Australian Journal of Crop Science AJCS

Aust J Crop Sci. 19(12):1164-1169 (2025) | https://doi.org/10.21475/ajcs.25.19.12.p77 ISSN:1835-2707

Impact of *Nitrospirillum amazonense* inoculation on the competitiveness of sugarcane pre-sprouted seedlings

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Submitted: 16/07/2025

Revised: 06/09/2025

Accepted: 16/10/2025

Abstract: The pursuit of sustainability in the sugarcane-energy sector has intensified interest in bacterial inoculants. Among these, *Nitrospirillum amazonense* has been identified as a promising agent for enhancing crop competitive ability against weeds. This study evaluated the influence of *N. amazonense* inoculation on pre-sprouted sugarcane seedlings (var. RB966928) under competition with three major weed species: *Merremia aegyptia*, *Urochloa decumbens*, and *Cyperus rotundus*. For each weed, a completely randomized 4 × 2 factorial design was employed, with the following factors: (i) four weed densities (10, 20, 40, and 80 plants·m^{-2}) and (ii) the presence or absence of *N. amazonense* inoculation. Growth parameters (height, leaf area, shoot dry mass, and root dry mass) were assessed 90 days after transplanting. Inoculation significantly increased seedling height only when the plants were in competition with *U. decumbens*. Conversely, for *M. aegyptia*, inoculation intensified the negative competitive effects, as evidenced by a progressive decrease in leaf area with increasing weed density. No significant effects were detected under competition with *C. rotundus*. Although inoculation did not offset the adverse effects of weed competition, the findings underscore critical crop–weed–microbe interactions. The differential responses observed among weed species indicate that the effectiveness of *N. amazonense* may be context-dependent, highlighting the need for further investigation to refine inoculation methodologies or integrate them with complementary practices to fully realize its potential within integrated crop management.

Keywords: Plant growth-promoting rhizobacteria; Plant-microbiota interactions; *Saccharum officinarum*; Sustainable management; Weed competition.
Abbreviations: DAP_days after planting; DAT_days after transplanting; PGPB_plant growth-promoting bacteria; PSS_ pre-sprouted seedlings.

Introduction

Sugarcane (*Saccharum officinarum* L.) is one of the most important crops worldwide and serves as a key raw material for sugar, ethanol, and various bioproducts, such as bioelectricity, biogas, and bioplastics (Zhang et al., 2025). With the growing global demand for sustainable agricultural practices, strategies that increase productivity in the sugarcane-energy sector while reducing dependence on chemical inputs are urgently needed (Huang, 2024).

In this context, plant growth-promoting bacteria (PGPB) have gained attention as promising alternatives to synthetic fertilizers, offering the potential to reduce environmental impacts and align with low-carbon agriculture principles. Among these, *Nitrospirillum amazonense* is a notable rhizobacterium that provides physiological benefits to sugarcane, including enhanced bud sprouting, modified root architecture, increased tillering, and increased leaf area—factors that may contribute to up to an 18% increase in stalk yield (EMBRAPA, 2018). Field trials have further demonstrated the agronomic potential of *N. amazonense*. Inoculation with a precommercial product containing this bacterium increased the stalk yield by 27.5 t ha^{-1} (20%) and the sugar yield by 4.6 t ha^{-1} (25%) in sandy soils under low nitrogen fertilization without significantly altering the nutrient content in leaves or stems. These results reinforce the role of *N. amazonense* in increasing sugarcane productivity while reducing the environmental impact of cropping systems (Sica et al., 2020). The strain *N. amazonense* CBAmC has been shown to preferentially associate with the surface of sugarcane root tissues propagated by single-node cuttings, rather than colonizing internal plant tissues or persisting in the soil (Schwab et al., 2023).

Despite these benefits, sugarcane performance under field conditions is limited by weed interference—a major biotic constraint that can severely limit productivity (Martinelli et al., 2019). Species such as *Cyperus rotundus*, *Merremia aegyptia*, and *Urochloa decumbens* are prevalent in sugarcane agroecosystems and act as aggressive competitors for water, light, and nutrients (Azania, 2018). For pre-sprouted seedling (PSS) systems, recent studies have defined critical weed management

Table 1. Chemical and physical analysis of soil samples used in the experiment.

