The T0 treatments received 100% and the T1, T2, and T3 40% of the recommendation. For micronutrient fertilization, all treatments received 100% of the recommendation (0.81 mg dm^{-3} of B, 1.33 mg dm^{-3} of Cu, 1.55 mg dm^{-3} of Fe, 3.66 mg dm^{-3} of Mn, 0.15 mg dm^{-3} of Mo, and 4.0 mg dm^{-3} of Zn). Reagents for analysis (p.a.) were used to supply macro and micronutrients.

The *Azospirillum brasilense* was obtained from a commercial inoculant that guaranteed 2 × 10^{11} CFU L^{-1} (colony-forming units per

Table 1
Analysis of the soils used in the experiments.

Attributes[a]		Soil and water management[b]			
		HNT + HSW	HCT + HSW	HNT + HRW	HCT + HRW
pH		5.32	5.45	5.54	5.48
P	mg dm^{-3}	43.4	53.6	30.8	25.4
K^+	mg dm^{-3}	87.2	91.3	68.4	75.4
Na^+	$cmol_c$ dm-3	0.0	0.0	0.0	0.0
Al^{3+}	$cmol_c$ dm-3	0.2	0.0	0.0	0.0
Ca^{2+}	$cmol_c$ dm-3	1.7	2.0	1.8	1.9
Mg^{2+}	$cmol_c$ dm-3	0.4	0.5	0.4	0.4
EC	mS m^{-1}	134.2	154.2	120.4	128.8
TOC	g kg^{-1}	14.2	12.3	12.9	10.9
C-soluble	mg kg^{-1}	23.0	19.8	9.7	8.0
MBC	mg kg^{-1}	484.3	481.2	465.6	469.0
MBN	mg kg^{-1}	75.7	35.9	45.7	34.5
β -G		92.9	65.2	81.4	68.4
DGS		138.2	103.4	136.3	104.3

[a] pH: Hydrogenionic Potential; P: Phosphorus available; K: Potassium available; Na: Exchangeable sodium; Al: Exchangeable aluminum; Ca: Exchangeable calcium; Mg: Exchangeable magnesium; EC: Electrical conductivity. TOC: Total Organic Carbon; C-soluble: Carbon soluble; MBC: Microbial Biomass Carbon; MBN: Microbial Biomass Nitrogen; β-G: β-Glucosidase Enzyme (μg *p*-nitrophenol h^{-1} g^{-1}); DGS; Enzyme Dehydrogenase (μg TPF g^{-1} soil).

[b] HNT: no-tillage system soil samples; HCT: conventional tillage soil samples; HSW: soil samples from plots with successive irrigations using swine wastewater; HRW: soil samples from plots with successive irrigations using raw water.

liter) and was applied by homogenizing 100 mL for every 60,000 corn seeds. The *Herbaspirillum seropedicae* (strain HRC54) was obtained from the Laboratory of Cell and Tissue Biology collection (UENF). The multiplication of *Herbaspirillum seropedicae* was carried out according to Döbereiner et al. [83], with bacterial dilution in HS [47] to obtain 40 mg L^{-1} of C in HS and 10^8 cells mL^{-1}. The C content of HS was obtained according to the method of Yeomans and Bremner [41], and the HS was extracted in a 1:10 proportion in water [48].

The corn seeds were inoculated with *Azospirillum brasilense* and left to air-dry in a cool place for 15 min and planted (five seeds per pot), and after one week of germination, we left two more homogeneous plants per pot. The application of *Herbaspirillum seropedicae* + HS was carried on by foliar spray (5 mL per plant) in V4 and V8 stages (4 and 8 fully expanded leaves, respectively) [49,50]. The co-inoculation was carried out according to the procedures mentioned above, where the two bacterial strains were introduced individually, however, on the same plant, that was exposed to the action of *Azospirillum brasilense* and *Herbaspirillum seropedicae* + HS.

Deionized water were added in sufficient quantity to make up around 50% of the total pore volume (TPV) [51]. To maintain 50% of TPV, similar to 365.0 g of deionized water by pot, we height the experimental unit (pots + soil + water needed to maintain 50% of the TPV) daily to maintenance of soil moisture.

2.4. Biometric parameters, leaf mineral contents, and physiological parameters

Regarding biometric parameters, the mesurements are taken em three repetitions from the greenhouse. The following parameters were evaluated: plant height (PH), shoot dry mass (SDM), root dry mass (RDM), and SDM/RDM ratio. Shoot dry mass, and RDM were evaluated after sectioning and weighing following drying in an oven. The biometric parameters and leaf mineral contents they were evaluated 50 days after emergence.

At the end of the experiment, gas exchange measurements were evaluated in fully expanded leaves using a portable infrared gas analyzer (IRGA), model LI 6400 XT Portable Photosynthesis System (LI-COR, Lincoln, NE, USA), with a fixed light source at 1000 mmol m^{-2} s^{-1} of photosynthetically active photon flux density. Measurements were taken in 3 leafs (three replicates) on one plant per experimental unit (three repetitions) between 7:30 and 11:30 a.m., and the following variables were obtained: A - net assimilation rate of CO_2 (μmol CO_2 m^{-2} s^{-1}); E – transpiration rate (mmol H_2O m^{-2} s^{-1}); gs - stomatal conductance (mol H_2O m^{-2} s^{-1}); Ci - internal CO_2 concentration (μmol CO_2 mol^{-1}). Instantaneous water use efficiency (WUE) (A/E) [(μmol CO_2 m^{-2} s^{-1})/(mmol H_2O m^{-2} s^{-1})] and instantaneous carboxylation efficiency (A/Ci) [(μmol CO_2 m^{-2} s^{-1})/(μmol CO_2 mol^{-1})] were also calculated [52]. Total chlorophyll (TC) was obtained using the portable chlorophyll meter ClorofiLOG (model CFL 1030 Falker).

In the corn leaf samples collected at the end of the experiment (50 days after emergence), the contents of K, P, Ca, Mg, and Zn were evaluated following by a microwave oven for leaf digestion using nitric acid. We weigh 0.5g of the sample in to the digestion vessel and add 10 mL of HNO_3. After approximately 15 min of pre-digestion time, the vessel are place in microwave oven and digested in following conditions: 15 min of ramp time until 200 °C and 15 min of hold time in 200 °C (900–1050 of microwave power and 800 psi of pressure). For total N, powdered leaf were digested in H_2SO_4 in presence of K_2SO_4 catalyst mixture (K_2SO_4: $Cu_SO_4 \cdot 5H_2O$: Se = 10:1:0.1) by Kjeldahl method [84]. N was collected by distillation with NaOH followed by titration with H_2SO_4. The analysis of N content followed the methodology of Galvani and Gaertner [53].

