



Combined microbial inoculation improves plant growth, nutrient acquisition, and soil biological functioning in *Opuntia ficus-indica* under water deficit

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Abstract

The productivity of *Opuntia ficus-indica* (L.) Mill. in arid and semi-arid environments is strongly constrained by water scarcity and limited soil biological activity. This study evaluated the effects of plant growth-promoting rhizobacteria (PGPR) consortia (Cs1 and Cs2), arbuscular mycorrhizal fungi (AMF), and their combined inoculations (Cs1&AMF and Cs2&AMF) on plant growth, nutrient acquisition, soil physicochemical properties, enzymatic activities, and microbial functional diversity under non-stressed and water-stressed conditions (95% and 20% of field capacity, respectively). Over a six-month greenhouse experiment, microbial inoculation significantly enhanced plant growth compared with the non-inoculated control, with the strongest responses observed under combined PGPR&AMF treatments. Microbial inoculation also improved plant mineral nutrition in the cladodes, particularly nitrogen, phosphorus, and potassium contents (up to +32.88%, +50.00%, and +12.74%, respectively, under non-stressed conditions), while reducing residual mineral accumulation in soil, indicating enhanced nutrient uptake. Soil organic matter content increased markedly in inoculated treatments, especially under combined inoculations, accompanied by significant stimulation of phosphatase (+282.27% for Cs2&AMF treatment), β -galactosidase (up to +789.25% for Cs2&AMF), and urease (up to +40.68% for Cs1&AMF) activities under both water regimes. Biolog EcoPlate analyses revealed higher average well color development (AWCD) under both non-stressed (+352.61% for Cs1) and water-stressed conditions (+129.04% for Cs2&AMF), broader carbon substrate utilization, and increased microbial metabolic functioning, reflected by significantly higher functional diversity indices (Shannon diversity and substrate richness) in inoculated soils. Combined inoculations exhibited the greatest metabolic versatility and resilience under water stress. Multifactor analysis of variance (ANOVA) identified microbial inoculation as the dominant factor driving variability in soil biological and functional traits, while water regime primarily influenced plant growth and nutrient parameters. Principal component analysis highlighted a clear functional shift from physico-chemically driven control soils toward biologically active soil–plant systems promoted by PGPR&AMF synergy. Overall, these results demonstrate that combined PGPR and AMF inoculation represents an effective and sustainable strategy to enhance soil biological functioning, nutrient cycling, and drought tolerance of *O. ficus-indica* in water limited environments.

Keywords Cactus · Plant growth-promoting rhizobacteria · Arbuscular mycorrhizal fungi · Water stress · Soil enzymes · Microbial functional diversity

Introduction

Arid, semi-arid, and dry sub-humid regions occupy a central position in contemporary challenges related to sustainable agriculture, as they are simultaneously characterized

by water scarcity, soil fragility, and high vulnerability to climate change (Wang et al. 2023). Drylands cover approximately 41.5% of the Earth's terrestrial surface and are home to nearly 2.5 billion people, making the sustainability of agricultural production in these regions a global priority

(Reynolds et al. 2007). However, agricultural productivity is severely constrained by water scarcity and progressive soil degradation, including organic matter depletion, nutrient imbalance, salinization, and reduced biological activity, all of which impair nutrient cycling, soil fertility, and crop performance (Hassani et al. 2024; Shokri et al. 2025). Within this context, identifying crops adapted to harsh environments and biological strategies capable of enhancing plant-soil performance under water-limited conditions has become a major scientific and agronomic priority.

Among stress-adapted plant species, cactus pear (*Opuntia ficus-indica* (L.) Mill.) has emerged as a strategic crop due to its crassulacean acid metabolism (CAM) and its capacity to maintain productivity under limited water availability (Nefzaoui 2018). Recent studies have shown that *Opuntia* exhibits ecophysiological traits consistent with efficient water use, including optimized energy fluxes, evapotranspiration patterns, and seasonal functioning (Jardim et al. 2025), reinforcing its suitability for forage-based and agro-ecological systems in semi-arid environments. However, the agronomic potential of *O. ficus-indica* is not determined solely by its physiological traits; it is also strongly influenced by soil properties, nutrient availability, and the structure and functionality of soil microbial communities. Our recent study comparing soils under *O. ficus-indica* cultivation reported significant differences in soil physicochemical properties, bacterial abundance, functional microbial diversity, and enzymatic activities (Zouitane et al. 2025), highlighting that crop performance is closely linked to soil biological functioning.

Although *O. ficus-indica* represents a resilient option under water-limited conditions, the long-term sustainability of its production systems requires approaches that extend beyond the plant itself and explicitly address plant-soil interactions. In this context, microbial bio-inoculants represent a promising strategy to simultaneously enhance plant nutrition, stress tolerance, and soil biological quality (Zouitane et al. 2026a). Plant growth-promoting rhizobacteria (PGPR) comprise a diverse group of microorganisms capable of stimulating plant growth through direct and indirect mechanisms, including improved nutrient acquisition (e.g., phosphorus solubilization and iron mobilization), phytohormone production, modulation of oxidative stress, and induction of plant defense responses (Goswami et al. 2016). Under drought conditions, recent studies emphasize the ability of PGPR to enhance stress tolerance through coordinated effects on root architecture, stomatal regulation, hormonal balance, and antioxidant systems (El-Saadony et al. 2024; Ferioun et al. 2024). Consequently, PGPR are increasingly regarded as key tools for the bio-optimization of crops under limiting environmental conditions, particularly were

reducing chemical inputs and improving resilience are priority objectives (El-Saadony et al. 2024).

Nevertheless, in arid and semi-arid soils where phosphorus availability is often limited and nutrient diffusion is restricted, the benefits of PGPR may be further enhanced when they operate in symbiosis with organisms capable of extending the soil exploration zone. This role is fulfilled by arbuscular mycorrhizal fungi (AMF), which establish mutualistic associations with most terrestrial plants. Through their extensive hyphal networks, AMF improve the uptake of poorly mobile nutrients, particularly phosphorus, while also influencing host plant physiology, including water relations, metabolism, and stress tolerance (Silvestri et al. 2019). Recent syntheses confirm that AMF play a critical role in enhancing plant resilience to abiotic stress and in structuring rhizosphere microbial communities, with significant implications for biogeochemical cycling and soil functionality (Ahmed et al. 2025; Zouitane et al. 2026b).

