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Nitrogen efficiency and agronomic responses of maize to pre-inoculation and sowing-time inoculation with plant growth-promoting bacteria under different N rates

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Abstract: This study evaluated the agronomic efficiency of *Bacillus licheniformis* applied as a seed inoculant in different pre-treatment periods and compared it with conventional nitrogen fertilization. Two experimental phases were conducted. In the main harvest (Candói and Lapa), four treatments were evaluated: T1 – absolute control (no inoculation and no topdressing nitrogen), T2 – nitrogen control (100% nitrogen at sowing plus 200 kg ha^{-1} in topdressing), T3 – seed inoculation with *Azospirillum brasilense* 90 days before sowing combined with 75% of the recommended nitrogen, and T4 – seed inoculation with *Bacillus licheniformis* on the day of sowing combined with 75% nitrogen. In the second harvest (Serranópolis do Iguaçu and São Miguel do Iguaçu), the treatments were: T1 – absolute control, T2 – nitrogen control (100% nitrogen plus 160 kg ha^{-1} in topdressing), T3 – seed inoculation with *Bacillus licheniformis* 90 days before sowing plus 75% nitrogen, and T4 – seed inoculation with *Bacillus licheniformis* on the day of sowing plus 75% nitrogen. Inoculation with *Bacillus licheniformis* showed agronomic efficiency comparable to the nitrogen control and to *Azospirillum brasilense* inoculation during the main harvest period. In the second harvest, inoculating seeds with *Bacillus licheniformis* 90 days before sowing allowed a 25% reduction in nitrogen fertilizer application without negatively affecting maize grain yield, demonstrating that this practice maintains crop performance even under reduced N inputs.

Keywords: Plant Growth Promoting Rhizobacteria, Sustainability, Productivity; *Zea mays* L.

Introduction

Among the most important cereals produced globally are wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.) (Erenstein et al., 2022). Owing to its wide genetic diversity, maize adapts to tropical, subtropical, and temperate environments, which has resulted in numerous cultivation systems and consequently in greater dependence on external inputs (Sousa et al., 2021). Due to its nutritional composition (approximately 72% starch, 10% protein, and 4% fat), the crop represents a strategic food source for both humans and animals (Nuss and Tanumihardijo, 2010; Aguiar et al., 2022).

Although maize is highly responsive to fertilization, achieving high yields often requires substantial nutrient inputs, particularly nitrogen (Ranum et al., 2014; Nakatani et al., 2024). This element is a central component of molecules such as chlorophyll, ATP, NADH, NADPH, enzymes, and proteins, which directly influence plant development (Arenhardt et al., 2017; Pereira et al., 2018). However, excessive application of nitrogen, especially under inefficient use conditions, may cause environmental damage (Móring et al., 2021). Historically, intensive fertilizer use has been linked to soil acidification and reduced nutrient availability (Guo et al., 2010), as well as disturbances in soil microbial balance (Zhong et al., 2010).

To address these challenges, technological innovations have been designed to combine productivity gains with environmental sustainability, providing farmers with efficient alternatives (Sousa et al., 2021). Within this framework, plant growth-promoting rhizobacteria (PGPR) have received attention for their beneficial effects on various crops (Moreno et al., 2021). These

microorganisms, particularly endophytic strains, enhance plant development through nitrogen fixation (Sun et al., 2022), improved nutrient uptake (Kushwaha et al., 2022), synthesis of bioactive compounds (Kumar et al., 2019), and increased resistance to stress conditions (Aziz et al., 2022). Among PGPR, bacteria of the *Bacillus* genus have shown promising results in crops such as soybeans (Chagas Junior et al., 2022), beans (Araujo et al., 2010), and maize (Aguiar et al., 2022).
In addition, other PGPR genera, such as *Pseudomonas*, *Azospirillum*, and *Rhizobium*, are also widely used in agriculture for their ability to produce phytohormones, fix nitrogen, solubilize nutrients, and induce systemic resistance. However, unlike *Bacillus*, many of these genera are non-sporulating and can be more sensitive to environmental stresses and storage conditions (Bhat et al., 2022; Andrade et al., 2023). The success of *Bacillus* strains is partly due to their capacity to form spores resistant to heat and chemicals, favoring both field use and the production of durable commercial inoculants (Rocha et al., 2019). Nevertheless, there is still limited knowledge regarding their effectiveness as complementary tools to conventional fertilization practices in maize. Therefore, this study aimed to evaluate the agronomic performance of maize inoculated with *Azospirillum brasilense* or *Bacillus licheniformis* applied either 90 days before sowing or at sowing, and to assess their effectiveness in enhancing nitrogen use efficiency under reduced nitrogen fertilization across two growing seasons.