2.5. Statistical analysis

We performed the analysis of variance (ANOVA) and F-test ($p \leq 0.05$) to verify the significance between effects and their interactions. Normal distribution was evaluated by skewness, kurtosis, and the Shapiro-Wilks normality test. When the normal distribution was not achieved, logarithmic transformations were performed to carry out the data in a normal distribution. In case of significant effect for the interactions, the Tukey test was applied to compare the averages at 5% probability.

Factor Analisys (FA) [54] was performed using the Principal Components method to obtain eigenvalues, eigenvectors, factorial loads, samples scores and groups of interrelated variables. Factors with eigenvalues greater than one were selected and then rotated using the orthogonal Varimax method [54]. Samples scores were evaluated by mean and standard deviation and multivariate scatter plots with factorial loads.

Table 2
Summary ANOVA (P-value) for the attributes of vegetative development, mineral nutrition and gas exchange evaluated according to the treatments.

Source of Variation[a]	PH	SDM	RDM	N	P	K	Ca	Mg	CT	A/E	Gs
Soil	0.10	0.61	0.23	**0.00**	0.88	**0.01**	0.24	0.76	0.24	0.73	0.69
Water	0.69	0.17	**0.01**	**0.01**	0.54	**0.00**	**0.00**	**0.00**	**0.01**	0.09	0.55
Treatment	0.24	**0.00**	0.31	**0.00**	**0.00**	**0.00**	0.83	0.83	**0.00**	0.63	**0.01**
Soil*Water	0.05	0.25	0.54	0.77	0.22	0.84	0.13	0.55	0.50	0.35	0.51
Soil*Treat	0.07	0.96	0.23	**0.00**	0.31	0.02	0.83	0.13	0.07	0.72	**0.00**
Water*Treat	0.31	0.04	0.22	**0.00**	0.03	0.42	0.17	0.09	0.06	**0.00**	0.32
Treat*Soil*Water	0.13	0.33	**0.01**	0.13	0.57	**0.01**	0.36	0.08	0.57	0.18	0.90

[a] Soil – Effect of diferent historical of soil management, Water – Effect of diferent historical of irrigation water, Treatments – Microbial bio-inputs treatments and fertilizations. PH - Plant height, SDM – shoot dry mass, RDM - root dry mass, N - nitrogen leaf content, P- phosphorus leaf content, K-potassium leaf content, Ca-calcium leaf content, Mg – magnesium leaf content, TC – Total Chlorophyll, Gs – stomatal conductance, A/E – Instantaneous water use efficiency.

The statistical procedures were performed using the Open Source software R and Sisvar [55], using the DescTools package [56] and the Psych package [57].

3. Results

The statistical summary of ANOVA for each variable is presented in Table 2. Soil management (Soil, Table 2) presented significant main effects only in N and K leaf mineral contents: 19.9 g of N kg^{-1} in no-tillage system comparated with 17,1 g of N kg^{-1} in conventional tillage. Regarding K leaf contents, we observed 20.4 of K kg^{-1} in the no-tillage system and 19.1 of K kg^{-1} in conventional tillage.

The main effect of irrigation water (Water, Table 2) significantly influenced maize plants' biometric parameters and leaf mineral

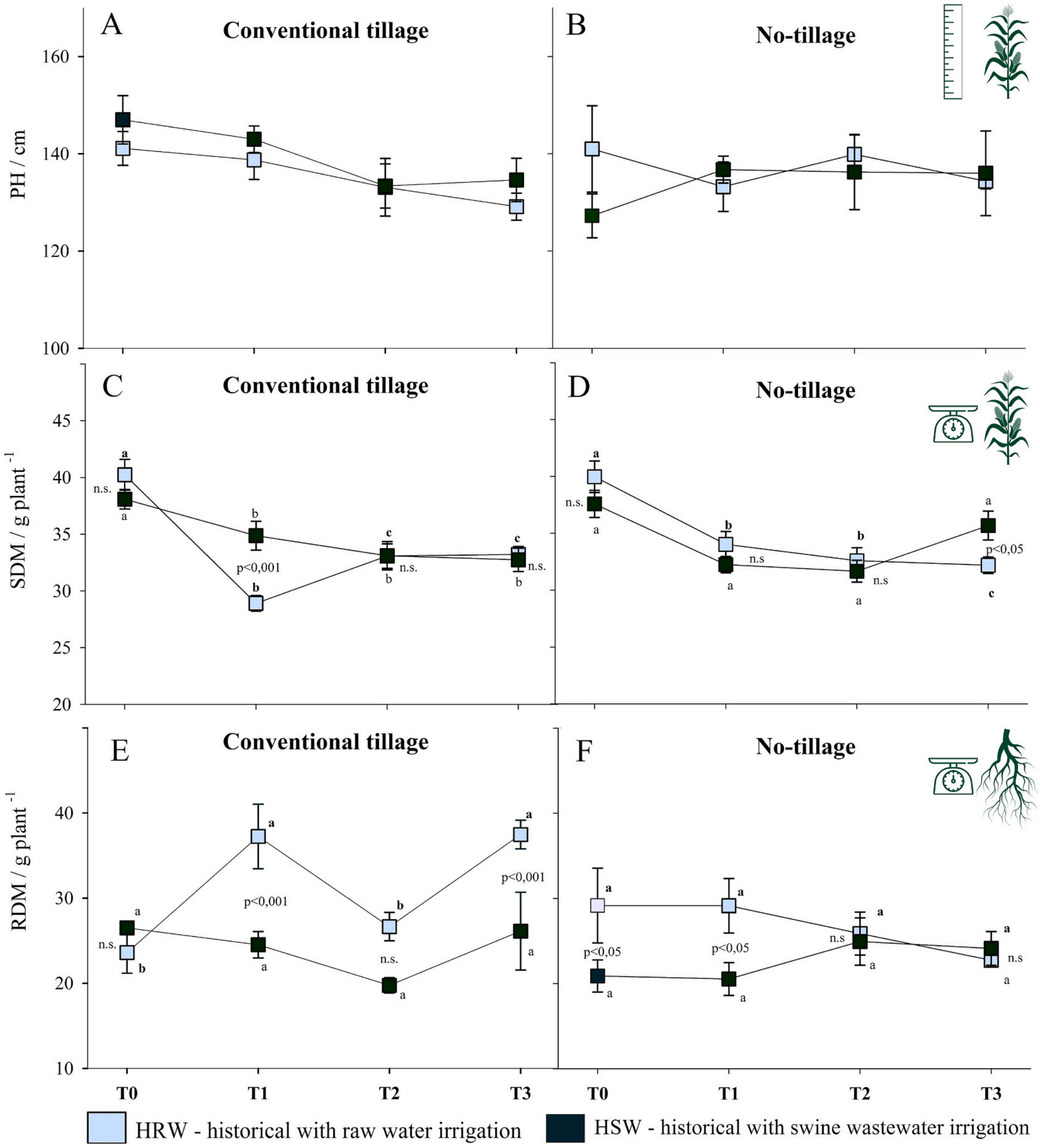

Fig. 1. Biometric parameters of plant height (PH)(A and B), shoot dry mass (SDM) (C and D), root dry mass (RDM) (E and F), according to the historical of soil management types [HNT - historical with no-tillage system (B,D and F), HCT - historical with conventional tillage system (A,C and E)] and water used in irrigation (HSW - historical with swine wastewater, HRW - historical with raw water). Letters compare treatments in the level of historical managment (land uses and irrigation) at the 5% significance level. The comparation of the types of water (HRW x HSW), within each treatment (T0 - without bioinputs and 100% fertilization, T1 – *Herbaspirillum seropedicae* + SH with 40% of the fertilization, T2 – *Azospirillum brasilense* with 40% of the fertilization and T3 - co-inoculation of *H. seropedicae* + SH with *A. brasilense* and 40% of the fertilization) is represented by n.s (not significant) and P-values ($p < 0.001$, $p < 0.01$ and $p < 0.05$).

