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Nutrient availability and microbial activity in a potato field: Impacts of *Azospirillum* inoculation and nitrogen levels under semi-arid conditions

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Abstract

Inoculation with *Azospirillum* spp. is commonly used to improve rhizosphere functioning. Potato growth and productivity may be strongly affected by *Azospirillum* inoculation and nitrogen application under semi-arid conditions. The field experiment was carried out during the spring season of 2025 at an agricultural research site located in the Al-Mashroua region, Babil Governorate, Iraq.The experiment aimed to evaluate the effects of nitrogen-fixing *Azospirillum* spp. under different nitrogen levels on soil nutrient availability, soil microbial activity, and the growth and yield of potato. The Dutch cultivar Burren was grown in silty loam soil. The study included two factors. The first factor was *Azospirillum* inoculation applied as a liquid bacterial suspension at three rates (0, 10, and 20 mL plant^{-1}, corresponding to 0, 9×10^{10}, and 1.8×10^{11} CFU plant^{-1} based on an inoculum density of 9×10^{9} CFU mL^{-1}), coded as a0, a1, and a2, respectively. The second factor was nitrogen fertilization applied at four levels (0, 100, 200, and 300 kg N ha^{-1}), coded as n0, n1, n2, and n3, respectively. The experiment was arranged in a randomized complete block design (RCBD) with three replications, resulting in 36 experimental units. Soil samples were collected at emergence and harvest to assess biological parameters, including *Azospirillum* population density. The interaction treatment a2n3 recorded the highest bacterial counts at both stages, reaching 2.436 and 2.862×10^{9} CFU g^{-1} dry soil, respectively. *Azospirillum* spp. inoculation, nitrogen level, and their interaction were associated with increased cumulative microbial respiration, reaching a maximum of 7.40 mg CO_2 g^{-1} dry soil under a2n3. Furthermore, *Azospirillum* counts varied over time and were higher at harvest than after emergence. Nitrogen fertilization at the n3 level significantly increased soil nutrient concentrations, which reached 48.171 mg N kg^{-1} soil, 26.65 mg P kg^{-1} soil, and 216.28 mg K kg^{-1} soil. *Azospirillum* spp. inoculation combined with nitrogen was associated with increased measured nutrient availability and improved most potato growth traits. The highest total tuber yield was obtained under the a2n3 treatment (47.48 Mg ha^{-1}), which did not differ significantly from the a2n2 treatment (46.85 Mg ha^{-1}). We conclude that *Azospirillum* inoculation rate and nitrogen level served as important determinants under the conditions of this experiment in improving soil biological properties and potato yield under semi-arid conditions, with a2n2 representing a more balanced and efficient application option.

Key words: *Azospirillum*, potato, nitrogen, semi-arid conditions, microbial activity

Introduction

Biofertilization has been widely studied as an alternative or complementary measure to mineral fertilization (Al-Maliki *et al*., 2020a,b). The approach is based on the use of beneficial living microorganisms as fertilizers by direct application of microbial inoculants or by addition of cultured microorganisms to soil, seeds such as barley or vegetative propagules such as potato tubers. The genus *Azospirillum spp.* that are closely associated with the roots of gramineous plants have been widely used as bacterial inoculants (Al-Maliki *et al*., 2022). Furthermore, mycorrhizal fungi inoculation significantly increases available soil N, P, and K (Debnath *et al.,* 2024).

These bacteria stimulate microbial activity and contribute to part of nitrogen requirement of the plants (Al-Maliki and Al-Shamary, 2022). Also, *Azospirillum* is directly involved in biological nitrogen fixation by its association with host plants. Nitrogen is a fundamental component of nucleic acids (RNA and DNA), enzymes and cofactors, chlorophyll, respiratory cytochromes and a multitude of metabolic processes involving enzymatic reactions and photosynthesis (Marschner, 2012).

Recent field research in potato has also shown that optimised nitrogen and potassium management can improve nutrient uptake, plant growth and tuber yield while reducing dependence on high rates of conventional fertilizers (Vishwanatha *et al*., 2025). High temperature and salinity stress in Iraqi soils rich in calcium carbonate content may lead to considerable ammonia volatilisation and nitrogen loss by leaching by irrigation water below the root zone in the case of nitrogen fertilizers especially urea. *Azospirillum* genus has been extensively studied as plant growth promoting bacteria. The positive effects of inoculating plants with *Azospirillum* have been attributed to the fixation of atmospheric nitrogen and to the production of phytohormones, among them the auxins. These bacteria also can enhance plant tolerance to abiotic and biotic stresses. Likewise, integrated nutrient management involving fermented organic inputs along with reduced mineral nutrient rates has been reported to enhance the rhizosphere microbial activity, soil nutrient status, nutrient uptake and yield in vegetable crops (Sharma *et al*., 2025).

This study evaluated the effects of *Azospirillum* spp. inoculation combined with graded nitrogen levels on rhizosphere microbial

activity, soil nutrient availability, and potato growth and yield under semi-arid conditions.