Results and Discussion

Inoculant efficiency on main harvest period
The variance analysis for the main harvest (Table 1) revealed a significant interaction between treatment and environment for grain yield, total dry mass, and grain nitrogen content. Treatment effects were also significant for plant height, grain rows per ear, thousand grain weight, and total plant nitrogen. Environmental influence was detected on plant height, ear insertion height, grain rows and grains per row, grains per ear, and thousand grain weight. The coefficient of variation ranged from 2.05% to 8.62%, values classified as low (<10%) (Pimentel-Gomes, 2022), indicating high experimental precision.
In the main harvest period (Table 2), the absolute control (T1) showed the lowest grain yield, with averages of 15481 kg ha^{-1} in Candói-PR and 13584 kg ha^{-1} in Lapa-PR. No statistical difference was observed between nitrogen control (T2) and the treatment combining nitrogen fertilization with *Bacillus licheniformis* on the day of sowing (T4), suggesting that high N availability may mask PGPR effects (Hungria et al., 2016). The use of *Azospirillum brasilense* 90 days before sowing (T3) resulted in 17018 kg ha^{-1} in Candói-PR, significantly higher than the 15490 kg ha^{-1} recorded in Lapa-PR. These findings indicate that supplementing nitrogen fertilization with PGPRs is feasible and does not compromise yield or biomass. Similar responses were described by Sousa et al. (2021), who reported yield increases ranging from 281.43 to 2914.53 kg ha^{-1} with *Bacillus* inoculation compared to uninoculated controls. Such effects are consistent with PGPR mechanisms that enhance nutrient uptake and root development (Fukami et al., 2018).
For total dry mass, no statistical difference was observed among T2, T3, and T4 across environments (Table 2). The absolute control (T1) showed the lowest values, with 26737 kg ha^{-1} in Candói-PR and 24981 kg ha^{-1} in Lapa-PR. Regarding grain nitrogen content, T1 reached 40.0 g kg^{-1} in Candói-PR, significantly higher than 37.4 g kg^{-1} in Lapa-PR. In contrast, treatments T2, T3, and T4 recorded averages of 45.6, 43.4, and 45.7 g kg^{-1} in Lapa-PR, respectively, surpassing those obtained in Candói-PR, likely reflecting environmental modulation of microbial activity.
With no significant T x E interaction, Candói-PR showed higher averages for all traits influenced by the environment (Table 3). This suggests more favorable edaphoclimatic conditions that may also enhance PGPR performance. In this location, plant height and ear insertion height reached 261.25 cm and 157.27 cm, with 576.65 grains per ear and a thousand grain weight of 405.92 g. In Lapa-PR, the corresponding values were 237.54 cm, 136.37 cm, 515.83 grains per ear, and 364.68 g. Among treatments, T3 produced the tallest plants (254.85 cm), while T1 recorded the lowest average (241.51 cm), being statistically different. Superior plant height in T3 may reflect more effective early rhizosphere colonization. The highest number of grain rows occurred under T2, whereas inoculation treatments showed no difference, with means of 15.53 and 15.51 for T3 and T4, respectively. Plant nitrogen concentration peaked with T4 (38.8 g kg^{-1}), followed by T2 (36.9 g kg^{-1}) and T3 (35.9 g kg^{-1}), which did not differ statistically. The improvements observed in agronomic traits, including nitrogen accumulation, may be linked to bacterial growth-promotion mechanisms. *Bacillus licheniformis* produces enzymes capable of solubilizing chitin polymers, enhancing organic matter decomposition and nutrient availability (Shafi et al., 2017).

Inoculant efficiency on second harvest period
The variance analysis for the second harvest period indicated a significant Treatment x Environment interaction for grain nitrogen content (Table 4). Treatment effects were significant for plant population, grain yield, thousand grain weight, and total plant nitrogen, while the environment influenced ear index, plant height, grain rows per ear, grain yield, total dry mass, and total plant nitrogen. No significant differences were detected for ear insertion height, grains per row, or grains per ear. The coefficients of variation ranged from 4.23% to 14.42%, values classified as low (<10%) to medium (10–20%) (Pimentel-Gomes, 2022).
In São Miguel do Iguaçu-PR, all treatments differed significantly from the absolute control (T1) for grain nitrogen content, a pattern not observed in Serranópolis do Iguaçu-PR (Table 5). The highest values were recorded in São Miguel do Iguaçu-PR, being 24.1 g kg^{-1} for nitrogen control (T2), 21.9 g kg^{-1} for inoculation with *Bacillus licheniformis* 90 days before sowing (T3), and 21.7 g kg^{-1} for inoculation with *Bacillus licheniformis* on the day of sowing (T4). These results indicate that complementing

Table 1. Summary of the analysis of variance for the effect of four treatments (T), two environments (E) and their interaction (T x E), in the main harvest period (Phase 1).