contents. Pronounced impacts are evident, particularly for the variables root dry matter (RDM), K, Ca, and Mg. RDM is greater under swine water conditions (28.1 g plant^{-1}) compared to raw water (23.4 g plant^{-1}). Additionally, Ca and Mg leaf mineral contents are higher under raw water conditions (3.2 and 2.6 g kg^{-1}, respectively) compared to swine wastewater (1.89 and 1.5 g kg^{-1}, respectively).

Fertilization significantly affected maize plants' biometric parameters variables, leaf mineral contents and physiological parameters. The most significant p-values in N, P, and K leaf mineral contents occur in treatment with 100% fertilization.

Interaction effects do not exist between water and soil managements (Table 2). However, when evaluating fertilization and bioinput treatments (Treatment, Table 2), notable interaction effects are observed between Treatment and Soil as well as Treatment and Water for N contents. In the case of co-inoculation of *Herbaspirillum seropedicae* + SH with *Azospirillum brasilense* and 40% fertilization (Treatment 3), and evaluating the Treatment and Soil interaction (sliced by Treatment), a significant difference is found in N leaf

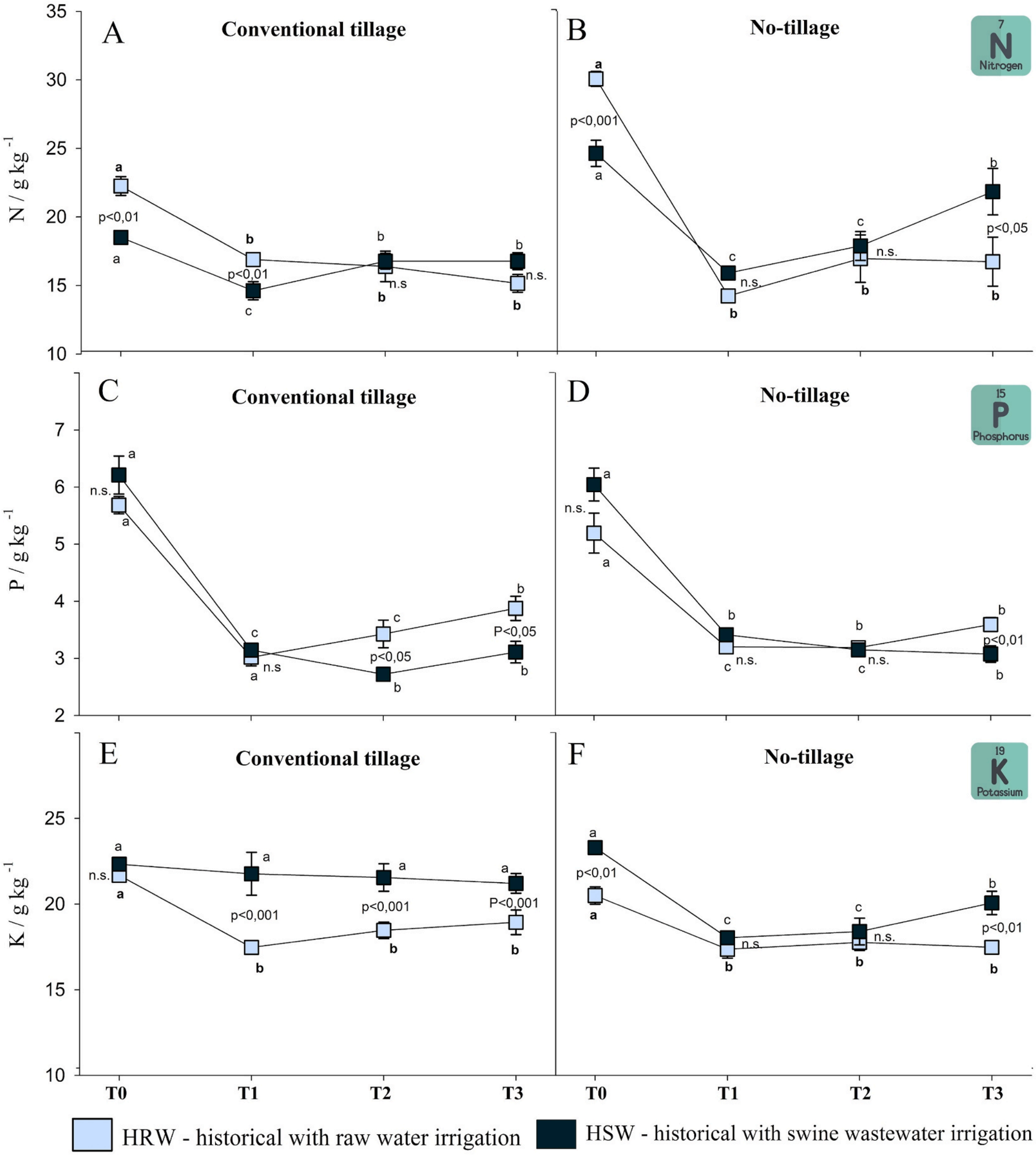

Fig. 2. Leaf mineral contents of nitrogen (N) (A and B), phosphorus (P) (C and D) and potassium (K) (E and F) according to the historical of soil management types [HNT - historical with no-tillage system (B,D and F), HCT - historical with conventional tillage system (A,C and E)] and water used in irrigation (HSW - historical with swine wastewater, HRW - historical with raw water). Letters compare treatments in the level of historical managment (land uses and irrigation) at the 5% significance level. The comparation of the types of water (HRW x HSW), within each treatment (T0 - without bioinputs and 100% fertilization, T1 – *Herbaspirillum seropedicae* + SH with 40% of the fertilization, T2 – *Azospirillum brasilense* with 40% of the fertilization and T3 - co-inoculation of *H. seropedicae* + SH with *A. brasilense* and 40% of the fertilization) is represented by n.s (not significant) and P-values ($p < 0.001$, $p < 0.01$ and $p < 0.05$).

contents (15.9 g kg^{-1} in Conventional Tillage/Treatment 3 and 19.3 g kg^{-1} in No-Tillage/Treatment 3, $p < 0.01$). Similarly, for the Treatment and Water (sliced by Treatment), a significant difference is observed (16.1 g kg^{-1} in RW/Treatment 3 and 19.6 g kg^{-1} in SW/Treatment 3, $p < 0.01$) in N leaf contents.