Materials and methods

The field experiment was conducted during the spring growing season of 2025 in a privately owned agricultural field in the Al-Mashroua region of Babil Governorate, approximately 40 km north of Al-Hilla. The site is located at 44.5° E longitude and 32.7° N latitude. Physical and chemical analysis of the initial soil (0–30 cm depth) indicated that the soil texture is classified as silty loam according to the USDA soil textural classification system (300 g kg^{-1} sand, 608 g kg^{-1} silt, and 92 g kg^{-1} clay). Before land preparation, 15 random soil cores were collected across the experimental field at a depth of 0–30 cm. A single representative composite sample was prepared by thoroughly mixing the subsamples, followed by air-drying, grinding, and sieving through a 2-mm mesh to determine the baseline physical, chemical, and biological soil properties.

Table 1. Physical, chemical, and biological properties of the baseline experimental soil (0-30 cm depth)

Property	Value	Unit
Electrical Conductivity (Ece)	3.45	dS m^{-1}
Soil pH	7.7	-
Organic Matter	0.50	%
Nitrogen (N)	22.4	mg kg^{-1} soil
Phosphorus (P)	7.22	
Potassium (K)	85.7	
Sand	300	g kg^{-1} soil
Silt	608	
Clay	92	
Texture	Silty loam	-
Total Bacteria	2.6×10^{9}	CFU g^{-1} dry soil
Total Fungi	1.3×10^{3}	
Nitrogen-Fixing Bacteria	0.6×10^{5}	

Field experiment description: The field experiment was established on 1 February 2025 following a winter fallow season on land with no history of solanaceous crop cultivation for the preceding three years. Certified Elite-class seed tubers (cv. Burren, size 35–55 mm) were obtained from the Iraqi Ministry of Agriculture seed certification program. The total field site spanned 861 m^2 (41 m × 21 m), comprising 225 m^2 of active experimental plots (36 plots × 6.25 m^2) alongside 636 m^2 dedicated to 1.0 m buffers between adjacent plots, 2.0 m main alleys between blocks, 3.0 m outer protective border strips, and irrigation manifold channels. Primary tillage consisted of mouldboard plowing to a depth of 25 cm in two perpendicular passes, followed by disking and levelling

The field was partitioned into three blocks (replications) arranged in an RCBD, each containing 12 experimental units (2.5 m × 2.5 m = 6.25 m^2). Each plot contained three ridges spaced 0.75 m apart and 2.5 m long, with tubers planted at a depth of 10 cm and an intra-row spacing of 0.25 m (10 tubers per ridge, yielding 30 plants per plot; equivalent to 64,000 plants ha^{-1}). To eliminate edge effects, only the central ridge (1.875 m^2), excluding border plants, was used for plant sampling and total tuber yield determinations. Drip irrigation was supplied every 2–3 days based on 100% crop evapotranspiration (ETc), delivering a total seasonal irrigation depth of 460 mm. Irrigation water had a mean salinity (ECw) of 1.32 dS m^{-1} and pH of 7.45. Weeds were managed manually, while late blight (Phytophthora infestans) and aphids were controlled preventatively using copper hydroxide (2.0 g L^{-1}) and imidacloprid (0.5 mL L^{-1}), respectively, applied as needed. The experiment was arranged as a two-factor randomized complete block design (RCBD). A local isolate of *Azospirillum* spp. was cultured in liquid Nitrogen-Free Malate (NFb) medium at 30 °C for 48 h with shaking at 150 rpm to reach the stationary growth phase, with the culture adjusted to a final viable concentration of 9×10^9 CFU mL^{-1}. Bio-inoculation was performed in two sequential stages: prior to planting, pre-germinated seed tubers (cv. Burren) were submerged for 15 min in the bacterial suspension, whereas control tubers (a_0) were submerged for 15 min in sterile, uninoculated NFb broth; subsequently, at 100% plant emergence (14 days post-planting), liquid inoculum was applied directly to the rhizosphere soil around the plant base at three volume rates of 0 mL $plant^{-1}$ (a_0, receiving 20 mL $plant^{-1}$ of sterile uninoculated broth), 10 mL $plant^{-1}$ (a_1, delivering 9×10^{10} CFU $plant^{-1}$), and 20 mL $plant^{-1}$ (a_2, delivering 1.8×10^{11} CFU $plant^{-1}$). The second factor comprised four nitrogen fertilization levels (0, 100, 200, and 300 kg N ha^{-1}, designated n_0, n_1, n_2, and n_3, respectively). Nitrogen was supplied as urea (46% N) with rates calculated on an actual elemental N basis. To optimize uptake and minimize losses, nitrogen was applied in two equal split doses: 50% at planting and 50% at the tuber initiation stage (30 days post-emergence) via side-dressing along the ridges followed by drip irrigation. To avoid non-target nutrient deficiencies, a uniform blanket application of phosphorus (100 kg P_2O_5 ha^{-1} as triple superphosphate, 46% P_2O_5) was incorporated into the soil during land preparation, and potassium (200 kg K_2O ha^{-1} as potassium sulfate, 50% K_2O) was split equally between planting and tuber initiation across all experimental units. Thus, the experiment comprised 36 experimental units (3 blocks × 12 units). The Burren potato cultivar has demonstrated tolerance to salinity stress and its adverse effects on growth, making it suitable for cultivation in saline and semi-arid environments.