Chemical Analysis									
P	**M.O**	**pH**	**K**	**Ca**	**Mg**	**H+Al**	**SB**	**CEC**	**V**
mg/dm³	**g/dm³**	**Ca Cl_2**	**mol_c/dm³**			**%**	**mmolc.dm^{-3}**		**%**
11	22	4.8	1.1	13	9	33	23	56	41
Micronutrients					Physical Analysis				
B	**Cu**	**Fe**	**Mn**	**Zn**	**Total Sand**	**Clay**	**Silt**	**Textural Classification**	
mg.dm^{-3}(ppm)						**g.dm^{-3}**		Clayey	

thresholds. For example, with *Cyperus rotundus*, the period prior to interference is approximately 26 days after planting (DAP), whereas the critical period for weed control falls between 26 and 68 DAP. Within this window, each day of weed coexistence can reduce yield by up to 8.22%, underscoring the high cost of delayed management (Giraldeli et al., 2022).
The competitive pressure from specific weeds is substantial. Interference from *Ipomoea* spp. and *Merremia* spp. can reduce sugarcane stalk yield by 27% to 36% (Bhullar et al., 2012). Similarly, *Urochloa decumbens* competes intensely, negatively impacting all the evaluated sugarcane growth parameters. In one study, 120 days of coexistence with *U. decumbens* resulted in a total loss (100%) of sugarcane plants, highlighting the aggressive nature of this species and the necessity of early and effective control measures (Amaral et al., 2018).
Crop–weed competition is a complex, bidirectional process in which the growth and development of each plant are mutually influenced (Massenssini et al., 2014). The outcome of these interactions depends on species-specific traits, resource availability, and each plant's ability to establish an effective root system (Galon et al., 2012). Therefore, practices that enhance crop root development may confer a competitive advantage against weeds. In this context, PGPB inoculation has emerged as a promising strategy to modulate these competitive dynamics by strengthening plant–microbiome symbiosis and improving root architecture, thereby potentially enhancing the competitive ability of crops during early growth stages (Matos, 2017).
While the role of plant–microbe interactions in promoting crop growth is well documented, their influence on crop–weed competition remains poorly understood—a critical knowledge gap in agricultural science.
Therefore, this study was designed to evaluate the effect of *N. amazonense* inoculation on the competitive ability of sugarcane pre-sprouted seedlings (PSSs) against three economically important weed species. Employing an additive experimental design (Radosevich, 1987), we assessed how increasing weed density affects early PSS development under controlled conditions. This research aims to provide a deeper understanding of crop–weed–microbiota interactions, which is essential for developing more sustainable and integrated weed management strategies.

Results

Interaction with Urochloa decumbens
For sugarcane in competition with *Urochloa decumbens*, inoculation with *N. amazonense* and weed density independently influenced growth parameters, as no significant interaction was detected between these factors ($p \geq 0.05$; Table S1).
Specifically, inoculation significantly increased plant height ($p \leq 0.0261$) from 79.55 cm in noninoculated plants to 83.30 cm in inoculated plants. However, it did not significantly alter fresh shoot biomass, leaf area, or root dry biomass (Table 2).
Increasing weed density significantly reduced plant height ($p \leq 0.0113$), fresh shoot biomass ($p \leq 0.0074$), and leaf area ($p \leq 0.0001$). The most pronounced effects occurred at the highest density (80 plants m^{-2}), which significantly suppressed these variables compared with the weed-free control. Root dry biomass was not significantly affected by weed density ($p \geq 0.1604$; Table 3).

Interaction with Merremia aegyptia
When competing with *M. aegyptia*, a significant interaction effect on leaf area was detected between inoculation and weed density ($p \leq 0.0078$). Weed density also had a main effect on leaf area ($p \leq 0.001$) and root dry biomass ($p \leq 0.0323$). Inoculation alone did not have a significant effect on any variable (Table S2).
The interaction revealed that the positive effect of *N. amazonense* on leaf area was evident only in the absence of weed competition (924.69 cm^2 in inoculated plants vs. 711.29 cm^2 in noninoculated plants). This benefit was progressively negated as weed density increased, with the leaf area of inoculated plants declining sharply at relatively high densities (Table 5). In contrast, the leaf area of uninoculated plants remained relatively stable across the different densities. Neither inoculation nor weed density significantly affected plant height nor fresh shoot biomass (Tables 4 and 5).