In *O. ficus-indica*, several studies have demonstrated the agronomic relevance of AMF symbiosis (Lahbouki et al. 2022, 2023; Greco et al. 2025). Beyond plant performance, AMF also influence soil biological quality: soil enzymatic activities such as phosphatase, dehydrogenase, and urease, which are widely used as indicators of microbial activity and nutrient cycling, contributing to the maintenance of functional soils under water stress (Li et al. 2025).

While PGPR and AMF each exhibit beneficial mechanisms, a key question in ecological intensification is whether their combined application can generate synergistic effects on both plant performance and soil functioning, particularly under contrasting irrigation regimes. Co-inoculation of PGPR and AMF is increasingly recognized as a strategy that promotes functional complementarity (Hnini et al. 2024). In other plant systems, several experimental studies have demonstrated that the combined application of AMF and PGPR enhances plant growth and improves soil-related parameters more effectively than either microbial inoculant applied alone (Sbbar et al. 2025; Tahiri et al. 2022; Zhang et al. 2024). These synergistic interactions have been shown to improve drought tolerance by promoting root development, increasing phosphorus and nitrogen uptake, stimulating antioxidant defense systems, regulating phytohormone balance, and enhancing osmotic adjustment, ultimately resulting in greater plant growth and stress resilience compared with single inoculations.

This functional complementarity is based on reciprocal interactions between the two microbial partners (Hnini et al. 2024). AMF enhance phosphorus and water acquisition through their extensive extraradical hyphal network, creating a more favorable rhizosphere environment for PGPR establishment and activity. In turn, PGPR stimulate AMF colonization by producing phytohormones, organic acids,

siderophores, and enzymes that improve nutrient availability, modify root architecture, and facilitate fungal development. Together, these reciprocal interactions promote greater nutrient acquisition, improved root functioning, and enhanced plant resilience under abiotic stress. Moreover, recent research indicates that such interactions can reshape rhizosphere microbial communities and improve soil fertility indicators, including organic carbon, total nitrogen, and available phosphorus, suggesting that microbial inoculants act as ecological engineers of the rhizosphere (Yang et al. 2025). This perspective is particularly relevant for dry-land systems, where soil fertility is tightly constrained by phosphorus availability, organic carbon dynamics, and the capacity of soils to sustain enzymatic and microbial activity under water stress (Kabato et al. 2025).

Despite the growing body of literature on microbial bio-inoculants, important knowledge gaps remain regarding the combined application of plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi in *O. ficus-indica*, particularly under contrasting water regimes. Most previous studies have focused on the individual application of either AMF or PGPR, or have investigated their combined effects in crop species other than *O. ficus-indica* (Ferioun et al. 2025; Lahbouki et al. 2023; Sbbar et al. 2025). Furthermore, integrated assessments simultaneously evaluating plant growth, nutrient acquisition, soil physicochemical properties, soil enzymatic activities, and microbial functional activity remain lacking for this CAM species. To the best of our knowledge, this is the first study to evaluate the individual and combined application of a native PGPR consortium isolated from the rhizosphere of *O. ficus-indica* together with AMF under greenhouse conditions and contrasting irrigation regimes. Unlike previous studies, the present work integrates plant growth, nutrient acquisition, soil physicochemical properties, soil enzymatic activities, and microbial functional activity to provide a comprehensive assessment of plant-soil system responses to microbial inoculation under water stress.

Therefore, the objective of this study is to evaluate the effects of microbial inoculation based on a consortium of PGPR and AMF, applied individually and in combination, on (i) the growth and nutrient uptake of *O. ficus-indica*, (ii) soil physicochemical properties, (iii) enzymatic activities indicative of nutrient cycling, and (iv) functional microbial diversity, under different irrigation regimes. This work aims to test the hypothesis that PGPR&AMF co-inoculation exerts a synergistic effect that enhances plant-soil system functionality under water-limited conditions.

Materials and methods

Microbial inocula

The study evaluated two bacterial consortia and one AMF complex for their growth-promoting potential. The AMF inoculum was obtained from the mycorrhizal collection of the National School of Agriculture of Meknes (ENAM, Morocco). The inoculum consisted of an indigenous consortium composed of *Rhizoglossum irregulare*, *Claroideoglossum etunicatum*, and *Funnelformis mosseae*, originally isolated from the rhizosphere of date palm in the Tafilalt oasis (Morocco) and multiplied by trap culture using sorghum as the host plant according to (El Hilali et al. 2022). The first bacterial consortia (Cs1), provided by the Faculty of Sciences and Technologies of Fez (FST-USMBA), included four previously characterized strains: *Achromobacter insolitus* (MFF3), *Pseudomonas putida* (MFE1), *Providencia rettgeri* (MFE3), and *Alcaligenes* sp. (MFE4) (Ferioun et al. 2023; 2024). The second consortium (Cs2) comprised four isolates from the *O. ficus-indica* rhizosphere: *P. frederiksborgensis* (ZN3), *P. koreensis* (ZN4), *Bacillus halotolerans* (ZN12), and *Priestia megaterium* (ZSSp3) (Zouitane et al., 2026a; Zouitane et al. 2025). All isolates exhibited multiple plant growth-promoting (PGP) traits, including phosphate solubilization, nitrogen fixation, and the production of IAA, siderophores, ammonia, HCN, and exopolysaccharides. Furthermore, they demonstrated drought tolerance, maintaining growth under osmotic pressures ranging from -0.05 to -0.73 MPa. Compatibility tests confirmed the absence of antagonistic interactions within each consortium. To prepare the inoculants, strains were cultured in LB broth at 30°C for 48 h to obtain a standardized inoculum of approximately 10^8 CFU.mL $^{-1}$ (Fig. 1), prior to inoculation, harvested by centrifugation ($6,000 \times g$, 20 min), and washed with 0.85% NaCl. Bacterial isolates were individually adjusted to a concentration of approximately 10^8 CFU/mL before being mixed in equal proportions to form the final consortium.

Plant material and experimental design

Cladodes of *O. ficus-indica* (L.) Mill. cv. Dellahia were collected in February 2023 from the Aknoul region in Taza Province, Morocco ($34^{\circ} 39' 39'' \text{N}$; $3^{\circ} 52' 08'' \text{W}$). Mother cladodes were collected during the rainy season from healthy plants. To minimize biological variability, all cladodes were selected from the same cladode tier and exhibited similar size and developmental stage, ensuring comparable physiological age. After harvesting, cladodes were air-dried in the shade at ambient temperature before planting. One mother cladode was planted per plastic pot.