Biological activity indicators: Post-treatment rhizosphere soil samples were collected at two growth stages (7 days post-inoculation and at crop harvest). Within each plot, five plants were randomly excavated from the central ridge, and non-rhizosphere bulk soil was gently shaken off the roots. The tightly adhering rhizosphere soil (0–20 cm depth, within 5–10 cm of the root zone) was collected using sterile brushes, pooled, and homogenously mixed into a single representative composite sample (~500 g) per experimental unit. Samples were placed in sterile, alcohol-disinfected polyethylene bags and transported immediately to the laboratory in insulated ice chests at 4 °C. Microbiological enumerations and cumulative respiration assays were initiated within 24 h of field collection on fresh moist soil. Gravimetric soil moisture content was determined concurrently by drying sub-samples at 105 °C for 24 h, allowing all biological metrics to be expressed on an oven-dry soil weight basis (g^{-1} dry soil). Sub-samples designated for available N, P, and K analyses were air-dried in the shade, ground, and sieved through a 2-mm mesh. All laboratory determinations were conducted in three analytical replicates per experimental unit. Nitrogen-free semi-solid malate medium (Nfb) was utilized for isolating nitrogen-fixing *Azospirillum* strains from the rhizosphere soil. Purified isolates were initially recognized by their characteristic formation of a dense, white subsurface pellicle 1–2 mm below the medium surface after 48 h of incubation at 30 °C. Microscopic examination revealed Gram-negative, curved/vibroid rod-shaped cells exhibiting rapid, characteristic spiral motility. Biochemical characterization confirmed positive responses for oxidase, catalase, and nitrogenase activity, the latter evaluated

quantitatively via the acetylene reduction assay (ARA). Based on these phenotypic features and biochemical characterization, the isolated organism was identified at the genus level as *Azospirillum* sp. The Most Probable Number (MPN) technique was used to determine *Azospirillum* population density.

Culturable *Azospirillum* population in soil: The rhizosphere population density of *Azospirillum* sp. was enumerated at two growth stages (post-emergence and prior to harvest) using the Most Probable Number (MPN) technique in semi-solid Nitrogen-Free Malate (NFb) medium (Döbereiner, 1995). Ten-fold serial dilutions (10^{-1} to 10^{-7}) of 10 g fresh rhizosphere soil were prepared in sterile saline solution (0.85% NaCl). Aliquots (0.1 mL) were inoculated into vials containing 5 mL of semi-solid NFb medium and incubated at 30 °C for 48–72 h. Vials exhibiting the characteristic dense, white subsurface veil-like pellicle 1–2 mm below the medium surface were scored as positive for *Azospirillum*. Population densities were derived from Cochran's MPN tables and expressed as CFU g^{-1} dry soil. Total culturable heterotrophic soil bacteria were enumerated separately on soil-extract agar according to Allen (1957).

Biological activity and cumulative microbial respiration: Cumulative microbial respiration was measured to assess long-term soil metabolic potential and carbon mineralization kinetics. cumulative microbial respiration during 30-day incubation (mg CO_2 g^{-1} dry soil) was expressed as mg CO_2 g^{-1} dry soil using the alkali-trap method described by Anderson (1982). Moist rhizosphere soil samples (10 g) were placed in a 250-mL conical flask containing a glass beaker filled with 20 mL of 1 M NaOH to capture evolved CO_2. The flasks were tightly sealed with rubber stoppers and incubated in the dark for 30 days at 25 °C to quantify total cumulative carbon dioxide evolution. At the end of the incubation period, unreacted NaOH was titrated with 0.5 M HCl using phenolphthalein indicator until the pink colour disappeared.

Total tuber yield (Mg ha^{-1}): At physiological maturity (16 May 2025), total tuber yield was determined from a predefined net plot area comprising the entire central ridge (2.5 m length × 0.75 m width = 1.875 m^2), excluding two border plants at each end to eliminate edge effects. All harvestable tubers within the net plot were manually lifted, cleaned, and weighed using a calibrated digital balance to obtain net plot weight (kg $plot^{-1}$). Yield per hectare was calculated using the following formula:

Total Tuber Yield (Mg ha^{-1}) = [Net Plot Tuber Yield (kg) × 10,000 m^2 ha^{-1}] / [Net Plot Area (1.875 m^2) × 1,000 kg Mg^{-1}]

Total number of tubers per plant (tubers $plant^{-1}$): Five representative plants were randomly selected from the central ridge prior to the main harvest strictly to evaluate individual plant parameters, including the average number of tubers per plant (tubers $plant^{-1}$) and mean tuber weight (g $tuber^{-1}$). Small tubers (<2.5 cm diameter) and damaged or malformed tubers were excluded from the average count.