SV	DF	Mean Square					
		POP	EI	PH	EIH	NGR	NGPR
T	3	13428416.57	0.01	284.50*	212.87	0.73*	3.14
E	1	18605.44	0.012*	4496.05*	3495.18*	1.99*	62.19*
Block	3	6589551.82	0.00	101.58	54.26	0.10	9.27
T x E	3	8141493.85	0.00	71.39	41.30	0.03	6.05
Residual	21	6260111.44	0.00	86.68	70.09	0.10	7.87
CV (%)	-	3.41	5.08	3.73	5.70	2.05	8.11
Mean	-	73326.58	1.022	249.4	146.82	15.78	34.58
SV	DF	Mean Square					
		NGPE	GY	TGW	DM	NCP	NCG
T	3	2505.48	8075217.40*	827.39*	52670940*	46.15*	43.20*
E	1	29588.33*	9676396.80*	13605.64*	22130543*	8.00	22.99*
Block	3	3306.97	882371.10	552.72	2372946	9.41	7.82
T x E	3	1881.08	1052024.10*	403.85	13478230*	0.00	21.38*
Residual	21	2214.94	243030.00	212.96	2899047	9.17	3.03
CV (%)	-	8.62	3.08	3.79	5.73	8.36	4.12
Mean	-	546.24	16014.84	385.3	29698.86	36.21	42.18

POP: Plant population (plants ha^{-1}); EI: ear index; PH: plant height (cm); EIH: ear insertion height (cm); NGR: number of grain rows (units); NGPR: number of grains per row (units); NGPE: number of grains per ear (units); GY: grain yield (kg ha^{-1}); TGW: thousand grain weight (g); DM: total dry mass (kg ha^{-1}); NCP: total nitrogen content in the plant (g kg^{-1}); NCG: total nitrogen content in grains (g kg^{-1}); SV: source of variation; DF: degrees of freedom; CV: coefficient of variation; *significant at 5% probability by the F test.

Table 2. Breakdown of the interaction between environment and treatment in the main harvest period (Phase 1).

Treatment	GY		DM		NCG	
	Environment		Environment		Environment	
	Candói-PR	Lapa-PR	Candói-PR	Lapa-PR	Candói-PR	Lapa-PR
T1	15481 bA	13584 bB	26737 bA	24981 bA	40.0 aA	37.4 bB
T2	16483 aA	16412 aA	28743 abB	32836 aA	41.4 aB	45.6 aA
T3	17018 aA	15489 aB	30656 aA	31780 aA	42.6 aA	43.4 aA
T4	16918 aA	16375 aA	29332 abB	32523 aA	41.3 aB	45.7 aA

T1: Absolute control; T2: Nitrogen control; T3: Inoculation with *Azospirillum brasilense* 90 days before sowing; T4: Inoculation with *Bacillus licheniformis* on the day of sowing; GY: Grain yield (kg ha^{-1}); DM: total dry mass (kg ha^{-1}); NCG: total nitrogen content in grains (g kg^{-1}). Means followed by the same lowercase letter in the column and capital letter in the row do not differ from each other at 5% probability using the Tukey test.

topdressing N-fertilizer with *Bacillus licheniformis* effectively enhanced nitrogen uptake and translocation to the grains. Elevated plant nitrogen concentration benefits stem N storage, remobilization during grain filling, and may increase photosynthetic efficiency (Debruin et al., 2017), supporting the idea that inoculation strengthens internal N cycling. Studies have shown that plant growth-promoting rhizobacteria (PGPB), including *Bacillus* spp., can enhance nutrient uptake, root growth, and overall nitrogen assimilation in maize (Sousa et al., 2021; Andrade et al., 2023; Nakatani et al., 2024).

Significant differences were observed in plant population (Table 6), averaging 68312 plants ha^{-1} in Serranópolis do Iguaçu-PR and 61093 plants ha^{-1} in São Miguel do Iguaçu-PR. Ear index, also known as prolificacy, was higher in Serranópolis do Iguaçu-PR (1.13) than in São Miguel do Iguaçu-PR (0.97). Low soil nitrogen can limit prolificacy, a source of reproductive plasticity at low densities, helping sustain grain yield (Debruin et al., 2017; Parco et al., 2020; Ross et al., 2020). However, values above 1.2 may reduce thousand grain weight and overall yield. Plant height and ear insertion height were greater in Serranópolis do Iguaçu-PR, averaging 219.85 cm and 129.18 cm, respectively. In contrast, São Miguel do Iguaçu-PR exhibited a higher number of grain rows (16.98) compared to Serranópolis do Iguaçu-PR (16.39).