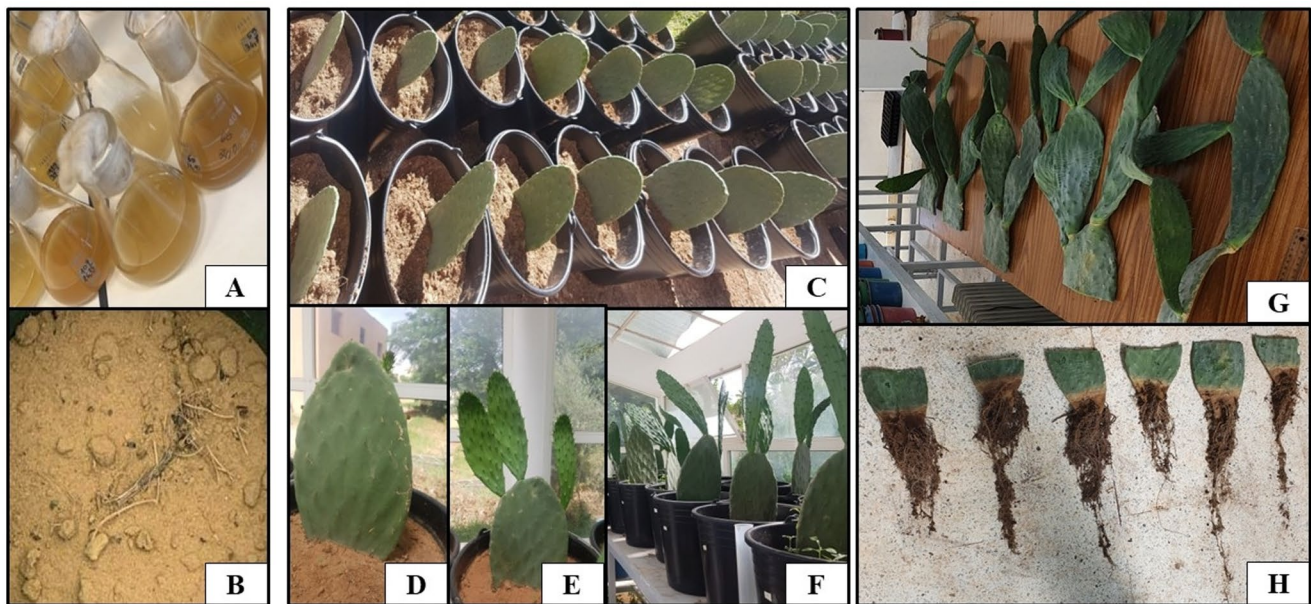


Fig. 1 Experimental workflow illustrating microbial inoculation, greenhouse cultivation, plant growth monitoring, and harvest of *Opuntia ficus-indica* under contrasting irrigation regimes. (A) Preparation of the bacterial inoculum; (B) Arbuscular mycorrhizal fungi inocu-

lum applied at planting; (C) Planting of cladodes; (D, E, and F) Plant growth one, three, and four month after planting, respectively; (G) Harvest of the aerial parts (cladodes); and (H) Root systems collected at harvest

Plastic pots were filled with a 1:1 (v/v) mixture of agricultural soil and sand (Fig. 1). This substrate composition follows the standard protocol commonly adopted in cactus ecophysiological studies (Lopez and Nobel, 1991), providing adequate drainage and aeration while maintaining sufficient water-holding capacity for CAM species under greenhouse conditions. The soil mixture exhibited alkaline conditions and moderate fertility, with a pH (H₂O) of 7.87, electrical conductivity of 204.67 $\mu\text{S cm}^{-1}$, and organic matter content of 4.93%. The nutrient and mineral composition included available phosphorus (74.18 $\mu\text{g g}^{-1}$), ammonium (5.43 $\mu\text{g g}^{-1}$), nitrate (46.60 $\mu\text{g g}^{-1}$), calcium (288.60 $\mu\text{g g}^{-1}$), magnesium (38.10 $\mu\text{g g}^{-1}$), sodium (5980.33 $\mu\text{g g}^{-1}$), iron (41.82 $\mu\text{g g}^{-1}$), and potassium (23,457 $\mu\text{g g}^{-1}$). The experiment was conducted under greenhouse conditions under natural light with an average photoperiod of approximately 16 h day⁻¹. Two irrigation regimes were applied: a well-watered condition corresponding to 95% of field capacity (FC) and a drought-stress condition corresponding to 20% FC. Throughout the experiment, pots were weighed before each irrigation event and the amount of water required to restore the target moisture level was added. The experimental setup followed a completely randomized block design (CRBD) with five replicates per treatment. The treatments included: (i) control; (ii) inoculation with the first bacterial consortium (Cs1); (iii) inoculation with the second bacterial consortium (Cs2); (iv) inoculation with an arbuscular mycorrhizal fungi (AMF) complex; (v) co-inoculation with Cs1 and AMF, and (vi) co-inoculation with Cs2 and AMF. The bacterial

inoculum volume (100 mL plant⁻¹) was selected according to previously published greenhouse PGPR inoculation protocols (Jetiyanon and Kloepper, 2002), while the AMF dose (10 g plant⁻¹) followed successful inoculation procedures reported in previous studies (Maulana et al. 2019). Plant harvest was performed six months after planting (Fig. 1).

Growth kinetics monitoring

Plant growth was monitored throughout the experimental period from March to September (six months) by measuring key morphological parameters, including plant height, number of cladodes, and total cladode area (Fig. 1). Measurements were conducted on a monthly basis to assess growth dynamics under the different treatments. Plant height, cladode length, and width were measured using a graduated ruler and the number of cladodes was recorded by direct counting. Total cladode area per plant was estimated using the elliptical mathematical equation, which has been validated for *O. ficus-indica* cladodes (Tiznado-Hernández et al. 2010), according to the following formula:

$$\text{Total cladode area per plant} = \sum_{i=1}^n \left(\frac{Li}{2} \right) * \left(\frac{wi}{2} \right) * \pi (\text{cm}^2)$$

where $\pi=3.141592$, Li and Wi represent the length and width of the i cladode, respectively, and n is the total number of cladodes per plant.

Plant nutrient analysis

At harvest, cladodes of *O. ficus-indica* were sampled from each experimental unit for subsequent nutrient analysis. Samples were thoroughly washed with tap water, followed by distilled water, to remove any soil or dust particles. The plant tissues were then air-dried and oven-dried at 70 °C until constant weight was reached. Dried samples were ground to a fine powder using a laboratory mill to ensure homogeneity for subsequent analysis. Total nitrogen (N) content in plant tissues was determined using the Kjeldahl method (Nelson and Sommers 1980), which involves digestion of the sample in concentrated sulfuric acid, distillation of the released ammonia, and titration. Total phosphorus (P) was measured by colorimetry after acid digestion of the plant material (Wolf 1982). Total potassium (K) was quantified using flame photometry after sample digestion (Asch et al. 2022).