Interaction with Cyperus rotundus
In the presence of *C. rotundus*, only weed density had a significant effect, reducing fresh shoot biomass ($p \leq 0.0147$) and leaf area ($p \leq 0.0030$). No significant effects of inoculation or an interaction between the factors were observed for any growth parameter (Table S3).
N. amazonense inoculation did not lead to significant differences in any of the measured variables (Table 6). Conversely, increasing *C. rotundus* density suppressed sugarcane aerial development, with significant reductions in fresh shoot biomass and leaf area at the highest density (80 plants m^{-2}) compared with those of the control (Table 7).

Table 2. Height (cm), shoot biomass (g), leaf area (cm^2), and root dry biomass (g) of sugarcane pre-sprouted seedlings (PSS) under competition with *Urochloa decumbens* as a function of inoculation with Aprinza (*Nitrospirillum amazonense*).

Treatment	Height (cm)	Shoot Biomass (g)	Leaf Area (cm^2)	Root Biomass (g)
With Inoculation	83.30 a	154.08 a	669.16 a	46.69 a
Without Inoculation	79.55 b	138.20 a	571.80 a	42.75 a
CV (%)	11.24	17.92	22.48	29.80

*Tukey's test applied to rows (lowercase letters). Means followed by the same letter do not differ significantly at the 5% significance level.

Table 3. Height (cm), Aerial Biomass (g), Leaf Area (cm^2), and Root Dry Biomass (g) of sugarcane PSS under competition with *U. decumbens* plant density.

Plant Density (plants/m^2)	Height (cm)	Shoot Biomass (g)	Leaf Area (cm^2)	Root Biomass (g)
0	86.00 a	154.65 a	817.99 a	37.99 a
10	90.00 a	160.46 a	726.43 ab	38.89 a
20	87.38 a	157.53 a	649.71 ab	51.82 a
40	82.50 ab	141.71 ab	544.65 bc	40.87 a
80	73.75 b	116.34 b	363.62 c	54.07 a
CV (%)	10.48	16.54	20.73	35.82

*Tukey's test applied to rows (lowercase letters). Means followed by the same letter do not differ significantly at the 5% significance level.

Discussion

This study revealed that inoculation with *N. amazonense* did not enhance the early development of pre-sprouted sugarcane seedlings (PSPs) or their competitive ability against the weed species *C. rotundus*, *M. aegyptia*, and *U. decumbens*. Although sporadic increases in plant height and leaf area were observed, these increases were insufficient to counteract the suppressive effects of weed interference, particularly at high densities.

This lack of a positive response aligns with previous research. Ferreira and Magri (2021) reported no significant benefits of *N. amazonense* inoculation on PSS growth. Similar outcomes have been documented for related diazotrophic bacteria. For example, Gonçalves et al. (2020) reported negative impacts of *Azospirillum brasilense* on PSS, including reduced biomass accumulation. Furthermore, Matos et al. (2024) reported that *A. brasilense* inoculation not only failed to improve maize performance under weed competition but also, in some cases, intensified the adverse effects of interference from *Ipomoea nil* and *C. rotundus*.

The variable efficacy of inoculation can be attributed to a complex interplay of biotic and abiotic factors. While the *N. amazonense* CBAmC strain is adapted to the acidic soil conditions of Brazilian agroecosystems (Paiva et al., 2024), the successful establishment of exogenous microorganisms is often limited by competition with native soil microbiota and environmental constraints such as pH, moisture, and nutrient availability (Veen et al., 1997). This interpretation is corroborated by Oliveira et al. (2006), who documented variable responses to *N. amazonense* across different sugarcane genotypes, underscoring the critical influence of genotype–strain–environment interactions.

Furthermore, the propagation method and developmental stage of the host plant are critical determinants of inoculation success. The literature predominantly reports the growth-promoting effects of *N. amazonense* in sugarcane propagated traditionally via stalks (EMBRAPA, 2018; Reis et al., 2020; Sica et al., 2020; Mascarenhas, 2021). In contrast, research on PSS, which possesses a more developed root system at planting, is limited. The distinct physiology, root exudate profile, and rhizosphere environment of PSS compared with those of newly developing roots from stalks likely influence microbial colonization (Lopes et al., 2021; Matoso et al., 2021). The importance of the inoculation technique is further highlighted by Schwab et al. (2023), who demonstrated superior colonization efficiency when inoculation occurred via stalk immersion compared with other methods.