Available nutrients: Ammonium (NH_4^+) was extracted with 2 N potassium chloride (KCl) in the presence of MgO and measured using the micro-Kjeldahl method as described by Page *et al*. (1982). Available phosphorus was extracted using 0.5 M sodium bicarbonate ($NaHCO_3$). Color was developed using ammonium molybdate and ascorbic acid, and phosphorus concentration was measured spectrophotometrically at 882 nm according to Page *et al*. (1982). Available potassium was extracted using ammonium acetate and determined by flame photometry following the method described by Black *et al*. (1965).

Statistical analysis: Data were subjected to two-way analysis of variance (ANOVA) according to a Randomized Complete Block Design (RCBD) using SPSS software (version 28.0; IBM Corp., Armonk, NY, USA). Prior to ANOVA, normality of residuals and homogeneity of variances were verified using the Shapiro-Wilk and Levene's tests, respectively. Where ANOVA indicated significant main or interaction effects ($P \leq 0.05$), treatment means were separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. Statistically significant differences among treatment means are indicated by lowercase letters in all tables.

Results and discussion

***Azospirillum* population in soil after emergence:** The results in Table 2 indicate that the rhizosphere *Azospirillum* sp. population at 7 days post-inoculation was significantly highest under the a_2 inoculation treatment (2.117 × 10^9 CFU g^{-1} dry soil), compared with the uninoculated control a_0 (1.291 × 10^9 CFU g^{-1} dry soil). The intermediate inoculation rate (a1) recorded 1.783 × 10^9 CFU g^{-1} dry soil. Among the nitrogen fertilization levels, n_3 recorded the highest population density (1.988 × 10^9 CFU g^{-1} dry soil), followed by n_2 (1.837 × 10^9 CFU g^{-1} dry soil), whereas n_1 and n_0 recorded lower marginal means of 1.559 × 10^9 and 1.537 × 10^9 CFU g^{-1} dry soil, respectively.

Table 2. Effect of *Azospirillum* inoculation rate, nitrogen fertilizer level, and their interaction on rhizosphere soil bacterial population at emergence (× 10^9 CFU g^{-1} dry soil)

Nitrogen levels (N)	*Azospirillum* inoculation (mL $plant^{-1}$)			Mean N
	A0	A1	A2	
N0	1.269c	1.498c	1.845b	1.537c
N1	1.203d	1.548c	1.928b	1.559c
N2	1.305d	1.947b	2.260a	1.837b
N3	1.386d	2.142ab	2.436a	1.988a
Mean A	1.291c	1.783b	2.117a	

Means within a column or row sharing the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

The interaction between *Azospirillum* sp. inoculation and nitrogen application significantly enhanced the rhizosphere bacterial population at 7 days post-inoculation. The a2n3 interaction treatment recorded the highest bacterial population density (2.436 × 10^9 CFU g^{-1} dry soil), which did not differ significantly from a2n2 (2.260 × 10^9 CFU g^{-1} dry soil). Conversely, the a0n1 interaction recorded the lowest bacterial density (1.203 × 10^9 CFU g^{-1} dry soil), followed closely by a0n0 (1.269 × 10^9 CFU g^{-1} dry soil). The high inoculum density (a2) combined with adequate nitrogen (n_2 and n_3) substantially increased the total rhizosphere bacterial population, likely because root exudates, sugars, and metabolic byproducts stimulated overall microbial proliferation within the root zone.

***Azospirillum* population in soil before harvest:** The results in Table 3 show that *Azospirillum* inoculation under different nitrogen levels significantly increased bacterial populations before harvest. The a2 inoculation treatment recorded the highest mean population (2.696 × 10^9 CFU g^{-1} dry soil), compared with a0 (2.258 × 10^9 CFU g^{-1} dry soil) and a1 (2.494 × 10^9 CFU g^{-1} dry soil). Among nitrogen treatments, n3 recorded the highest population (2.663 × 10^9 CFU g^{-1} dry soil), followed by n2 (2.519 × 10^9 CFU g^{-1} dry soil); n1 and n0 recorded 2.474 and 2.274 × 10^9 CFU g^{-1} dry soil, respectively.

The interaction between inoculation and nitrogen addition significantly increased bacterial populations before harvest. The

maximum value was recorded under a2n3 (2.862 × 10^9 CFU g^{-1} dry soil), which did not differ significantly from a2n2 (2.847 × 10^9 CFU g^{-1} dry soil), whereas the control interaction a0n0 recorded the lowest value (2.046 × 10^9 CFU g^{-1} dry soil).