Physicochemical analysis of soil

After harvest, *O. ficus-indica* soil physicochemical properties were thoroughly characterized to evaluate the effects of microbial inoculation and irrigation treatments on nutrient availability. Soil pH was measured both in distilled water (H₂O) and 1 M KCl using a benchtop pH meter (Fisherbrand™ accumet™ FE150) to assess both active and exchangeable acidity. Electrical conductivity (EC), reflecting the total soluble salts, was determined using a benchtop multiparameter conductivity meter (Milwaukee MW150 MAX, Ref. 40,111.10) in a 1:2.5 (w/v) soil-to-deionized water suspension. Soil moisture content was evaluated by oven-drying the samples at 105 °C ± 5 °C overnight according to the NF ISO 11465 standard, providing a measure of water retention capacity and soil texture influence on plant water availability.

The organic carbon content, as an indicator of soil organic matter, was determined using the Walkley and Black method (Walkley and Black 1934), which relies on the oxidation of carbon by potassium dichromate in a sulfuric medium. Macro- and micronutrient concentrations, including calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), molybdenum (Mo), and cobalt (Co), were quantified after Mehlich 2 digestion (Mehlich 1953) using inductively coupled plasma atomic emission spectrometry (ICP-AES) (Arenas et al. 2011). Soil nitrogen dynamics were evaluated by measuring ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) concentrations following ISO/TS 14256–1 standards, providing insight into available inorganic nitrogen for plant uptake.

Soil enzymatic activities

Soil enzymatic activities were assessed to evaluate the functional potential of the microbial community and nutrient cycling. Phosphatase, β-D-galactosidase, and urease activities were measured following established protocols with slight modifications for microplate assays. Phosphatase activity was determined using 4-nitrophenyl phosphate (0.05 M) as a substrate (Tabatabai and Bremner 1969), while β-D-galactosidase activity was assessed using p-nitrophenyl β-D-galactopyranoside (0.02 M) (Eivazi and Tabatabai 1988). Urease activity was measured by incubating soil samples with 0.4 M urea (Kandeler and Gerber 1988; Sinsabaugh et al. 2000). For each enzyme, activities were quantified by comparison with calibration curves prepared from standard solutions: p-nitrophenol for phosphatase and β-D-galactosidase, and ammonium chloride (NH₄Cl) for urease. Enzymatic activities were expressed as μmol product h⁻¹ g⁻¹ dry soil.

Microbial functional diversity

The functional diversity of microbial communities in the *O. ficus-indica* rhizospheric soils was evaluated using the Biolog EcoPlate™, which allows assessment of substrate utilization patterns across a range of carbon sources (Grzadziel et al. 2019). For each soil sample, 1 g of soil was suspended in 9 mL of sterile physiological water (0.85% NaCl) and shaken for 30 min at 180 rpm to extract microbial cells. From this suspension, 1 mL was further diluted in 9 mL of sterile saline, and 150 μL of the resulting solution was inoculated into each of the 96 wells of the EcoPlate™, which contains 31 distinct carbon substrates plus a control well with water. Each treatment was replicated three times to ensure reproducibility. The 31 carbon sources are categorized into five chemical groups: carbohydrates, polymers, amino acids, carboxylic acids, and amines/amides, allowing the evaluation of functional capabilities of the soil microbial communities across different metabolic types. Plates were incubated at 28 °C for 7 days, and optical density (OD) at 590 nm was measured every 24 h using a microplate reader (Multiskan FC, ThermoFisher Scientific).

The overall metabolic activity of the microbial communities was quantified using the Average Well Color Development (AWCD) (Garland 1997), calculated as: $AWCD = \sum (C - R)/n$.

Substrates with an OD_{590 nm} below 0.25 were considered as non-metabolized (Grzadziel et al. 2019). Substrate-specific AWCD (S-AWCD) and diversity metrics were calculated on the fifth day of incubation, when color development was optimal. For each substrate category, substrate-specific AWCD (S-AWCD) was calculated to assess the

metabolic potential within different functional groups (Garland and Mills 1991): $S\text{-AWCD} = \sum (S-C - R)/n$.

The richness index (S) was defined as the number of substrates with $OD_{590\text{ nm}} \geq 0.25$, representing metabolically active substrates. The Shannon diversity index (H') and Shannon evenness index (E) were calculated using the following formulas (Hill et al. 2003; Jatowiecki et al. 2016; Zak et al. 1994): $H' = -\sum p_i \cdot \ln(p_i)$ and $E = H'/\ln(S)$.

- C: reading of the well OD
- S-C: reading of the well of each substrate group OD
- R: reading of the control well OD
- n: the number of substrates on an EcoPlate™ (31)
- p_i : proportional color development of the well over the total color development of all wells of a plate
- S: the number of wells with color development (substrate utilization richness)

Statistical analysis

All experimental data, including growth kinetics monitoring, soil physicochemical properties, plant nutrient contents, microbial metabolism, substrate-specific utilization patterns, calculated functional indices, and soil enzymatic activities, were subjected to statistical analysis. Data were first analyzed using multifactor analysis of variance (ANOVA) to assess the effects of experimental factors and their interactions. When significant effects were detected, comparisons of means were performed using Tukey's Honestly Significant Difference (HSD) test at a significance level of $P \leq 0.05$, implemented in Minitab® 21.2 software (64-bit). Significant differences among treatments or soil types were indicated by different letters. To explore relationships among all measured parameters and identify underlying multivariate patterns, Principal Component Analysis (PCA) was conducted using XLSTAT 2016.02.28451. In addition, variability in microbial substrate utilization across the 31 carbon sources in each soil was visualized using a heatmap generated with the *pheatmap* package in RStudio. Together, these analyses provided an integrated view of treatment effects and functional interactions within the rhizosphere of *Opuntia ficus-indica*, facilitating the interpretation of microbial and plant responses under different experimental conditions.