Grain yield was lower in São Miguel do Iguaçu-PR (5899.32 kg ha^{-1}) compared to Serranópolis do Iguaçu-PR (7112.32 kg ha^{-1}), as described in Table 6. No significant difference was observed for total dry mass. Total nitrogen in the plant was higher in Serranópolis do Iguaçu-PR (23.94 g kg^{-1}) than in São Miguel do Iguaçu-PR (21.31 g kg^{-1}). Similarly, Bueno et al. (2022) reported

Table 3. Test of comparison of means for the main effects of environment and treatment in the main harvest period (Phase 1).

Environment	EI	TGW	PH	EIH	NGR	NGPR	NGPE
Candói-PR	1.04 a	405.92 a	261.25 a	157.27 a	16.03 a	35.98 a	576.65 a
Lapa-PR	1.00 b	364.68 b	237.54 b	136.37 b	15.53 b	33.19 b	515.83 b
Mean	1.02	385.30	249.4	146.82	15.78	34.58	546.24
Treatment		PH		NGR		NCP	
T1		241.51 b		15.97 ab		33.10 b	
T2		253.05 ab		16.10 a		36.99 ab	
T3		254.85 a		15.53 bc		35.91 ab	
T4		248.18 ab		15.51 c		38.84 a	
Mean		249.40		15.78		36.21	

T1: Absolute control; T2: Nitrogen control; T3: Inoculation with *Azospirillum brasilense* 90 days before sowing; T4: Inoculation with *Bacillus licheniformis* on the day of sowing; EI: ear index; TGW: thousand grain weight (g); PH: plant height (cm); EIH: ear insertion height (cm); NGR: number of grain rows (units); NGPR: number of grains per row (units); NGPE: number of grains per ear (units); NCP: total nitrogen content in the plant ($g\ kg^{-1}$). Means followed by the same lowercase letter do not differ from each other using the Tukey test at 5% probability.