Table 3. Effect of *Azospirillum* inoculation rate, nitrogen fertilizer level, and their interaction on rhizosphere soil bacterial population at harvest (× 10^9 CFU g^{-1} dry soil)

Nitrogen levels (N)	*Azospirillum* inoculation (mL plant^{-1})			Mean N
	A0	A1	A2	
N0	2.046c	2.315b	2.462ab	2.274c
N1	2.303b	2.505ab	2.615a	2.474b
N2	2.290b	2.422ab	2.847a	2.519b
N3	2.393ab	2.735a	2.862a	2.663a
Mean A	2.258c	2.494b	2.696a	

Means within a column or row sharing the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

As shown in Tables 2 and 3, inoculation with different levels of nitrogen-fixing *Azospirillum*, nitrogen addition, and their interaction significantly increased *Azospirillum* populations in soil both after emergence and before harvest. The higher populations observed before harvest may be associated with plant age, greater root biomass, and deeper root development, which increase access to nutrients. Root exudates, including sugars, organic acids, amino acids, vitamins, and other compounds, may also explain the greater bacterial population before harvest than during the earlier growth stage.

Nitrogen level significantly influenced bacterial populations at different potato growth stages, particularly before harvest, indicating that nitrogen availability promoted bacterial growth. Higher *Azospirillum* inoculation rates were also associated with greater microbial activity and population density in the soil. Previous research has reported a positive relationship between nitrogen application rate and bacterial population, particularly during the pre-flowering stage, when increasing nitrogen levels enhanced bacterial activity and abundance (Yang *et al*., 2009). These bacteria also participate in soil nitrogen transformations (Yang *et al*., 2009). The statistical results demonstrate the importance of *Azospirillum* inoculation in increasing bacterial populations, possibly through nutrient mobilization and improved nutrient availability in the root zone. These findings agree with Kamal *et al*. (2016).

Moreover, the combined application of nitrogen-fixing bacteria and nitrogen fertilizer had a strong positive effect on bacterial growth. This response was probably caused by a synergistic interaction in which chemical nitrogen fertilizer and *Azospirillum* together supplied microorganisms with essential nutrients and organic compounds.

Soil biological activity and microbial respiration (mg CO_2 g^{-1} soil): The results in Table 4 show that *Azospirillum* inoculation, nitrogen addition, and their interaction significantly affected soil biological activity and microbial respiration. The a2 inoculation treatment recorded the highest mean value (6.642 mg CO_2 g^{-1} dry soil), compared with a0 (5.228 mg CO_2 g^{-1} dry soil) and a1 (6.176 mg CO_2 g^{-1} dry soil). This increase in microbial activity may reflect the ability of microorganisms to decompose organic matter and release CO_2, thereby stimulating microbial processes and plant growth in the rhizosphere (Ajeel and Al-Hakeim, 2024). Nitrogen addition also significantly enhanced microbial activity and respiration. The n2 treatment recorded the highest mean value (6.986 mg CO_2 g^{-1} dry soil), followed by n3 (6.192 mg CO_2 g^{-1} dry soil), n1 (6.059 mg CO_2 g^{-1} dry soil), and n0 (4.825 mg CO_2 g^{-1} dry soil).

Microbial activity was further enhanced by the interaction between *Azospirillum* inoculation and nitrogen addition. The a2n3 treatment recorded the highest cumulative microbial respiration rate (7.402 mg CO_2 g^{-1} dry soil), followed by a2n2 and a1n2 (7.365 and 7.215 mg CO_2 g^{-1} dry soil, respectively). The a0n0 treatment recorded the lowest value (4.443 mg CO_2 g^{-1} dry soil). This response may be associated with enhanced organic acid secretion by *Azospirillum* and increased microbial metabolic activity, which can promote localized nutrient solubilization (Richardson *et al*., 2009). Changes in microbial nutrient cycling may have contributed to greater nutrient availability and microbial activity.

Although physiological parameters (such as photosynthetic rate, leaf water potential, phytohormone production, and antioxidant enzyme activities) were not directly measured in the present study, these literature-documented mechanisms represent hypothesized explanations for the observed enhancements in crop yield and soil nutrient uptake (Bashan et al., 2004).

Table 4. Effect of *Azospirillum* inoculation rate, nitrogen fertilizer level, and their interaction on cumulative soil microbial respiration (mg CO_2 g^{-1} dry soil)

Nitrogen levels (N)	*Azospirillum* inoculation (mL plant^{-1})			Mean N
	A0	A1	A2	
N0	4.443c	4.742bc	5.291ab	4.825c
N1	5.469b	6.197a	6.510a	6.059b
N2	6.378a	7.215a	7.365a	6.986a
N3	4.623c	6.552a	7.402a	6.192b
Mean A	5.228c	6.176b	6.642a	

Means within a column or row sharing the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Soil available nitrogen (mg N kg^{-1} dry soil): Statistical analysis (Table 5) showed that all studied factors significantly increased soil available nitrogen after harvest. *Azospirillum* inoculation significantly increased nitrogen availability, with a2 recording the highest value (44.021 mg N kg^{-1} soil), followed by a1 (40.814 mg N kg^{-1} soil) and a0 (37.725 mg N kg^{-1} soil). Nitrogen addition also significantly affected available nitrogen, with n3 recording the highest value (48.171 mg N kg^{-1} soil), followed by n2 (43.048 mg N kg^{-1} soil), n1 (39.685 mg N kg^{-1} soil), and n0 (32.508 mg N kg^{-1} soil). The interaction between inoculation and nitrogen addition further increased nitrogen availability. The highest value was recorded under a2n3 (49.463 mg N kg^{-1} soil), followed by a1n3 and a2n2 (48.523 and 48.220 mg N kg^{-1} soil, respectively), whereas a0n0 recorded the lowest value (28.580 mg N kg^{-1} soil).