3.1. Biometric parameters

Overall, treatments did not influence plant height (PH) (Fig. 1A and B). These results indicate that treatments with 40% of NPK fertilizer (T1, T2, and T3, mean = 135.8 cm) did not differ significantly ($P < 0.05$) from T0 (mean = 139 cm) with 100% of NPK fertilization.

For shoot dry mass (SDM) (Fig. 1C and D), 100% of NPK fertilization (T0) historically stood out statistically independent of irrigation and soil management. It should be noted that SDM production, in general, in treatments T1, T2, and T3 (mean = 39 g $plant^{-1}$) was, on average, 16.5 % lower than T0 (100% fertilization dose) (mean = 32.5 g $plant^{-1}$) (Fig. 1C and D). For corn plants grown in soil with a no-tillage system and impacted by a long-term history of irrigation with wastewater (HSW), the co-inoculation (*Azospirillum* + *Herbaspirillum*) treatment (T3) was statistically superior (Fig. 1D).

The root dry mass (RDM) positively responded to the bioinput treatments, particularly when irrigated with raw water and under conventional tillage (Fig. 1E and F). In soil impacted by conventional tillage (HCT) (Fig. 1E), the roots exhibited more significant development when *Herbaspirillum* strain (T1 and T3) was used, either in co-inoculation or isolated (Fig. 1E).

3.2. Leaf mineral contents

For leaf N content, even with the same dose of mineral fertilizer used, plants grown in no-tillage soil (mean = 28.4 g kg^{-1}) (Fig. 2B) increased by approximately more than 50% of their N content compared to conventional tillage soil (mean = 20.5 g kg^{-1}) (Fig. 2A) when 100% fertilization was applied (T0). The effect of microbial inoculants was more evident when plants were grown in no-tillage

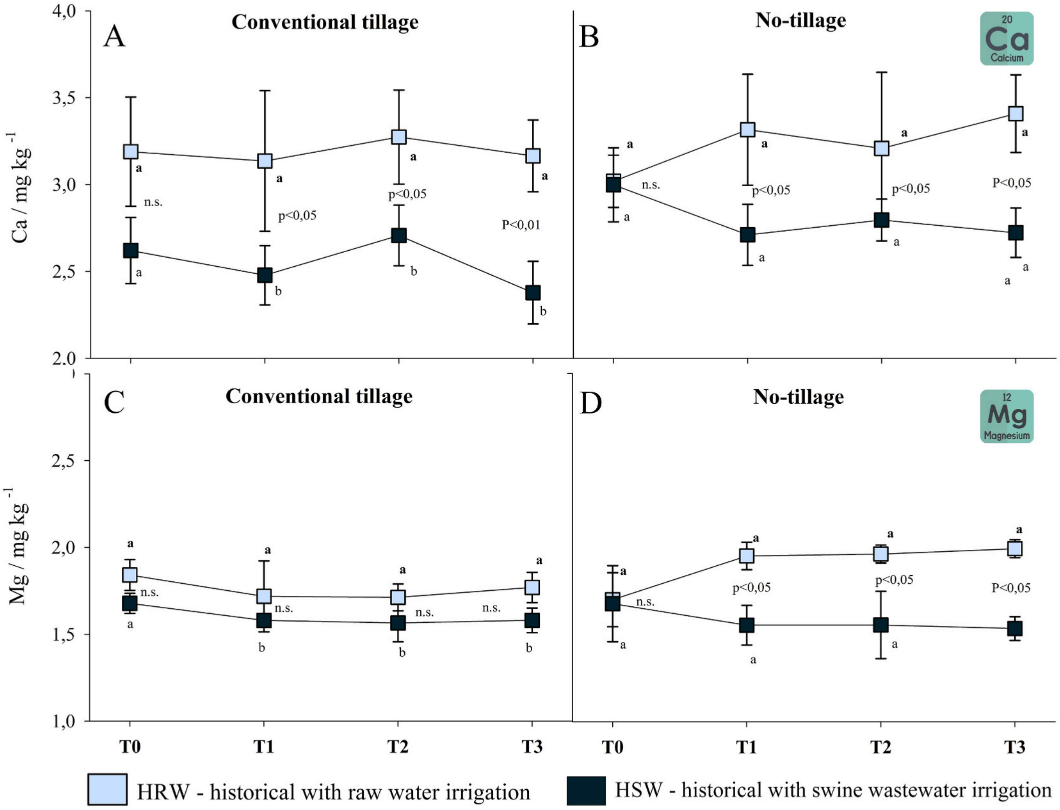

Fig. 3. Leaf mineral contents of Calcium (Ca) (A and B) and Magnesium (Mg) (C and D) according to the historical of soil management types [HNT - historical with no-tillage system (B and D), HCT - historical with conventional tillage system (A and C)] and water used in irrigation (HSW - historical with swine wastewater, HRW - historical with raw water). Letters compare treatments in the level of historical managment (land uses and irrigation) at the 5% significance level. The comparation of the types of water (HRW x HSW), within each treatment (T0 - without bioinputs and 100% fertilization, T1 – *Herbaspirillum seropedicae* + SH with 40% of the fertilization, T2 – *Azospirillum brasilense* with 40% of the fertilization and T3 - co-inoculation of *H. seropedicae* + SH with *A. brasilense* and 40% of the fertilization) is represented by n.s (not significant) and P-values ($p < 0.001$, $p < 0.01$ and $p < 0.05$).

soil (Fig. 2B). Co-inoculation (*Azospirillum* + *Herbaspirillum*) with 40% fertilization (T3) resulted in greater values for N contents (mean = 21.75 g kg^{-1} N), slightly lower than the treatment with 100% fertilization (T0) (mean = 24.53 g kg^{-1} N) (Fig. 2B). Treatment T3 was statistically superior to T1 and T2 in no-tillage system (Fig. 2B).

The 100% fertilization treatment stood out regarding P leaf contents, and with 40% fertilization, higher P contents were observed in Co-inoculation (*Azospirillum* + *Herbaspirillum*) treatments (T3) (Fig. 2C and D). Overall (Fig. 2C and D), among the studied nutrients, P presented the most significant decrease in contents when comparing treatments that received 100% of the fertilization (T0) (5.4 g kg^{-1}) with soils treated with microbial inoculants, where 40% of the P dose was applied (T1 = 3.0 g kg^{-1}, T2 = 2.9 g kg^{-1}, and T3 = 3.21 g kg^{-1}). In these treatments, the mean reduction of leaf P contents was 43.9% (Fig. 2C and D).