Results and discussion

Analysis of variance

Analysis of variances (ANOVA) was adopted as a statistical tool to evaluate the effect of each factor (water regime

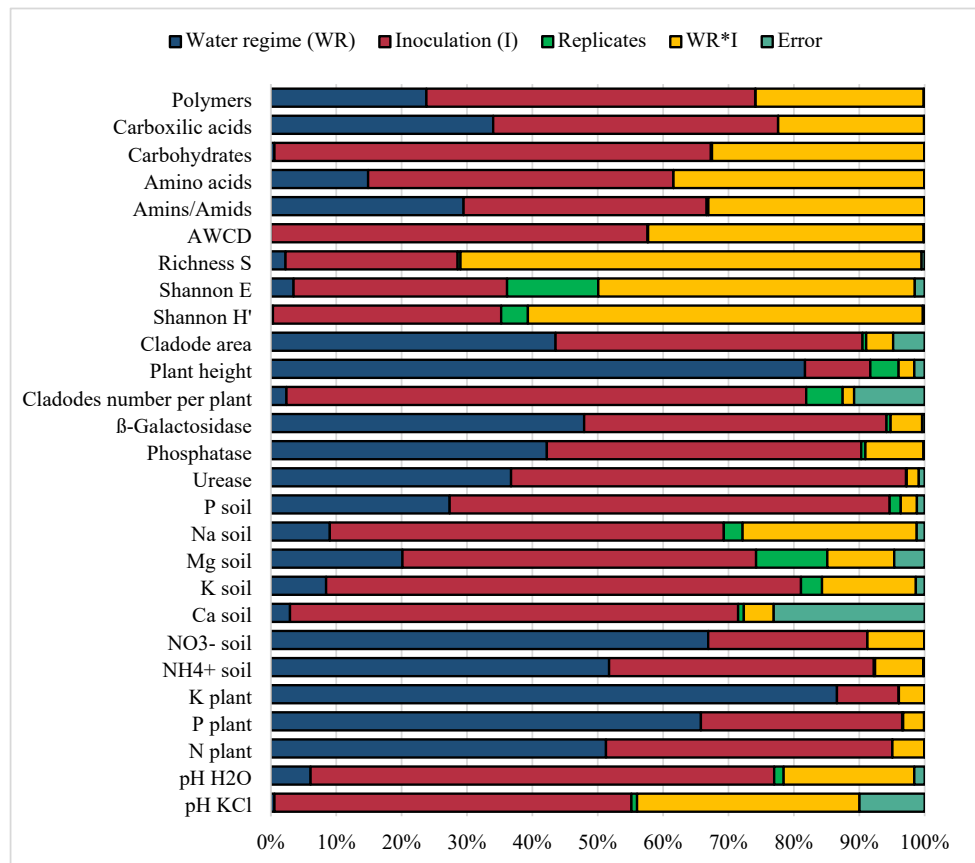
and inoculation) as well as their combination on the variability of plant and soil traits (Fig. 2 and Table S1). The sources of variability differed depending on the parameters studied. For most traits, the inoculation factor was the primary driver of variability, explaining more than 60% of the total variance in carbohydrate metabolism, AWCD, urease activity, soil P, Na, Mg, K, and Ca contents, as well as pH H₂O and pH KCl. The water regime (WR) ranked second, contributing between 5 and 40%, and the WR × inoculation interaction accounted for less than 35%. In contrast, for cladode area, plant height, β-galactosidase and phosphatase activities, NO₃⁻ and NH₄⁺ contents, and plant N, P, and K contents, WR replaced inoculation as the dominant source of variability, explaining more than 45% of the total variance, with inoculation as the second contributor. For Richness (S), Shannon evenness (E), and Shannon diversity index (H'), the interaction between WR and inoculation was the primary source of variability, accounting for more than 50% of the total variance, and where inoculation factor is the second source of variability, explaining less than 30% of the total variability.

Growth kinetics

The temporal evolution of *O. ficus-indica* growth parameters, including cladode number, plant height, and cladode surface area over six months, reveals an overall stimulation by microbial inoculation, although the magnitude of this response was strictly dependent on the water regime (Fig. 3A–C). Under non-stressed conditions, a progressive and steady increase in all parameters was recorded across all treatments; however, inoculated plants consistently outperformed the non-inoculated control. The superiority of the combined treatments was particularly marked at the end of the experiment in September, where the Cs1&AMF treatment exhibited the highest performance, notably for cladode number and surface area, with increases of + 81.81% and + 44.21% respectively compared to the control. Plant height was also synergistically stimulated, achieving a superiority of + 10.70% for the same treatment.

Under water-stress conditions, although overall growth was slowed by the water deficit, inoculation played a crucial role in mitigating the adverse effects of stress. The control consistently displayed the lowest values, confirming its sensitivity to water scarcity. Conversely, the Cs2&AMF and Cs1&AMF combinations were distinguished by a significant maintenance of growth. For the number of cladodes, the Cs2&AMF treatment even reached an increase of + 54.54% compared to the stressed control in September. Regarding height and surface area, the combined treatments maintained tissue expansion and vertical growth significantly superior to single inoculations such as Cs1, Cs2, or

Fig. 2 Sources of the variability of measured traits based on ANOVA two-way Mean squares



AMF alone. By September, Cs2&AMF and Cs1&AMF displayed heights that were + 33.84% and + 29.52% greater than the control, respectively. These results indicate that the interaction between bacterial consortia and arbuscular mycorrhizal fungi creates a favorable synergy for tissue initiation and expansion. This suggests that the PGPR&AMF complex improves nutrient uptake and physiological adjustment, enabling *O. ficus-indica* to maintain robust vegetative development even under unfavorable osmotic pressure.

The enhancement of vegetative growth observed in *O. ficus-indica* following microbial inoculation, and particularly under PGPR&AMF co-inoculation, was consistently reflected across cladode number, plant height, and cladode area. Together, these responses indicate a coordinated stimulation of photosynthetic organ development and biomass accumulation, even under water-deficit conditions, where non-inoculated control plants systematically exhibited the lowest performance. Similar trends have been reported in potted *O. ficus-indica*, where AMF inoculation promoted greater cladode production, canopy expansion, and overall plant vigor compared with non-inoculated controls, confirming that mycorrhizal symbiosis can accelerate cladode emission and vegetative growth over time (Greco et al. 2025). These growth improvements were associated with a marked increase in mycorrhizal colonization parameters

(frequency, intensity, and arbuscule frequency) in AMF-inoculated treatments compared with the control. Notably, co-inoculation with PGPR did not significantly modify colonization levels, indicating that the observed growth promotion was primarily driven by effective AMF establishment. Under drought stress, the general slowdown in cladode appearance, plant elongation, and tissue expansion is a well-documented response in cactus pear (Lahbouki et al. 2022). However, several studies have demonstrated that AMF inoculation, either alone or combined with bio-stimulants, mitigates these limitations by improving growth performance and physiological status under water deficit (Lahbouki et al. 2022, 2023). The maintenance of greater plant height, higher numbers of cladodes, and larger cladode surface areas in inoculated treatments observed in the present study is therefore consistent with previous findings showing that AMF enhances biomass production, water-use-related physiological traits, and photosynthetic performance of *O. ficus-indica* across different irrigation regimes (Kebede et al. 2024).