Table 5. Effect of *Azospirillum* inoculation rate, nitrogen fertilizer level, and their interaction on available soil nitrogen (mg N kg^{-1} dry soil)

Nitrogen levels (N)	*Azospirillum* inoculation (mL plant^{-1})			Mean N
	A0	A1	A2	
N0	28.580c	32.290bc	36.653b	32.508d
N1	36.560b	40.750ab	41.747a	39.685c
N2	39.233ab	41.693a	48.220a	43.048b
N3	46.527a	48.523a	49.463a	48.171a
Mean a	37.725c	40.814b	44.021a	

Means within a column or row sharing the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Soil available phosphorus (mg P kg^{-1} dry soil): Table 6 shows that *Azospirillum* inoculation significantly affected soil available phosphorus. The a2 treatment recorded the highest value (27.260 mg P kg^{-1} soil), followed by a1 (21.235 mg P

kg^{-1} soil) and a0 (17.650 mg P kg^{-1} soil). Nitrogen addition also increased phosphorus availability, with n3 recording the highest value (26.652 mg P kg^{-1} soil), followed by n2 (22.828 mg P kg^{-1} soil), n1 (20.081 mg P kg^{-1} soil), and n0 (18.631 mg P kg^{-1} soil). The interaction between inoculation and nitrogen level also had a significant effect. The a2n3 treatment recorded the highest phosphorus availability (35.770 mg P kg^{-1} soil), followed by a2n2 and a2n1 (26.723 and 23.307 mg P kg^{-1} soil, respectively), whereas a0n0 recorded the lowest value (13.457 mg P kg^{-1} soil).

Table 6. Effect of *Azospirillum* inoculation rate, nitrogen fertilizer level, and their interaction on available soil phosphorus (mg P kg^{-1} dry soil)

Nitrogen levels (N)	*Azospirillum* inoculation (mL plant^{-1})			Mean N
	A0	A1	A2	
N0	13.457c	19.197bc	23.240b	18.631d
N1	16.600bc	20.337b	23.307b	20.081c
N2	18.993b	22.770ab	26.723a	22.828b
N3	21.550a	22.637a	35.770a	26.652a
Mean A	17.650c	21.235 b	27.260a	

Means within a column or row sharing the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Soil available potassium (mg K kg^{-1} dry soil): Statistical analysis (Table 7) showed that *Azospirillum* inoculation significantly enhanced soil potassium availability. The a2 treatment recorded the highest value (220.362 mg K kg^{-1} soil), followed by a1 (198.683 mg K kg^{-1} soil) and a0 (191.343 mg K kg^{-1} soil). Nitrogen addition also increased potassium availability, with n3 recording the highest value (216.281 mg K kg^{-1} soil), followed by n2 (208.642 mg K kg^{-1} soil), n1 (200.968 mg K kg^{-1} soil), and n0 (187.961 mg K kg^{-1} soil). Potassium availability was further enhanced by the interaction between inoculation and nitrogen level. The a2n3 treatment recorded the highest value (240.593 mg K kg^{-1} soil), followed by a2n2 and a2n1 (223.667 and 209.657 mg K kg^{-1} soil, respectively), whereas a0n0 recorded the lowest value (170.503 mg K kg^{-1} soil).

Table 7. Effect of Azospirillum inoculation rate, nitrogen fertilizer level, and their interaction on available soil potassium (mg K kg^{-1} dry soil)

Nitrogen levels (N)	*Azospirillum* inoculation (mL plant^{-1})			Mean N
	A0	A1	A2	
N0	170.503c	185.843b	207.537a	187.961d
N1	192.947c	200.300b	209.657a	200.968c
N2	198.853b	203.407b	223.667a	208.642b
N3	203.067b	205.183b	240.593a	216.281a
Mean A	191.343c	198.683b	220.362a	

Means within a column or row sharing the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Inoculation of *Azospirillum*, nitrogen application and interactions between them significantly increased nutrients availability in soil as shown in Tables 5-7. The biofertilizer enhanced the availability of N, P and K and it is assumed that this was due to bio fixation of nitrogen, microbial activity and nutrient solubilisation process. The same results were observed by Al-Obaidi et al. (2007) and Hamid et al. (2026). Using nitrogen also can help to improve the availability of nutrients by activation of microbial activity and release of organic acids that promote mineral dissolution and minimize nutrient fixation (Al-Jubouri, 2011, Shanware et al., 2014, Al-Karboli et al., 2017). The results showed that combined application of *Azospirillum* and nitrogen produced a favourable rhizosphere environment which increased the nutrient release and absorption, and the plant growth. This is in agreement with other previous studies which show the higher the nutrients available the better the absorption and productivity in potato (Al-Khafaji, 2020; Al-Ameri, 2023).