Regarding leaf K content, treatments with soil impacted by swine wastewater showed higher K content (Fig. 2E and F), corroborating the higher available K^+ values found in these soils (Table 1). This impact was less pronounced under no-tillage soils (Fig. 4F).

In the foliar contents of Ca and Mg (Fig. 3A–D), there was no difference between treatments and historical soil usage. We observed only the impact of irrigation history (HRW and HSW), and the main highlight is related to the reduction of the absorption of Ca (Fig. 3A and B) and Mg (Fig. 3C and D) in soil impacted by swine water irrigation, considering that all treatments received the same dose of limestone (Fig. 3A–D).

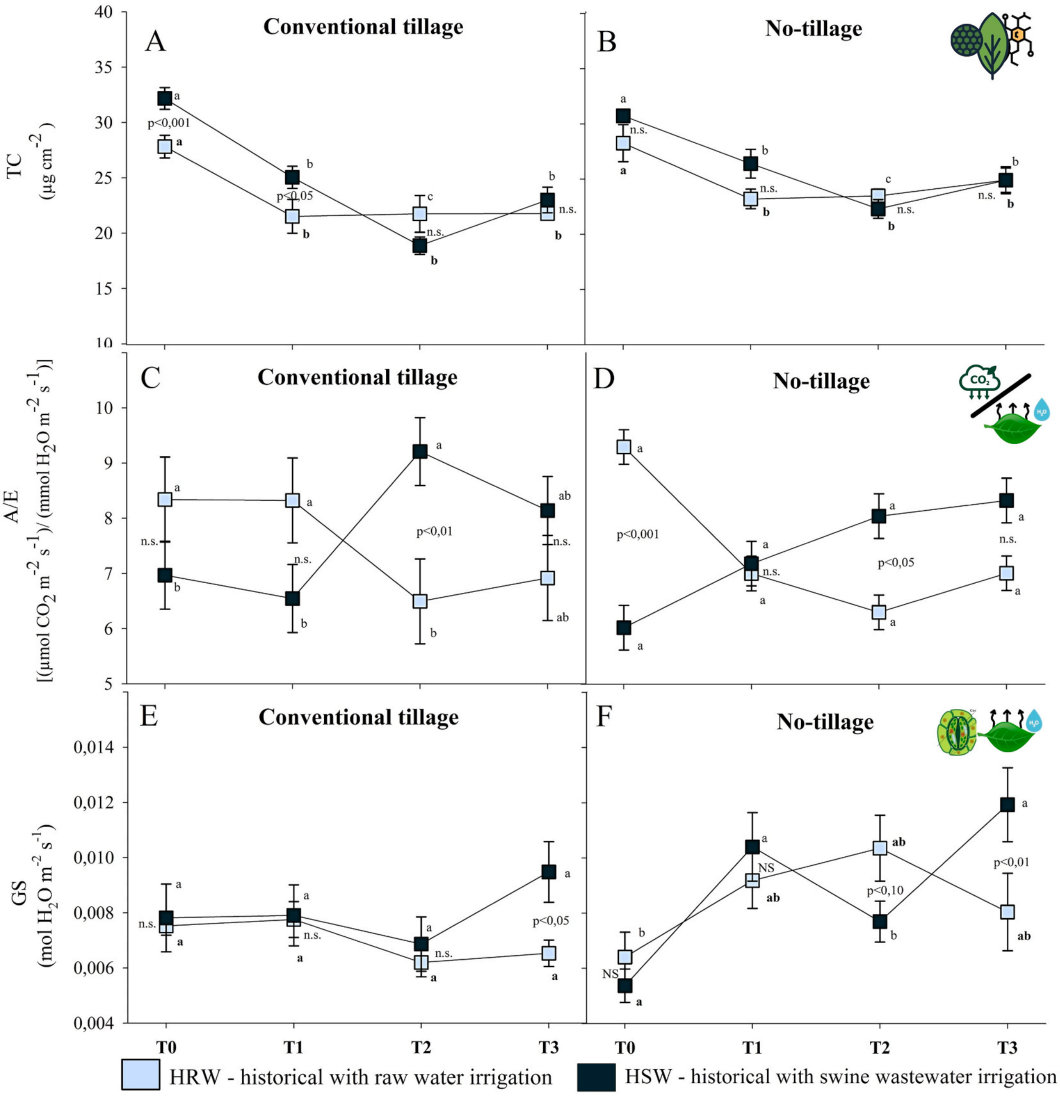

Fig. 4. Total Chlorophyll (TC) (A and B), Instantaneous water use efficiency (A/E) (C and D) and Stomatal conductance (Gs) (E and F) according to the historical of soil management types [HNT - historical with no-tillage system (B,D and F), HCT - historical with conventional tillage system (A,C and E)]. Letters compare treatments in the level of historical managment (land uses and irrigation) at the 5% significance level. The comparation of the types of water (HRW x HSW), within each treatment (T0 - without bioinputs and 100% fertilization, T1 – *Herbaspirillum seropedicae* + SH with 40% of the fertilization, T2 – *Azospirillum brasilense* with 40% of the fertilization and T3 - co-inoculation of *H. seropedicae* + SH with *A. brasilense* and 40% of the fertilization) is represented by n.s (not significant) and P-values ($p < 0.001$, $p < 0.01$ and $p < 0.05$).

3.3. Photosynthetic parameters

Total chlorophyll (TC) contents were higher with 100% fertilization (T0), and there were slight differences within the microbial inoculant treatments (T2, T3, and T4) (Fig. 4A and B). The instantaneous water use efficiency (A/E) (Fig. 4C and D) is higher when microbial inoculants are applied, mainly when *Azospirillum brasilense* is used as a single strain (T2) or co-inoculated with *Herbaspirillum seropedicae* (T3) (Fig. 4C and D). It should be noted that this increase in A/E only occurs in soil impacted by swine water waste (HSW) (Fig. 4C and D). Microbial inoculants influenced data related to stomatal conductance (GS) (Fig. 4E and F), with a more pronounced effect in soil from the no-tillage system (HNT) (Fig. 4F) irrigated with swine wastewater (HSW), particularly in the co-inoculation (*Azospirillum* + *Herbaspirillum*) treatments (T3) (Fig. 4F).

3.4. Multivariate statistics

Factor Analysis (FA) (Table 3) evaluated the interrelated variables and their relationship with the treatments. The first six factors explained 76% of the total data variability and presented eigenvalues greater than 1 [54].

Factor 1 retained 19.69% of the total variability in the original data, and the highest positive factor loadings were related to the attributes shoot dry mass (SDM), N, P, and CT (Table 3), indicating the effect of fertilization on plant growth and nutrient uptake. There was a positive relationship between SDM and macronutrient levels (Table 3), indicating that the relative growth rate of dry matter follows the relative rates of nutrient uptake. The attributes A, GS, and E group had the highest negative loadings in Factor 2 (15.6% of the explained variance) (Table 3). Factor 2 is directly associated with physiological changes in the plant, which did not show a direct relationship with biometric parameters or nutritional attributes.