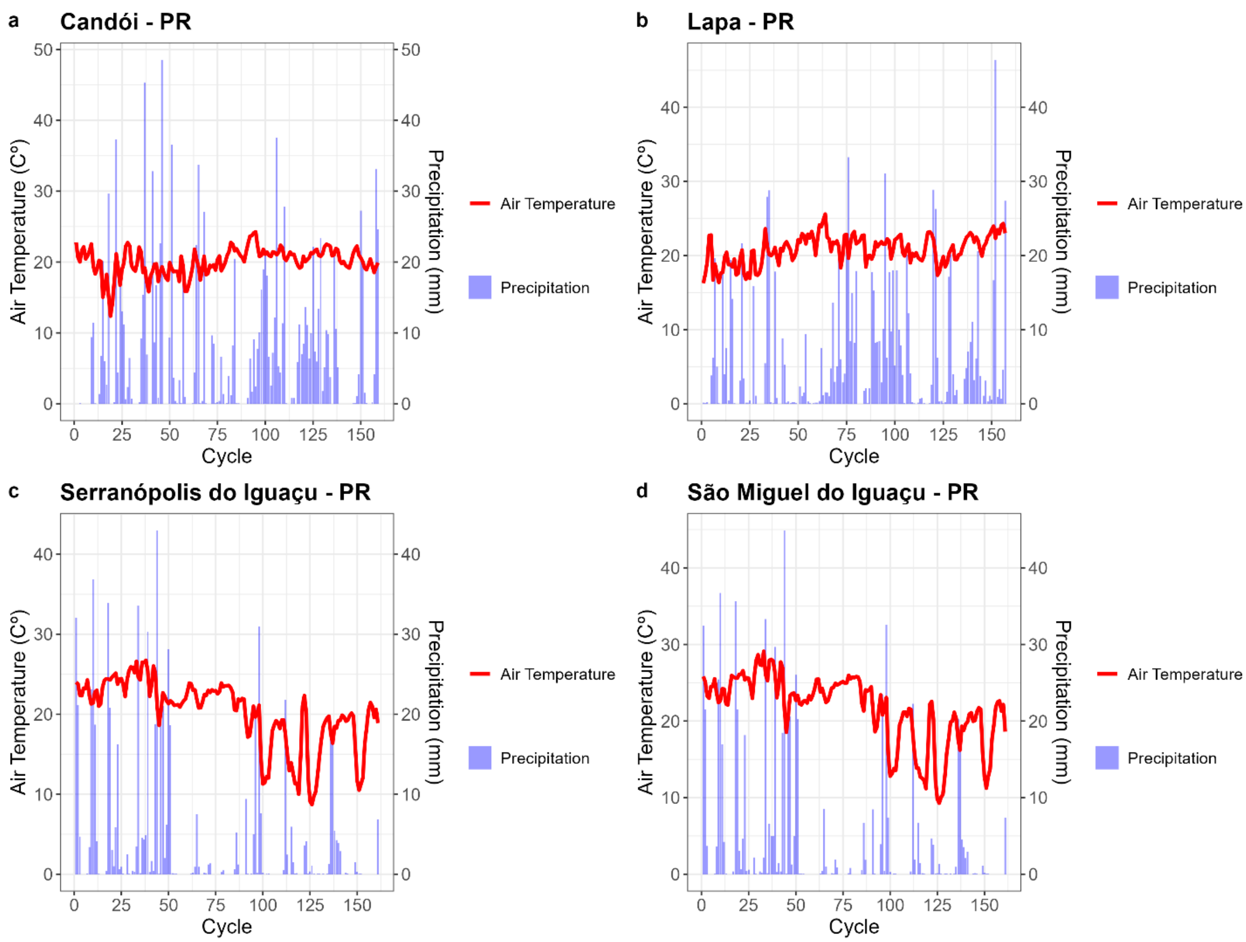

Fig 1. Mean air temperature (ºC) and precipitation (mm) during the maize cultivation cycle: main harvest in Candói–PR and Lapa–PR (a, b) and second harvest in Serranópolis do Iguaçu–PR and São Miguel do Iguaçu–PR (c, d).

that maize seeds inoculated with Bacillus reached 29.64 $g\ kg^{-1}$ nitrogen in plant tissues, whereas non-inoculated seeds accumulated only 11.33 $g\ kg^{-1}$. These parallels reinforce that the inoculation improves plant N status, although the magnitude depends on the environment.

Across treatments, the nitrogen control showed the highest grain yield, averaging 7000.58 $kg\ ha^{-1}$, while T1 and T4 were lower at 6109.29 and 6294.71 $kg\ ha^{-1}$, respectively. T3 provided an intermediate yield of 6637.4 $kg\ ha^{-1}$. The overall mean for the experiment was 6552.57 $kg\ ha^{-1}$, exceeding yields reported in other second-harvest maize studies, such as 6261 $kg\ ha^{-1}$ with *Azospirillum brasilense* (Smaniotto et al., 2023) and 6476 $kg\ ha^{-1}$ with 300 $kg\ ha^{-1}$ N-fertilizer (Albert et al., 2023). These results suggest that inoculation 90 days before sowing is a viable strategy to reduce N-fertilizer use. All treatments improved thousand

Table 4. Summary of the analysis of variance for the effect of four treatments (T), two environments (E) and their interaction (T x E), in the second harvest period (Phase 2).

SV	DF	POP	EI	PH	EIH	NGR	NGPR
		MS					
T	3	26556790*	0.01	304.54	131.63	0.24	7.08
E	1	546519832	0.22*	4550.14*	2707.90	3.18*	1.65
Block	6	283374800	0.02	263.43	149.68	0.33	3.10
T x E	4	28367522	0.03	27.76	57.75	1.25	0.34
Residual	26	13209494	0.02	136.50	78.76	0.52	2.74
CV (%)	-	5.64	13.21	5.58	7.33	4.32	6.34
Mean	-	64425.45	1.06	209.30	121.14	16.67	26.15
SV	DF	NGPE	GY	TGW	DM	NCP	NCG
		MS					
T	3	1897.20	1486101.47*	738.51*	3026104.40	29.08*	35.24*
E	1	684.68	16579754.92*	329.58	10285775.40*	80.33*	271.44*
Block	6	885.77	405004.31	12.12	4498239.80	9.21	9.99
T x E	4	1134.75	43427.09	296.91	328336.50	0.73	19.32*
Residual	26	772.50	319654.00	171.03	1676686.30	7.93	3.09
CV (%)	-	6.38	8.63	4.23	14.42	12.39	9.41
Mean	-	435.47	65.525.742	308.87	89.815.721	22.73	18.690

POP: Plant population (plants ha^{-1}); EI: ear index; PH: plant height (cm); EIH: ear insertion height (cm); NGR: number of grain rows (units); NGPR: number of grains per row (units); NGPE: number of grains per ear (units); GY: grain yield (kg ha^{-1}); TGW: thousand grain weight (g); DM: total dry mass (kg ha^{-1}); NCP: total nitrogen content in the plant (g kg^{-1}); NCG: total nitrogen content in grains (g kg^{-1}); SV: source of variation; DF: degrees of freedom; CV: coefficient of variation; *significant at 5% probability by the F test.

grain weight over T1 (294.06 g), with T2, T3, and T4 recording 312.03, 313.05, and 313.07 g, respectively. Although total plant nitrogen differed significantly only for T2, the consistent gains in grain weight and, in some environments, grain N content suggests that *Bacillus licheniformis* enhances plant N economy even under the variable conditions of the second harvest. Moreover, PGPR have been widely recognized for improving plant growth, nutrient availability, and stress tolerance (Kumar et al., 2019; Bath et al., 2022; Aziz et al., 2022), helping contextualize the positive outcomes of inoculation in this study.