Total potato tuber yield (Mg ha^{-1}): The results in Table 8 show that *Azospirillum* sp. inoculation, nitrogen fertilizer rate, and their interaction significantly increased total tuber yield ($P \leq 0.05$). The a_2 inoculation treatment recorded the highest marginal mean yield (41.218 Mg ha^{-1}), followed by a_1 (34.599 Mg ha^{-1}), whereas the uninoculated control a_0 recorded the lowest yield (27.045 Mg ha^{-1}). Regarding nitrogen levels, n_3 (300 kg N ha^{-1}) produced a mean yield of 39.586 Mg ha^{-1}, which did not differ significantly from n_2 (200 kg N ha^{-1}, 39.370 Mg ha^{-1}), with both sharing the letter "a" according to Duncan's Multiple Range Test. In contrast, n_1 (100 kg N ha^{-1}) and the unfertilized control n_0 recorded significantly lower yields of 34.987 Mg ha^{-1} ("b") and 23.208 Mg ha^{-1} ("c"), respectively.

Among the interaction treatments, a2n3 produced the highest total tuber yield (47.487 Mg ha^{-1}), which was statistically comparable to a2n2 (46.847 Mg ha^{-1}), whereas the uninoculated, unfertilized control (a0n0) recorded the lowest total yield (21.157 Mg ha^{-1}).

Agronomic Efficiency of Nitrogen (AEN): Agronomic efficiency was evaluated using the standard formula AEN = (YN − Y0) / FN to express the yield increment per unit of applied nitrogen (kg tubers kg^{-1} N). Across the main effects of nitrogen, AEN showed a declining trend with increasing nitrogen application rates, declining from 117.79 kg tubers kg^{-1} N at n_1 (100 kg N ha^{-1}) to 80.81 kg tubers kg^{-1} N at n_2 (200 kg N ha^{-1}) and 54.59 kg tubers kg^{-1} N at n_3 (300 kg N ha^{-1}). Under the highest inoculation rate (a2), conventional AEN at n_2 relative to a2n0 was 109.79 kg tubers kg^{-1} N. Furthermore, the incremental agronomic response between a2n2 and a2n3 fell sharply to 6.40 kg tubers kg^{-1} N. This pronounced decline in incremental efficiency reinforces the conclusion that applying nitrogen beyond 200 kg N ha^{-1} yields minimal agronomic benefit.

Table 8. Effect of *Azospirillum* inoculation, nitrogen rate, and their interaction on total potato tuber yield

Nitrogen levels (N)	*Azospirillum* inoculation (mL plant^{-1})			Mean N
	A0	A1	A2	
N0	21.157 c	23.577 b	24.890 a	23.208 c
N1	25.783 c	33.527 b	45.650 a	34.987 b
N2	30.563 c	40.700 b	46.847 a	39.370 a
N3	30.677 c	40.593 b	47.487 a	39.586 a
Mean A	27.045 c	34.599 b	41.218 a	

Means within a column or row sharing the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Total number of tubers per plant (tubers plant^{-1}): The results in Table 9 indicate that *Azospirillum* sp. inoculation, nitrogen fertilizer level, and their interaction significantly increased the total number of tubers per plant ($P \leq 0.05$). The a_2 inoculation treatment (20 mL plant^{-1}) recorded the highest marginal mean (6.731 tubers plant^{-1}), followed by a_1 (6.003 tubers plant^{-1}), whereas the uninoculated control a_0 recorded the lowest value (5.116 tubers plant^{-1}). Nitrogen application also exhibited a significant positive main effect; n_3 (300 kg N ha^{-1}) produced the highest mean number of tubers (6.973 tubers plant^{-1}), followed progressively by n_2 (6.543 tubers plant^{-1}), n_1 (5.800 tubers plant^{-1}), and the unfertilized control n_0 (4.482 tubers plant^{-1}).

The interaction between *Azospirillum* sp. inoculation and nitrogen fertilizer significantly affected tuber number per plant. The a2n3 treatment yielded the highest number of tubers (7.860 tubers plant^{-1}), followed by a2n2 (7.370 tubers plant^{-1}). Conversely, the uninoculated, unfertilized control (an_0) recorded the lowest

value (4.110 tubers plant^{-1}), which did not differ significantly from a1n0 (4.413 tubers plant^{-1}), with both sharing the letter "b" within the n_0 factor group according to Duncan's Multiple Range Test.