The multivariate scatter plot (Fig. 5) demonstrates the substantial contribution of treatments that received 100% fertilizer in the variable group of Factor 1 (SDM, N, P, and CT) (Fig. 5A). For Factor 2, where a negative factor loading of the E, GS, and A variable group is observed (Fig. 5A), there is a highlighted contribution of treatments with microbial inoculants (T1, T2, and T3) and soil from the no-tillage system (HNT) (Fig. 5A).

Isolating the effects and evaluating score variations in treatments (Fig. 6), there is a greater contribution from T0 and a smaller contribution from co-inoculation treatments (T3) to Factor 1 (Fig. 6A). Treatment T3 exhibits a stronger contribution to Factor 2 (Fig. 6B), which is associated with plant physiological attributes linked to the negative axis of Factor Analysis (Fig. 5A).

Factor 3 (12.7%) had an expressive negative factorial loading of leaf Ca and Mg contents (Fig. 5B). The positive scores attributed to swine wastewater irrigation (SW) treatments (Fig. 5B) indicate an inverse contribution, i.e., less Ca and Mg absorption by the plant in SW treatments (Fig. 5B). Factor 4 (9.6%) had a strong negative factor loading of root dry mass (RDM) and a positive factorial load of the SDM/RDM relationship (Fig. 5B). The multivariate scatter plot (Fig. 5B) demonstrates the higher contribution of microbial inoculants in raw water irrigation (Herb-RW and Co–In-RW) (Fig. 5B). No treatment effect was observed in the variance of Factor 3

Table 3

Factor loadings[a] of the biometric parameters, leaf mineral contents, physiological attributes, eigenvalues and explained variance of the factors after the Varimax orthogonal method.

Eigenvalue	3.35	2.65	2.16	1.83	1.64	1.29	Communality R^2
Explained Variance %	19.7	15.6	12.69	10.79	9.66	7.57	
Accumulated Variance %	19.7	35.3	45.0	58.8	68.4	76.1	
Variables[b]	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	
PH	0.20	−0.03	0.06	0.08	−0.10	**0.85**	0.33
SDM	**0.77**	0.07	0.05	0.25	0.11	0.26	0.75
RDM	0.01	0.09	−0.04	**−0.96**	0.04	0.02	0.92
SDM/RDM	0.28	−0.04	0.08	**0.92**	0.00	0.08	0.93
N	**0.72**	0.10	0.07	0.09	−0.24	0.05	0.53
P	**0.85**	0.28	−0.14	−0.06	0.12	0.06	0.81
K	0.60	0.13	0.43	0.17	0.11	0.03	0.55
Ca	−0.03	0.06	**−0.87**	0.02	−0.14	0.16	0.53
Mg	0.10	−0.02	**−0.82**	−0.09	0.18	−0.19	0.45
Zn	0.66	−0.10	−0.30	−0.28	0.08	0.20	0.48
TC	**0.75**	0.14	−0.01	0.20	0.03	−0.31	0.65
A	−0.11	**−0.86**	−0.01	0.06	0.44	0.01	0.96
GS	−0.13	**−0.82**	−0.02	0.12	−0.24	−0.12	0.69
Ci	−0.10	−0.58	0.22	−0.22	−0.45	−0.27	0.56
E	−0.19	**−0.86**	−0.04	0.04	−0.19	0.19	0.95
A/E	0.12	0.10	0.10	−0.01	**0.88**	−0.17	0.93
A/CI	0.14	−0.16	0.16	0.13	−0.40	−0.36	0.32

[a] Factor loadings: strong - ≥ 0.7 (in bold) and moderate - between 0.7 and 0.5 [54].

[b] PH - Plant height, SDM – Shoot dry mass, RDM - root dry mass, N – nitrogen leaf content, P- phosphorus leaf content, K- potassium leaf content, Ca- calcium leaf content, Mg – magnesium leaf content, Zn – Zinc leaf content, TC – Total Chlorophyll; A – net assimilation rate of CO_2, Gs – stomatal conductance, Ci – internal CO_2 concentration, E – transpiration rate, A/E – Instantaneous water use efficiency and A/Ci - instantaneous carboxylation efficiency.