From a mechanistic perspective, the simultaneous improvement of these morphological traits under PGPR&AMF co-inoculation can plausibly be attributed to complementary and synergistic processes. AMF enhance phosphorus and nutrient acquisition through their

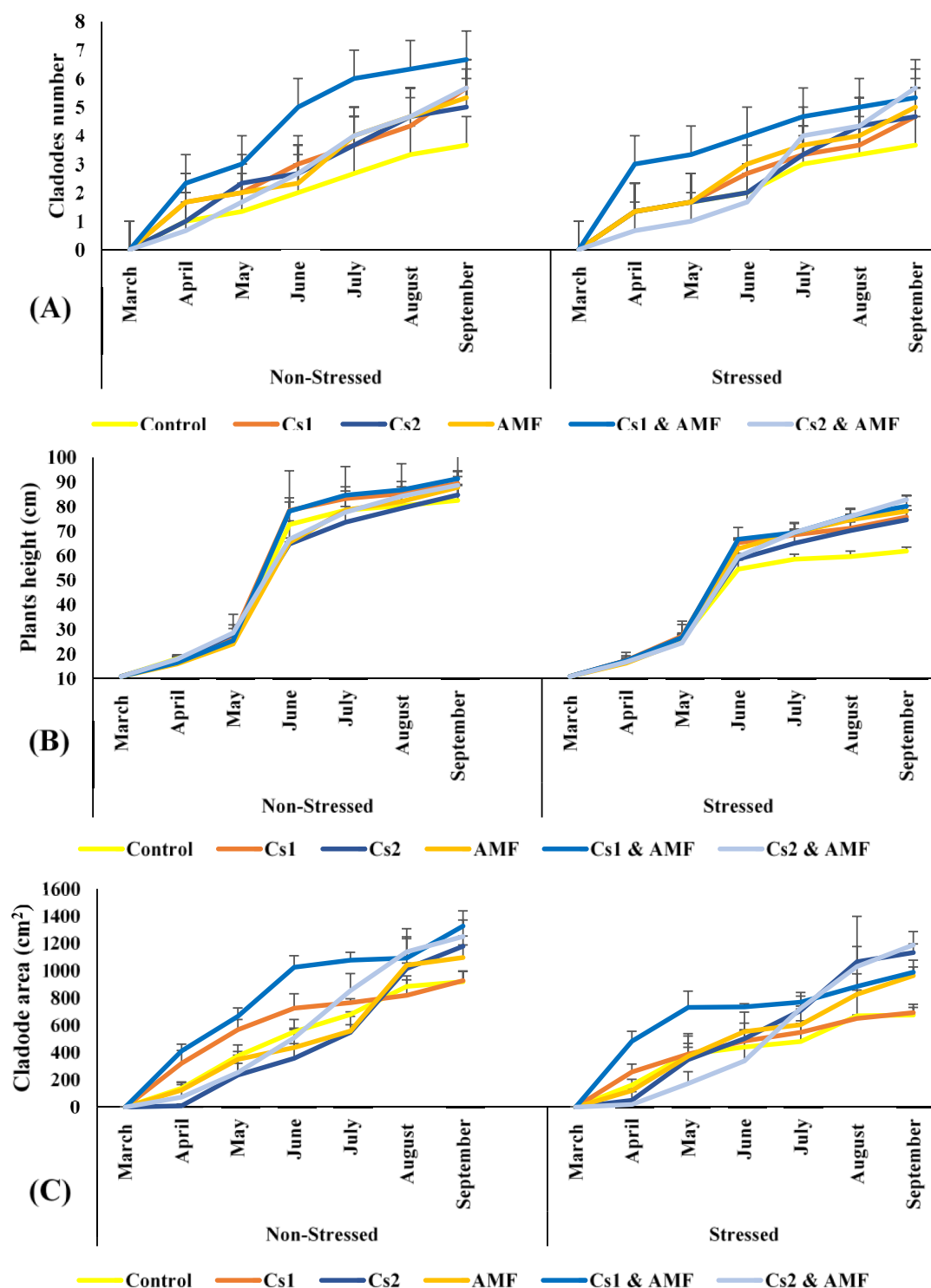


Fig. 3 Dynamics of cladode number (A), plants height (B), and cladode area (C) of *O. ficus-indica* in response to microbial inoculation under well-irrigated and drought conditions. Distinct letters indicate

significant differences among treatments at the same time point (one-way ANOVA followed by Tukey's test, $P \leq 0.05$)

extraradical hyphal networks, while PGPR contribute to improved rhizospheric functionality, root system development, and hormonal regulation (Backer et al. 2018; Hnini et al. 2024). These mechanisms collectively support sustained cell expansion, photosynthetic surface development, and shoot growth, particularly under limiting water availability. The increase in cladode area observed in co-inoculated plants is of particular physiological relevance, as cladodes constitute the primary photosynthetic organs and determine light interception capacity and CAM metabolic activity in *Opuntia*. Reports on AMF-inoculated *O. ficus-indica* similarly describe larger canopy development and increased shoot and root biomass relative to controls (Greco et al. 2025). Under drought conditions, the ability of inoculated plants to maintain greater cladode surface areas suggests improved water and mineral nutrition, enhanced osmotic adjustment, and increased protection against oxidative damage, responses that have been widely documented for PGPR and PGPR&AMF associations in both perennial and annual crops (Goswami et al. 2016; Khaliq et al. 2022; Khan et al. 2024; Tahiri et al. 2022).

Plant nutrient

The Table 1 presents the concentrations of N, P, and K (mg g⁻¹ DW of cladodes) in *O. ficus-indica* subjected to different microbial treatments under non-stressed and water-stressed conditions. Under non-stressed conditions, significant variations in nutrient contents were observed among treatments. Nitrogen content was highest in plants treated with Cs2 (+32.88%), whereas the control, Cs1, AMF alone, and combined treatments showed lower and statistically distinct values. Phosphorus concentration was significantly enhanced by AMF inoculation, either alone (+50.00%) or in combination Cs2&AMF (+45.45%), with the highest values recorded in AMF treated plants. Potassium content was

maximized in the Cs2&AMF treatment, increasing 12.74% compared with the control. Under water-stressed conditions, microbial inoculation significantly influenced plant nutrient status. Nitrogen content was highest in Cs2 treated plants (+31.63%), followed by AMF and combined treatments, while the control exhibited significantly lower values. Phosphorus content increased markedly in AMF and combined treatments, with AMF alone and Cs2&AMF showing the highest concentrations by 25.00% and 21.43%, respectively, compared to the control. Potassium accumulation was also significantly improved by microbial treatments, particularly Cs2 and Cs2&AMF (14.10% and 12.88%, respectively), compared with the stressed control.