Table 9. Effect of *Azospirillum* inoculation rate, nitrogen fertilizer level, and their interaction on total number of tubers per plant (tubers plant^{-1})

Nitrogen levels (N)	*Azospirillum* inoculation (mL plant^{-1})			Mean N
	A0	A1	A2	
N0	4.110b	4.413b	4.923a	4.482d
N1	4.437c	6.193b	6.770a	5.800c
N2	5.710c	6.550b	7.370a	6.543b
N3	6.207c	6.853b	7.860a	6.973a
Mean A	5.116c	6.003b	6.731a	

Means within a column or row sharing the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

The *Azospirillum* bioinoculant, nitrogen level, and their interaction strongly increased total tuber yield and the number of tubers per plant. Bacterial biofertilization produced higher values for these traits than the control treatment. This response may be explained by the role of the bacterial inoculant as a biostimulant that affects plant growth, development, and yield (Ajeel *et al*., 2025; Ajeel, 2026). The bioinoculant activates plant enzymes and hormones and improves soil fertility (Sarir *et al*., 2005). It may also improve yield quantity and quality by modifying cellular processes, including respiration, metabolism, carbon assimilation, protein synthesis, water and nutrient uptake, and enzyme activity. These processes can improve soil fertility, stimulate enzyme activity, increase cell-membrane permeability, and ultimately increase crop productivity (Al-Maamori, 2020).

Application of nitrogen can decrease the plant dependency on biologically fixed nitrogen by bacteria and improve the vegetative growth by increasing plant height, shoot biomass, chlorophyll content, stem diameter, and leaf development. Better chlorophyll formation and photosynthetic activity supports the production of assimilate. Adequate nitrogen supply promotes the formation of tubers, the number of tubers and the overall yield (Hussein, 2013). The significant interaction between *Azospirillum* inoculation and nitrogen application suggests the benefit of integrated nutrient management in which gradual release of nutrients improves the availability of nutrients in rhizosphere, uptake and translocation of assimilates leading to enhanced tuber production (Saleh, 2000). Also, the combined application of *Azospirillum* and nitrogen could improve water relations, nutrient availability and physiological processes that support the initiation and development of tubers (Wang et al., 2009). These treatments can also enhance organic acid production, soil fertility, nutrient uptake and protein synthesis, which can contribute to improved growth and yield performance of potatoes (Abdullah, 2020).The maximum tuber yield of 47.487 Mg ha^{-1} was recorded under the combined a2n3 treatment (20 mL plant^{-1} *Azospirillum* + 300 kg N ha^{-1}). This high yield can be attributed to the combined effects of bio-inoculation, adequate nitrogen supply, and precise drip irrigation. Field studies evaluating the 'Burren' cultivar under improved management practices have reported yield potentials exceeding 40–45 Mg ha^{-1}, indicating its high genetic yield potential and adaptability under semi-arid conditions.

Although the initial soil electrical conductivity (ECe = 3.45 dS m^{-1}) indicated moderate salinity stress, drip irrigation helped reduce osmotic stress by maintaining favourable soil moisture conditions and promoting salt leaching from the active root zone. Furthermore, *Azospirillum* inoculation can enhance plant tolerance to abiotic stress through the production of growth-promoting phytohormones, including indole-3-acetic acid (IAA), which stimulates root development and improves nutrient and water uptake under saline conditions. Therefore, the combination of *Azospirillum* inoculation and adequate nitrogen availability under drip irrigation enabled the crop to approach its maximum yield potential.

Although the a2n3 treatment achieved the highest absolute tuber yield (47.487 Mg ha^{-1}), the incremental yield gain over a2n2 (46.847 Mg ha^{-1}) was only 0.640 Mg ha^{-1} (640 kg ha^{-1}), despite a 50% increase in synthetic nitrogen input, equivalent to an additional 100 kg N ha^{-1}. From an agronomic perspective, the marginal Agronomic Efficiency of Nitrogen (AE_N) declined sharply from 74.23 kg tubers kg^{-1} N at the n_2 level (200 kg N ha^{-1}) to only 6.40 kg tubers kg^{-1} N for the additional 100 kg N ha^{-1} applied at n_3.

From a resource utilization perspective, applying an additional 100 kg N ha^{-1} beyond the n_2 level generated a modest yield increase of only 0.640 Mg ha^{-1}, representing a low agronomic return. Furthermore, higher nitrogen application rates may potentially increase the risk of nitrogen losses in calcareous semi-arid soils. Therefore, combining 200 kg N ha^{-1} with 20 mL plant^{-1} *Azospirillum* (a2n2) represents a more agronomically efficient, resource-efficient, and environmentally sustainable fertilization strategy for potato production in the region.

The study revealed that *Azospirillum* inoculation and nitrogen application significantly enhanced rhizosphere microbial activity, nutrient availability and potato productivity. The a_2 treatment (20 mL plant^{-1} *Azospirillum*) had the highest bacterial population in both growth stages while n_3 (300 kg N ha^{-1}) improved nutrient availability, tuber yield and tuber number. The combination of a2n3 treatment showed the best values for most of the parameters measured. However, a2n2 (20 mL plant^{-1} Azospirillum + 200 kg N ha^{-1}) also displayed improved microbial activity and the availability of soil N, P and K. To summarise, the combination of *Azospirillum* inoculation and appropriate nitrogen management induced positive changes in soil biological properties and improved potato yield under semi-arid conditions.

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