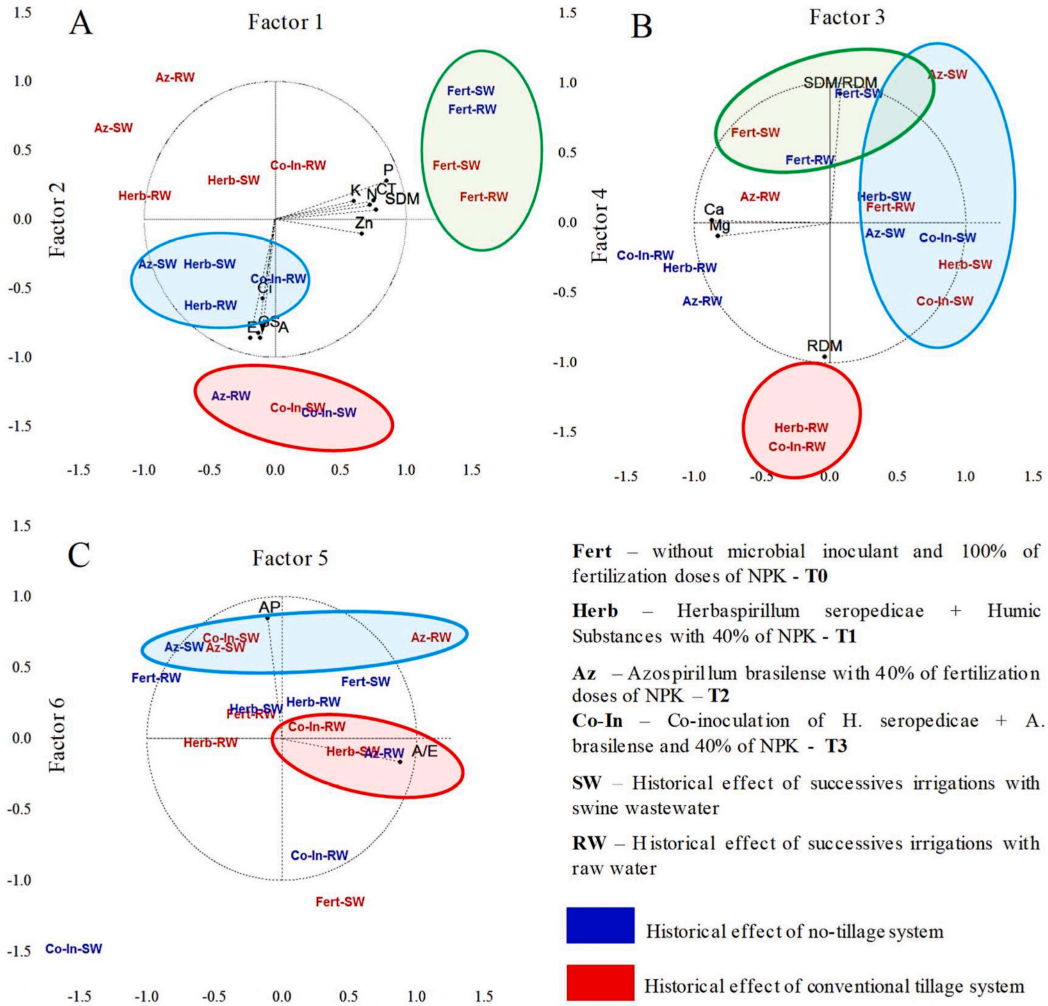

Fig. 5. Graphical representation of the factor loadings of the variables (≥0.7) and representation of the sample scores (average of the repetitions of the treatments) for the Factors 1 and 2 (A), Factor 3 and 4 (B) and Factor 5 and 6 (C). PH - Plant height, SDM - shoot dry mass, RDM - root dry mass, N – nitrogen leaf mineral content, P- phosphorus leaf mineral content, K- potassium leaf mineral content, Ca-calcium leaf mineral content, Mg – magnesium leaf mineral content, Zn – Zinc leaf mineral content, TC – Total Chlorophyll; A – net assimilation rate of CO_2, Gs – stomatal conductance, Ci – internal CO_2 concentration, E − transpiration rate, A/E − Instantaneous water use efficiency and A/Ci - instantaneous carboxylation efficiency.

(Fig. 6C), which was explained solely by the effect of irrigation management. For Factor 4 (Fig. 6D), the effects of T1 and T3 are highlighted, as well as on the negative axis of Factor 4 (Fig. 5B). Inoculations with Herbaspirillum seropedicae + HS (isolated or co-inoculated) resulted in higher RDM values when considering treatments with microbial inoculants.

Factor 5 (9.6% of explained variation) and Factor 6 (7.6% of explained variation) (Fig. 6E and F) had less participation in data variation, with less effect of treatments on the variance of these factors (Fig. 6E and F).

4. Discussion

The efficacy of microbial bio-inputs in corn is subject to multiple influencing factors [58,59], including the specific type and dosage of fertilization applied [60], as well as the selection of cultivars, varieties, and hybrids [22]. Other factors also directly affect the growth performance of host plants; soil type, moisture, salinity, pH variation and temperature [61]. The present study emphasizes cultivating a variety of creole corn under optimal soil moisture and base saturation conditions, with variations in NPK fertilization under the influence of soil management effects (conventional and no-tillage) and the impact of diferents types of irrigation water (swine wastewater and raw water). In this context, few studies centred on the interaction between soil management, irrigation types and microbial inoculants (Table 1). Interactions are more evident with lower NPK fertilization, as we observed greater nitrogen acquisition by plants in the no-tillage system irrigated with swine wastewater (Table 1).

The results of our study corroborate those described in the literature; strains of *Azospirillum brasilense* and *Herbaspirillum*

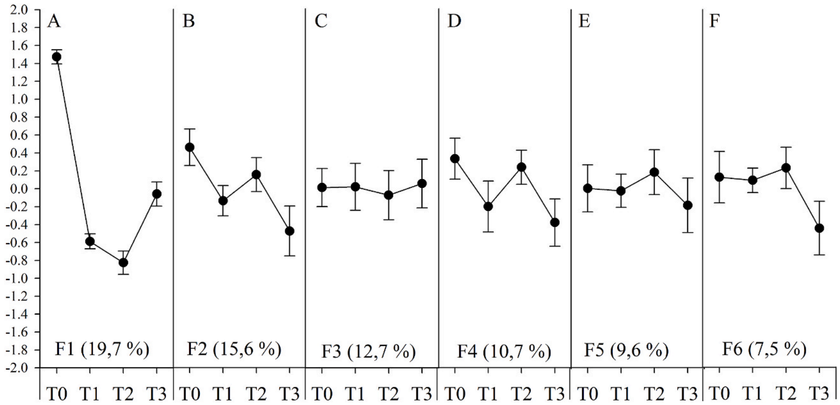

Fig. 6. Representation of the mean and standard error of the factor scores of each Factor 1 (A), Factor 2 (B), Factor 3 (C), Factor 4 (D), Factor 5 (E) and Factor 6 (F) and it is proportional explications of data variation, grouped only by treatments. T0 - without bioinputs and 100% fertilization, T1 – *Herbaspirillum seropedicae* + SH with 40% of the fertilization, T2 – *Azospirillum brasilense* with 40% of the fertilization and T3 - co-inoculation of *H. seropedicae* + SH with *A. brasilense* and 40% of the fertilization.

seropedicae, in single-strain formulation or co-inoculated, can induce physiological changes in plants, promoting alterations in root architecture and aboveground plant parts (Cassán et al., 2013 [25]). Even with a significant reduction in fertilization (lowered by 60%), the impact on some variables, such as plant height, shoot dry mass, and N–K content, was less significant due to the benefits of microbial inoculants. This reduction in fertilization dose led to approximately 11.8%–21.1% decreases in shoot dry mass (Fig. 1C and D). Leite et al. [62], evaluating phosphate fertilization and dose-response relationships for maize in pot experiments, indicated a reduction of approximately 48% in the shoot dry mass of maize when reducing phosphate fertilization doses by 60% while keeping other nutrients at optimal levels. Regarding a reduction of N and K, the impact on shoot dry mass is much greater [62].

The biofertilization effect was less expressive (Fig. 2). However, considering the low-input treatments with microbial inoculants and 40% of the fertilizer dose, only the co-inoculation (treatment T3) stands out in Factor 1 (Figs. 5A and 6A) of more significant explanation of data variance (Table 3) and where there is a strong relationship between SDM, N, P and CT (Table 3, Figs. 5A and 6A). Nitrogen acquisition stands out with the co-inoculation of bacteria (T3 treatment) and under the effects of no-tillage and swine wastewater management. *Herbaspirillum seropedicae* and *Azospirillum brasilense* co-inoculated have been associated with better agronomic effects in maize than inoculated alone [31].

The effect of co-inoculation may be related to the production of indole compounds (ICs), which according to Cortés-Patiño et al. [63], *Azospirillum* sp. and *Herbaspirillum* sp. can coexist and produce larger quantities of ICs when co-inoculated, such as auxin and indole-3-acetic acid (IAA). These compounds have physiological importance in bacteria-plant interactions, promoting photostimulation [64] and the formation of lateral roots, decreasing the length of the primary root and increasing the formation of fine root [65]. Therefore, allowing greater nutrient uptake and, consequently, higher biomass production.