The nuanced effects of inoculation were also highly dependent on the competing weed species. In combination with *U. decumbens*, inoculation promoted an increase in sugarcane height without a corresponding increase in biomass, suggesting a potential shift in resource allocation rather than a true competitive advantage. In the presence of *M. aegyptia*, inoculation appeared to intensify competition, possibly due to rhizospheric interactions where root proximity inadvertently favors weed resource acquisition (Fialho et al., 2016), a phenomenon previously observed in maize-weed systems (Fialho, 2013). Conversely, against the highly competitive *C. rotundus*, inoculation had no discernible effect, which was likely overwhelmed by the weed's formidable root system and superior resource capture efficiency (Azania, 2018).

Although this study did not find immediate benefits, the efficacy of inoculation should not be entirely dismissed. Potential long-term effects may become evident at later growth stages, allowing for an adaptation period for the establishment of plant–microbe symbiosis (Ferreira and Magri, 2021). Future research should explore synergistic effects by integrating inoculation with other agronomic practices, such as optimized fertilization or integrated weed management strategies (Oliveira et al., 2006). Ultimately, while *N. amazonense* did not mitigate weed competition under the conditions tested, microbial inoculation remains a valuable area of investigation. Further studies are essential to unravel the complex

Table 4. Height (cm), shoot biomass (g), leaf area (cm^2), and root dry biomass (g) of sugarcane pre-sprouted seedlings (PSS) under competition with *Merremia aegyptia* as a function of inoculation with Aprinza (*Nitrospirillum amazonense*).

Treatment	Height (cm)	Shoot Biomass (g)	Leaf Area (cm^2)	Root Biomass (g)
With Inoculation	80.25 a	138.08 a	924.69 a	40.36 a
Without Inoculation	76.95 a	132.05 a	711.29 b	42.17 a
CV (%)	12.36	21.02	21.11	19.41

*Tukey's test applied to rows (lowercase letters). Means followed by the same letter do not differ significantly at the 5% significance level.

Table 5. Height (cm), Aerial Biomass (g), Leaf Area (cm^2), and Root Dry Biomass (g) of sugarcane PSS under competition with *Merremia aegyptia* plant density.

Plant Density (plants/m^2)	Height (cm)	Shoot Biomass (g)	Leaf Area (cm^2)		Root Biomass (g)
			I	W.I	
0	86.00 a	154.65 a	924.69 Aa	711.29 Ba	37.99 ab
10	82.00 a	145.09 a	646.63 Aabc	555.42 Aa	29.14 b
20	68.88 a	118.07 a	440.76 Bbc	687.18 Aa	40.56 ab
40	77.63 a	128.48 a	705.28 Aab	449.89 Ba	41.79 ab
80	78.50 a	129.04 a	370.27 Ac	449.49 Aa	56.84 a
CV (%)	15.42	25.70	23.61		38.64

*I: With inoculation; W.I: Without inoculation. Tukey test in rows (lowercase letters) and columns (uppercase letters). Means followed by the same letter do not differ significantly at 5% significance level.

Table 6. Height (cm), shoot biomass (g), leaf area (cm^2), and root dry biomass (g) of sugarcane pre-sprouted seedlings (PSS) under competition with *Cyperus rotundus* as a function of inoculation with Aprinza (*Nitrospirillum amazonense*).

Treatment	Height (cm)	Shoot Biomass (g)	Leaf Area (cm^2)	Root Biomass (g)
With Inoculation	82.05 a	143.30 a	685.42 a	62.20 a
Without Inoculation	76.15 a	130.38 a	590.25 a	53.67 a
CV (%)	18.59	36.73	21.03	64.49

* Tukey's test applied to rows (lowercase letters). Means followed by the same letter do not differ significantly at the 5% significance level.

Table 7. Height (cm), Aerial Biomass (g), Leaf Area (cm^2), and Root Dry Biomass (g) of sugarcane PSS under competition with *Cyperus rotundus* plant density.

Plant Density (plants/m^2)	Height (cm)	Shoot Biomass (g)	Leaf Area (cm^2)	Root Biomass (g)
0	86.00 a	154.65 a	817.99 a	37.99 a
10	77.38 a	135.46 ab	659.92 ab	59.79 a
20	80.75 a	145.67 a	628.15 ab	66.24 a
40	80.13 a	140.93 ab	585.58 b	61.33 a
80	71.25 a	107.99 b	497.54 b	64.33 a
CV (%)	12.43	18.56	22.48	36.03

*Tukey's test applied to rows (lowercase letters). Means followed by the same letter do not differ significantly at the 5% significance level.

tripartite interactions among crops, weeds, and microbiota, particularly under diverse field conditions, to refine this sustainable technology.