Linear correlation coefficients

Correlation coefficients are categorized by strength, being null (r = 0.0), weak (0.10–0.30), medium (0.31–0.60), strong (0.61–0.90), very strong (0.91–0.99), and perfect (r = 1.00) (Carvalho et al., 2004). In this study (Table 7), plant height showed a medium negative correlation with plant population and grain nitrogen (r = -0.39 and r = -0.54), a strong positive correlation with ear insertion height (r = 0.73), and medium positive correlations with total plant nitrogen, total dry mass, grain yield, and ear index (r = 0.38, 0.53, 0.47, and 0.32). This suggests that higher nitrogen allocation to vegetative growth can reduce grain nitrogen content, consistent with a previous study that reported that vegetative tissue in modern hybrids accumulates more N than grains can receive (DeBruin et al., 2017). Positive correlations of medium to strong magnitude were observed between ear insertion height, total plant nitrogen, ear index, and grain yield (r = 0.60, 0.51, 0.61), while plant population correlated negatively with ear index (r = -0.69). Plant population strongly contributed to higher grain nitrogen (r = 0.64) but moderately reduced yield (r = -0.49). All of these correlations were significant at the 5% probability level by the t-test. These findings indicate that optimizing plant arrangement can enhance nitrogen accumulation in grains, although improper management may compromise yield.

During the main harvest period, treatments differed in their influences on agronomic traits. Treatments T1 and T4 showed little association with any measured variables. T2 notably affected the number of grains per row, while T3 influenced ear index, total plant nitrogen, and total dry mass. Total nitrogen content in grains and grain yield were the most relevant variables, contributing substantially to the observed differences, whereas plant population had the least impact. In the second harvest period, T4 was associated with the number of grain rows, while T3 showed similarity with thousand grain weight, total dry mass, and total nitrogen in grains. Studies by Katsenios et al. (2022) reported that *B. mojavensis*, *B. licheniformis*, and *B. amyloliquefaciens* promoted higher biomass accumulation at 84 days after sowing (315.6, 305.7, and 298.3 g, respectively), which also improved thousand grain weight. Traits such as thousand grain weight, total nitrogen in grains, grain yield, plant population, and ear index had the greatest influence on overall performance, while number of grains per ear, plant height, and ear insertion height had moderate effects, and grain rows contributed the least.

Table 5. Breakdown of the interaction between environment and treatment in the second harvest period (Phase 2).

Treatment	NCG	
	Environment	
	São Miguel do Iguaçu – PR	Serranópolis do Iguaçu – PR
T1	15.7 bA	15.5 aA
T2	24.1 aA	16.9 aB
T3	21.9 aA	16.0 aB
T4	21.7 aA	15.6 aB

T1: Absolute control; T2: Nitrogen control; T3: Inoculation with *Bacillus licheniformis* 90 days before sowing; T4: Inoculation with *Bacillus licheniformis* on the day of sowing; NCG: total nitrogen content in grains (NCG, %). Means followed by the same lowercase letter in the column and capital letter in the row do not differ from each other at 5% probability using the Tukey test.

Table 6. Test of comparison of means for the main effects of environment and treatment in the second harvest period (Phase 2).

Environment	POP	EI	PH	EIH	NGR	GY	DM	NCP
Serranópolis do Iguaçu – PR	68312.76 a	1.13 a	219.85 a	129.18 a	16.39 b	7112.51 a	9347.85 a	23.94 a
São Miguel do Iguaçu – PR	61093.47 b	0.97 b	196.99 b	111.76 b	16.98 a	5899.32 b	8554.25 a	21.31 b
Mean	64425.45	1.06	209.3	121.14	16.67	6552.57	8981.57	22.73
Treatment		GY		TGW		NCP		
T1		6109.29 b		294.06 b		21.04 b		
T2		7000.58 a		312.03 a		25.02 a		
T3		6637.94 ab		313.05 a		22.17 ab		
T4		6294.71 b		313.07 a		22.11 ab		
Mean		6552.57		308.87		22.73		

T1: Absolute control; T2: Nitrogen control; T3: Inoculation with *Bacillus licheniformis* 90 days before sowing; T4: Inoculation with *Bacillus licheniformis* on the day of sowing; POP: plant population (plants ha^{-1}); EI: ear index; PH: plant height (cm); EIH: ear insertion height (cm); NGR: number of grain rows (units); GY: grain yield (kg ha^{-1}); DM: total dry mass (kg ha^{-1}); NCP: total nitrogen content in the plant (g kg^{-1}); TGW: thousand grain weight (g). Means followed by the same lowercase letter do not differ from each other using the Tukey test at 5% probability.