The changes in cladode mineral composition (N, P, and K) observed in *O. ficus-indica* fall within the ranges reported in previous literature (Galizzi et al. 2004) and confirm that microbial inoculation can substantially modulate plant nutrient status. Our findings demonstrate that this response depends on both the inoculant type and water availability. In our experiment, the bacterial consortium Cs2 consistently maximized N concentrations under both non-stressed and water-stressed conditions, while the control remained significantly lower, indicating that Cs2 likely enhanced N acquisition and/or N assimilation capacity. Such improvements in N nutrition after PGPR inoculation are widely documented and are generally attributed to mechanisms including stimulation of root growth and activity, improvement of nutrient uptake efficiency, and, depending on the strains involved, facilitation of biological N inputs or enhanced N metabolism (Backer et al. 2018). Under drought, PGPR-driven induced systemic tolerance mechanisms, such as phytohormone modulation, exopolysaccharide production, and antioxidant stimulation, are also recognized as key processes that indirectly sustain nutrient uptake by maintaining root functionality and plant water relations (Kaushal and Wani 2016). Notably, similar PGPR and PGPR&AMF

Table 1 Effect of microbial treatments on nitrogen (N), phosphorus (P), and potassium (K) contents in *O. ficus-indica* cladodes under non-stressed and water-stressed conditions

	Treatments	N mg/g DW of cladodes	P mg/g DW of cladodes	K mg/g DW of cladodes
Non-stressed	Control	19.01 ± 0.19 ^h	2.20 ± 0.03 ^f	46.60 ± 0.06 ^e
	Cs1	19.90 ± 0.06 ^f	2.50 ± 0.04 ^e	47.20 ± 0.01 ^d
	Cs2	25.26 ± 0.06 ^b	2.60 ± 0.04 ^e	46.40 ± 0.03 ^{ef}
	AMF	19.89 ± 0.08 ^f	3.30 ± 0.05 ^{bc}	49.65 ± 0.07 ^b
	Cs1 & AMF	19.80 ± 0.09 ^{fg}	2.50 ± 0.12 ^e	48.65 ± 0.05 ^c
	Cs2 & AMF	19.59 ± 0.07 ^g	3.20 ± 0.03 ^c	52.54 ± 0.08 ^a
Stressed	Control	19.60 ± 0.03 ^g	2.80 ± 0.02 ^d	40.93 ± 0.07 ^j
	Cs1	20.60 ± 0.11 ^e	2.76 ± 0.05 ^d	41.46 ± 0.30 ⁱ
	Cs2	25.80 ± 0.08 ^a	3.20 ± 0.03 ^c	46.70 ± 0.05 ^e
	AMF	23.10 ± 0.07 ^c	3.50 ± 0.05 ^a	43.50 ± 0.09 ^g
	Cs1 & AMF	22.47 ± 0.03 ^d	3.21 ± 0.02 ^c	42.70 ± 0.09 ^h
	Cs2 & AMF	23.20 ± 0.02 ^c	3.40 ± 0.03 ^{ab}	46.20 ± 0.07 ^f

Distinct letters indicate significant differences among treatments at the same time point (one-way ANOVA followed by Tukey's test, $P \leq 0.05$)

mediated improvements in plant N, together with growth benefits, have been observed beyond cactus, including in maize, faba bean and wheat systems under stress, where co-inoculation improved plant performance and nutrient acquisition relative to uninoculated controls (Raklami et al. 2019; Zeng et al. 2024). Phosphorus uptake, in contrast, was clearly driven by AMF; under both irrigation regimes, P concentrations were markedly higher in AMF and combined treatments (AMF alone and Cs2&AMF showing the highest values), which matches the canonical role of AMF in enhancing P acquisition, especially under low moisture, where P diffusion in soil becomes strongly limiting. The mechanistic basis (hyphal foraging beyond root depletion zones, increased absorptive surface, and improved P transport to the host) is a central concept in mycorrhizal biology (Sally and David 2008). In addition, the broader literature shows that AMF can increase plant N and P content across species and conditions, with effect sizes often strengthened under environmental constraints (Wu et al. 2024). In annual crops, AMF&PGPR interactions are also repeatedly reported to enhance nutrient uptake and plant performance under stress, partly because PGPR can stimulate mycorrhizal establishment and function, producing additive or synergistic outcomes at the whole-plant level (Nadeem et al. 2014). Potassium showed its strongest enhancement in combined treatments, particularly Cs2&AMF, suggesting a synergistic interaction between rhizosphere bacteria and AMF in sustaining K acquisition and/or internal K homeostasis, especially relevant under drought, where maintaining ionic balance and osmotic adjustment is critical. The involvement of AMF in K nutrition is increasingly recognized, with syntheses explicitly addressing how mycorrhizal associations can improve plant K acquisition and the likely transport interfaces involved (Garcia and Zimmermann 2014). In parallel, the physiological importance of K under water deficit (osmotic adjustment, stomatal regulation, and drought response) is well established and reviewed in plant-stress literature (Nieves-Cordones et al. 2019). Evidence from other crops supports our pattern, co-inoculation has been shown to improve water relations and physiological functioning in maize under combined stresses (drought and heat) (Romero-Munar et al. 2023), and multiple systems report stronger nutrient uptake and performance under dual inoculation than under single inoculation, consistent with the idea that AMF-driven nutrient foraging and PGPR-driven rhizosphere mobilization/root stimulation can act complementarily.