Materials and Methods

Plant material and growth conditions

The study was conducted under greenhouse conditions at the Center for Agricultural Sciences of the Federal University of São Carlos (UFSCar), Araras, São Paulo, Brazil. The experimental units consisted of 30-L polyethylene pots filled with a dystrophic Red Latosol (Oxisol) collected from the topsoil (0–20 cm depth) of a native forest area with no prior history of cultivation or pesticide application. The soil chemical and physical characteristics were determined following the methodology of Raij et al. (2001) and are presented in Table 1. Pre-sprouted seedlings (PSS) of the sugarcane cultivar 'RB966928', produced via bud-chip technology as described by Landell et al. (2012), were used. The weed species selected for the study were *Merremia aegyptia, Urochloa decumbens*, and *Cyperus rotundus*. Seeds were procured from Agrocosmos (São Paulo, Brazil), a commercial supplier of research-grade weed seeds.

Experimental design and treatments

Three separate experiments were conducted, one for each weed species. Each experiment was arranged in a completely randomized design (CRD) with a 4 × 2 factorial scheme and four replications. The first factor was weed density at four levels: 1, 2, 4, and 8 plant $pots^{-1}$ (equivalent to 10, 20, 40, and 80 plants m^{-2}, respectively). The second factor was the inoculation status of sugarcane PSS: inoculated with *Nitrospirillum amazonense* or noninoculated. Two additional control treatments (weed-free controls) were included for comparison: inoculated PSS grown without weeds and noninoculated PSS grown without weeds.

Inoculation and crop establishment

Sixty days after sprouting, sugarcane PSSs were transplanted into the pots. Inoculation was performed immediately prior to transplanting by immersing the PSS root systems for five minutes in a commercial solution of Aprinza® containing *N. amazonense* strain BR11145 (1×10^8 CFU mL^{-1}). On the same day, weed seeds were sown at a depth of 1 cm. The seeding rates were calculated to exceed the target densities, and following emergence, the seedlings were thinned to establish the final desired plant density per pot. Any nontarget weeds were manually removed throughout the experiment. Pots were irrigated daily via a sprinkler system to maintain soil moisture near field capacity.

Data collection and statistical analysis

At 90 days after transplanting (DAT), the experiment was terminated, and sugarcane plants were harvested to assess growth parameters. The following variables were measured: plant height (from the soil surface to the insertion point of the uppermost leaf with a visible dewlap, cm); total green leaf area (cm^2), determined via a portable leaf area meter (LI-3000C, LI-COR Biosciences, Lincoln, NE, USA); shoot fresh biomass (g), measured after the plants were cut at the soil level; and root dry biomass (g), obtained after the root system was carefully washed from the soil and dried in a forced-air oven at 65 °C for 72 hours or until a constant weight was achieved.

Data from each experiment were analyzed separately. All the data were first checked for normality of residuals (Shapiro–Wilk test) and homogeneity of variances (Bartlett's test). The data were subsequently subjected to two-way analysis of variance (ANOVA). When the F test indicated a significant effect ($p \leq 0.05$), treatment means were compared via Tukey's honestly significant difference (HSD) test ($\alpha \leq 0.05$). All the statistical procedures were performed in R via the RStudio interface.

Conclusion

Inoculation with *Nitrospirillum amazonense* did not enhance the early growth of pre-sprouted sugarcane seedlings (variety RB966928) or improve their competitive ability against *Cyperus rotundus*, *Merremia aegyptia*, and *Urochloa decumbens*. The adverse effects of weed competition, particularly at high densities, were the dominant factor limiting seedling development, leading to significant reductions in height, shoot dry mass, and leaf area. While microbial inoculation remains a promising technology, our findings indicate that its standalone application under these conditions is insufficient. Effective implementation in pre-sprouted sugarcane seedling systems will likely require methodological adjustments or integration with complementary weed management strategies.

Acknowledgements

The authors are grateful to the São Paulo Research Foundation (FAPESP) for granting a scholarship to the first author (Process No. 2022/08100-3). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Authors' Contributions

All authors contributed to the study's conception and design. PAM and MMRM was responsible for Project Administration, Supervision and Resource Provision. Material preparation, data collection, and analysis were performed by LCGJ and CFF. The first draft of the manuscript was written by LCGJ, and all authors critically reviewed and edited previous versions. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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