Table 7. Pearson linear correlation coefficients for both phases of the study, evaluating the agronomic efficiency and feasibility of Bacillus licheniformis in seed treatment, storage, and as a complement to N-fertilizer topdressing.

Linear Correlation	Coefficient	Linear Correlation	Coefficient
PH x EIH	0.73*	NCP x NGR	-0.52*
PH x TNP	0.38*	NCP x GY	0.51*
PH x DM	0.53*	GY x POP	-0.49*
PH x GY	0.47*	GY x TGW	0.36*
PH x EI	0.32*	GY x NGPR	0.36*
PH x NCG	-0.54*	GY x EI	0.61*
PH x POP	-0.39*	EI x POP	-0.69*
EIH x NGR	-0.34*	EI x NCG	-0.45*
EIH x NCG	-0.37*	NGPE x NGR	0.43*
EIH x EI	0.43*	NGPE x NGPR	0.76*
EIH x GY	0.60*	TNG x POP	0.64*
EIH x NCP	0.41*		

POP: Plant population (plants ha^{-1}); EI: ear index; PH: plant height (cm); EIH: ear insertion height (cm); NGR: number of grain rows (units); NGPR: number of grains per row (units); NGPE: number of grains per ear (units); GY: grain yield (kg ha^{-1}); TGW: thousand grain weight (g); DM: total dry mass (kg ha^{-1}); NCP: total nitrogen content in the plant (g kg^{-1}); NCG: total nitrogen content in grains (g kg^{-1}); *significant at 5% by *t Student* test.

The ability of rhizobacteria to enhance root system development, especially in crops with high nutrient uptake like maize, enables greater agronomic efficiency of nitrogen use, highlighting the effectiveness and feasibility of the practice.

Materials and Methods

Meteorological data

During the main harvest period, temperatures were generally favorable for crop establishment, growth, and grain filling (Figure 1), with only brief periods below the optimal range. Lapa-PR experienced milder conditions than Candói-PR, particularly during early growth. In the second harvest period, mean temperatures ranged from 20 to 24 ºC in Serranópolis do Iguaçu-PR and 20 to 26 ºC in São Miguel do Iguaçu-PR. The highest temperatures coincided with topdressing nitrogen application, potentially reducing its efficiency. The accumulated precipitation ensured sufficient soil moisture for good maize establishment, with totals above 100 mm during the first harvest and nearly 200 mm in the second. However, high temperatures combined with rainfall at the time of nitrogen topdressing may have caused nutrient losses. Flowering and anthesis occurred during periods of low rainfall, supporting pollination, while grain filling benefited from mild temperatures and adequate precipitation. Consequently, despite potential top-dressing losses, maize maintained high yield potential.

Experimental design, seasons and treatments

The experiments were carried out during the main harvest period (Phase 1) in Candói – PR (25º 31' 57” S, 51º 48' 00” W, 920 m altitude) and Lapa – PR (25º 50' 52” S, 49º 39' 8” W, 855 m altitude), and during the second harvest period (Phase 2) in Serranópolis do Iguaçu – PR (25º 23' 08” S, 53º 58' 38” W, 325 m altitude) and São Miguel do Iguaçu – PR (25º 22' 28” S, 54º 12' 55” W, 321 m altitude). In Phase 1, trials were conducted in Candói from 15 September 2017 to 20 February 2018, and in Lapa from 15 October 2017 to 20 March 2018. The second-season experiments (Phase 2) were established later, with evaluations conducted in Serranópolis do Iguaçu from 10 February 2018 to 20 July 2018, and in São Miguel do Iguaçu over the same period. These timeframes represent the typical main and second growing seasons for maize in the region. According to Köppen climate caracterization, Candói – PR and Lapa – PR have a Cfb climate (temperate with mild summers), whereas Serranópolis do Iguaçu – PR and São Miguel do Iguaçu – PR present a Cfa climate (humid subtropical) (Alvares et al., 2014). The soil’s physical and chemical characteristics are presented in Supplementary Table 1.