Promoting growth effects can be intensified in soil environments that favour carbon input, enabling higher stability of the soil microbial community [59], as reported by Semenov et al. [66], who observed a positive influence of the soil carbon on the *Azospirillum* sp. population. In this work, the impact of historical management of no-tillage and swine wastewater use improve de efficiency of microbial bio-input in co-inoculation treatment (T3) (Figs. 1D and 2B). No-tillage management and swine wastewater irrigation water generate higher biological quality of the soil (Table 1), where higher values of TOC, C-soluble, MBN, β-G, and DGS are observed (Table 1), indicating good soil quality and active microorganisms associated with the N cycle [67]. The C-soluble indicator indicates labile C in the soil [42].

Thus, the deposition of residues in the no-tillage system (HNT), coupled with the positive impact of irrigation with swine wastewater, increases labile organic carbon in the soil and enhances biological activity. In this condition, bio-stimulation and/or biofertilization effects can also be generated by native bacteria [68]. Populations of *Azospirillum* spp. are widely distributed in tropical soils [69], particularly in environments with no soil disturbance, higher organic matter content, and live plants in the off-season [70]. Bacterial inoculation has provided significant increases in maize grain yield concerning soil attributes (organic matter, texture, tillage management), with clay soils and no-tillage management generally standing out (Barbosa et al., 2022).

The biostimulant effect was better expressed in Factors 2 and 4 (Table 3). Considering the influence of microbial inoculants on root development, treatments T1, T2, and T3 stand out, which presented higher RDM (Figs. 5B and 6D). This effect reinforces the hypothesis that microbial inoculants under abiotic stress conditions (lower fertility) tend to have more noticeable effects on root development when compared to a condition without microbial inoculants [71]. According to Azevedo et al. [27], applying *Herbaspirillum seropedicae* with humic substances causes physiological alterations in maize roots, increasing root length. A study by Barbosa et al. [72], which compiled 60 studies conducted in different regions of Brazil, showed that inoculating maize with *Azospirillum brasilense* increased root dry mass by an average of 12.1%.

The RDM responses were higher when the maize was cultivated in the soil with historical irrigation with raw water (HRW) (Fig. 1E) and when associated with a strain of *Herbaspirillum* sp. (Treatments T1 and T3, Fig. 1E and D). This bacteria is specialized in colonizing the inside of the plants, i.e. have better endophytic competence (Roesh et al., 2006). This represents an ecological advantage over bacteria such as *Azospirillum* sp., which usually colonize the rhizosphere. The biostimulant effect of this strain is associated with the produced compounds that generate the H^+ concentration gradient in the cell due to the hydrolysis of the ATP proton pump, allowing the symport movement of molecules and the flow of nutrients in the apoplast [2,73]. The relationship between RDM and nutrients was low (non-significant correlations), demonstrating that the direct effect of root growth and nutrient uptake was less evident.

The bio-stimulant effects on plant physiology were better expressed by Factor 2 (Table 3, Fig. 5A). Positive effects of treatments with microbial inoculants were observed compared to 100% fertilization (Fig. 6). In general, microbial inoculantes treatments (T3 and T2) under soil impacted by swine wastewater (HSW) (Fig. 4F) showed the highest values of stomatal conductance (GS) in the plant, which are directly correlated to Ci. This correlation can be explained by the higher photosynthetic rate, increasing the consumption of Ci, which may have stimulated stomatal opening, thus elevating GS [74]. It is a positive result, showing stomatal function controlling the absorption of CO_2 and, consequently, plant production [75]. In addition, microbial inoculants increased the instantaneous water use efficiency (A/E), meaning plants can produce more by consuming less water [76]. Oliveira [77] reported similar results when Herbaspirillum seropedicae is associated with humic substances.

Management practices focusing on using effective soil microbiomes under low fertility to promote healthy ecosystems and supporting the sustainable management of soil ensure world food security [78]. A more stable production system under moderate nutrient stress consuming fewer non-renewable resources like fertilizer may be reached in a no-tillage system with swine wastewater use. In our work, N contents in soil from conventional tillage (HCT) irrigated with raw water (HRW) with 100% of fertilizer (22.2 ± 1.37 g kg^{-1}) are the no-significant difference ($P < 0.001$) to soil from no-tillage (HNT) irrigated with swine wasterwater (HSW) with 40% of fertilizer (21.7 ± 1.68 g kg^{-1}). In the same way, shoot dry mass (SDM) in soil from conventional tillage (HCT) irrigated with raw water (HRW) with 100% of fertilizer (40.2 ± 1.4 g kg^{-1}) are the less expressive difference ($P < 0.05$) to no-tillage (HNT) irrigated with swine wasterwater (HSW) with 40% of fertilizer (35.7 ± 1.2 g kg^{-1}).

For leaf K content, swine wastewater serves as a source of K in the soil (Fig. 2F), corroborating the results found by Guidinelle et al. [79], who observed higher leaf K contents when irrigating maize with swine wastewater. Regarding foliar contents of Ca and Mg, swine wastewater (HSW) (Fig. 5) decreases their uptake. This effect is related to the high ion saturation in the soil solution, which can form ionic pairs with Ca^{2+} and Mg^{2+}, reducing plant uptake. These results can be corroborated by the high electrical conductivity value (Table 1) in HSW, which is rich in sulfates, nitrates, and low molecular weight organic acids, according to Guidinelle et al. [79]. High values of sulfate and nitrite can form ionic pairs such as $CaNO^{3+}$, $CaSO_4$, and $MgSO_4$, reducing the activity of the ion in the solution and its uptake by the plant [80]. We did not observe the effect of microbial inoculants ameliorating this higher salinity effect in the soil, as observed by Fukami et al. [81].

5. Conclusions

Using microbial inoculants presents a promising avenue to reduce reliance on chemical fertilization while enhancing production efficiency. The treatments with a 60% reduction in the application dosage of fertilizer associated with microbial inoculants exhibited satisfactory effects on biometric parameters and resulted in the improved physiological quality of corn.

The positive impact on the soil by the no-tillage management system irrigated with swine wastewater provided a more favorable edaphic environment, microbial activity, and biological quality, presumably maintaining the introduced microbial population and/or promoting, in conjunction with native bacteria, the action of bioestimulation effect in the rhizosphere. The beneficial effects of microbial inoculants on corn's vegetative development occur mainly when *Azospirillum* and *Herbaspirium* are co-inoculated as bio-inputs.

CRediT authorship contribution statement

Rebyson Bissaco Guidinelle: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Diego Lang Burak:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Otacilio José Passos Rangel:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Anderson Lopes Peçanha:** Writing – review & editing, Writing – original draft, Methodology. **Renato Ribeiro Passos:** Writing – review & editing, Writing – original draft, Formal analysis. **Letícia Oliveira da Rocha:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Fábio Lopes Olivares:** Writing – review & editing, Writing – original draft, Formal analysis. **Eduardo de Sá Mendonça:** Writing – review & editing, Writing – original draft, Formal analysis.

Declaration of competing interest

The authors declare that there is no competing interest in this research.

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