In both experimental phases, a randomized block design with a 4 x 2 factorial arrangement (four treatments x two environments) and four replications was employed. For the first phase, the treatments were: T1 – Absolute control (No inoculation; Without application of N-fertilizer in top dressing); T2 – Nitrogen control (100% N-fertilizer at sowing base + 200 kg ha^{-1} of N-fertilizer in top dressing); T3 – Inoculation with *Azospirillum brasilense* 90 days before sowing (*Azospirillum brasilense* + 75% of the recommended nitrogen [120 kg ha^{-1} of N-fertilizer in top dressing]); T4 – Inoculation with *Bacillus licheniformis* on the day of sowing (*Bacillus licheniformis* + 75% of the recommended nitrogen [120 kg ha^{-1} of N-fertilizer in top dressing]). For the second phase, the treatments were: T1 – Absolute control (No inoculation; Without application of N-fertilizer in top dressing); T2 – Nitrogen control (100% N-fertilizer at sowing base + 160 kg ha^{-1} of N-fertilizer in top dressing); T3 – Inoculation with *Bacillus licheniformis* 90 days before sowing (*Bacillus licheniformis* + 75% of the recommended nitrogen [120 kg ha^{-1} of N-fertilizer in top dressing]); T4 – Inoculation with *Bacillus licheniformis* on the day of sowing (*Bacillus licheniformis* + 75% of the recommended nitrogen [120 kg ha^{-1} of N-fertilizer in top dressing]). Each experimental unit consisted of 8 rows, 0.50 m apart, with 6.0 m length. For the main harvest period, sowing occurred in late September in Candói-PR and late October in Lapa-PR, using the hybrid P30F53VYH at a target population of 75000 plants ha^{-1}. In the second harvest period, sowing took place in late February at both locations, using the hybrid STATUS VIP3 T4F with a target population of 60 thousand plants per ha.

Inoculant and plant materials

In the base fertilization, 40 kg ha^{-1} of P_2O_5 and 40 kg ha^{-1} of K_2O were applied, without nitrogen. Phytosanitary management was performed preventively to avoid biotic interference. Topdressing N-fertilizer was applied once at the V_4 stage (Ritchie et al., 1993), according to the dosages for each treatment. The *Bacillus licheniformis* inoculant (strain CCTB 07 = CNPSo 3204) was a pre-commercial product called BTP 060-19 with $1x10^8$ CFU mL^{-1}. Inoculation with *Azospirillum brasilense* used the commercial product AzoTotal, containing strains Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084) at $2x10^8$ CFU mL^{-1}. Both bacteria were applied as seed treatment at 150 mL per 60 thousand seeds. Seeds inoculated with *Bacillus licheniformis* 90 days before sowing were stored at 15 ºC to maintain germination and vigor. All seeds received 35 mL of the fungicide Maxim (Thiamethoxam 350 g L^{-1}) per 60 thousand seeds.

Measured variables

The variables assessed in this study are described as follows. Plant population (POP, plants ha^{-1}) was determined by counting plants in five linear meters, and the ear index (EI) was calculated as the ratio of ears to plants per linear meter. Plant height (PH, cm) and ear insertion height (EIH, cm) were measured directly using a graduated ruler. The number of grain rows (NGR, units), grains per row (NGPR, units), and grains per ear (NGPE, units) were obtained as the average from ten ears per treatment. Grain yield (GY, kg ha^{-1}) was calculated from harvesting the four central rows of each experimental unit, followed by threshing, weighing, and correcting grain moisture to 13%. A subsample was used to determine thousand grain weight (TGW, g). Total dry mass (DM, kg ha^{-1}) was obtained by sampling five plants from the useful plot area at stage R1 (Ritchie et al., 1993) and drying them in a forced-air oven at 65 ºC for 72 hours. Total nitrogen content in plant (NCP) and grains (NCG) was determined according to Silva (2009), with results expressed in g kg^{-1}. To complement the analysis, satellite meteorological data on mean air temperature (ºC) and precipitation (mm) were obtained using the NASA POWER tool (NASA POWER, 2023).

Statistical analysis

The collected data were first analyzed for outliers and then checked for statistical assumptions, including normality of errors, homogeneity of variances, and independence of errors, using the Shapiro-Wilk, Bartlett, and Durbin-Watson tests, respectively. Once the assumptions were satisfied, analysis of variance (ANOVA) was performed to evaluate the effects of treatments, environments, and their interaction on the measured variables at 5% probability ($p < 0.05$). For variables showing significant effects, simple and main effects were further analyzed using Tukey's mean comparison test at the same probability level. Additionally, linear correlations were calculated to assess the strength and direction of relationships among variables. All analyses were conducted using R software (R Core Team, 2023) with the AgroR version 1.3.7 (Shimizu et al., 2023) and metan version 1.18.0 (Olivoto and Lúcio, 2020) packages.

Conclusion

Inoculation with *Bacillus licheniformis* showed agronomic efficiency comparable to nitrogen control and *Azospirillum brasilense* inoculation during the main harvest period. Furthermore, inoculating with *Bacillus licheniformis* 90 days before sowing can reduce N-fertilizer application by 25% in the second harvest period without negatively affecting maize grain yield.

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