Soil physicochemical characteristics

Table 2 reports the concentrations of major mineral elements in the soil, including ammonium, nitrate, calcium,

potassium, magnesium, sodium, and phosphorus, measured under non-stressed and water-stressed conditions following different microbial treatments. Under non-stressed conditions, soil NH_4^+ concentrations showed only slight variations among treatments, with no strong differentiation between the control and inoculated soils. In contrast, NO_3^- content was significantly higher in control and soils associated with Cs1, and Cs2 treatments, while AMF and combined treatments (Cs1 & AMF and Cs2 & AMF) exhibited significantly lower nitrate levels. For calcium, no significant differences were observed among treatments, indicating that microbial inoculation did not markedly affect soil Ca availability under non-stressed conditions. Similarly, magnesium content did not differ significantly among most treatments, except for the Cs1&AMF combination, which showed a significantly lower Mg concentration compared with the control and single inoculations. Potassium availability was significantly reduced in soils associated with Cs1&AMF and Cs2&AMF treatments compared with the control, whereas single inoculations showed intermediate values. Soil phosphorus content also tended to decrease in inoculated treatments, with significantly lower values observed for Cs1&AMF and Cs2&AMF compared with the control. Under water stress, soil mineral contents were more strongly affected by microbial treatments. The control soil exhibited the highest NO_3^- concentration, while all inoculated treatments showed significantly lower nitrate levels, indicating increased nitrate removal from the soil. NH_4^+ concentrations were less affected and showed minor differences among treatments. Calcium and magnesium contents were generally lower in inoculated soils than in the control, particularly in soils associated with AMF and combined treatments, although differences were not significant statistically. Potassium levels were significantly reduced in inoculated soils, especially in Cs1&AMF compared with the stressed control. Phosphorus content also decreased significantly in soils receiving microbial inoculation, with the lowest values observed in Cs1&AMF and Cs2&AMF treatments. Other elements (Mn, Zn, Mo, Co, Cu, and Fe) were detected at very low concentrations ($<0.07 \mu\text{g g}^{-1}$ soil) under all treatments and water regimes.

The present study demonstrates that microbial inoculation significantly modulates soil nutrient dynamics in *O. ficus-indica*, particularly under water-stressed conditions. Beyond the general enhancement of nutrient uptake already highlighted, the differential responses observed among bacterial consortia, AMF, and their combinations provide insight into the underlying mechanisms governing soil–plant nutrient transfer in cactus systems. The marked reduction in soil nitrate concentrations in AMF and combined treatments, especially under water stress, suggests an enhanced nitrate acquisition and assimilation by *O. ficus-indica*. Mycorrhizal

Table 2 Effect of microbial treatments on soil mineral composition under non-stressed and water-stressed conditions

Treatments	NH ₄ ⁺ μg/g of soil	NO ₃ ⁻ μg/g of soil	Ca μg/g of soil	K μg/g of soil	Mg μg/g of soil	Na μg/g of soil	P μg/g of soil
Non-stressed							
Control	1.09 ± 0.03 ^b	8.57 ± 0.02 ^d	6506.00 ± 153.00 ^a	30.46 ± 0.60 ^a	264.10 ± 19.00 ^a	117.43 ± 7.20 ^a	4.34 ± 0.14 ^{ab}
Cs1	1.06 ± 0.01 ^{bc}	7.12 ± 0.01 ^h	6451.00 ± 210.00 ^a	28.39 ± 2.84 ^{abc}	245.60 ± 19.60 ^a	75.62 ± 7.63 ^b	3.44 ± 0.13 ^{ef}
Cs2	1.00 ± 0.01 ^{ef}	7.66 ± 0.04 ^g	6407.00 ± 160.50 ^a	25.57 ± 1.52 ^{bcd}	230.50 ± 19.20 ^{ab}	118.88 ± 6.05 ^a	3.86 ± 0.08 ^{cd}
AMF	0.96 ± 0.01 ^f	6.31 ± 0.01 ⁱ	6409.00 ± 216.00 ^a	24.27 ± 1.51 ^{cde}	250.00 ± 18.70 ^a	89.69 ± 4.97 ^b	4.01 ± 0.09 ^{ac}
Cs1 & AMF	0.88 ± 0.01 ^g	6.02 ± 0.06 ^j	6284.30 ± 142.80 ^a	17.20 ± 1.56 ^f	180.88 ± 12.96 ^b	76.79 ± 5.42 ^b	3.21 ± 0.07 ^f
Cs2 & AMF	0.78 ± 0.01 ^h	5.48 ± 0.01 ^k	6101.00 ± 195.00 ^a	20.96 ± 0.97 ^{ef}	218.60 ± 19.9 ^{ab}	76.62 ± 4.57 ^b	3.60 ± 0.05 ^{de}
Stressed							
Control	1.14 ± 0.01 ^a	16.38 ± 0.02 ^a	6427.00 ± 231.00 ^a	30.57 ± 0.79 ^a	259.20 ± 20.90 ^a	114.89 ± 3.59 ^a	4.37 ± 0.24 ^a
Cs1	1.05 ± 0.04 ^{bcd}	8.23 ± 0.02 ^c	6385.00 ± 270.00 ^a	23.36 ± 1.79 ^{de}	250.50 ± 18.60 ^a	90.88 ± 5.67 ^b	3.79 ± 0.08 ^{cd}
Cs2	1.02 ± 0.01 ^{cde}	14.24 ± 0.02 ^b	6357.50 ± 55.00 ^a	26.52 ± 0.85 ^{abcd}	252.75 ± 12.05 ^a	77.05 ± 4.98 ^b	4.07 ± 0.08 ^{abc}
AMF	1.05 ± 0.01 ^{bcd}	8.76 ± 0.01 ^c	6367.50 ± 119.00 ^a	29.62 ± 1.24 ^{ab}	236.65 ± 15.14 ^a	84.82 ± 3.88 ^b	4.06 ± 0.14 ^{abc}
Cs1 & AMF	1.01 ± 0.01 ^{de}	6.37 ± 0.01 ⁱ	6352.00 ± 273.00 ^a	17.75 ± 1.15 ^f	217.80 ± 18.80 ^{ab}	78.74 ± 5.93 ^b	3.53 ± 0.11 ^{def}
Cs2 & AMF	0.96 ± 0.02 ^f	7.78 ± 0.01 ^f	6149.20 ± 104.60 ^a	26.29 ± 2.32 ^{abcd}	237.00 ± 17.40 ^a	80.82 ± 2.49 ^b	3.74 ± 0.05 ^{cde}

Distinct letters indicate significant differences among treatments at the same time point (one-way ANOVA followed by Tukey's test, $P \leq 0.05$)

Other measured elements (Mn, Zn, Mo, Co, Cu and Fe) showed low concentrations ($< 0.01 \mu\text{g/g of soil}$), which corresponds to the detection limit of the apparatus (analytical range: 0.01 to 1000 $\mu\text{g/g}$)

fungi are known to improve nitrogen uptake efficiency by increasing soil exploration and facilitating nitrate transport to host plants (Smith and Read 2008). In cacti, improved nitrogen uptake has been linked to enhanced CAM metabolism and drought tolerance, allowing plants to maintain productivity under arid conditions (Cui and Nobel 1992; Nobel et al. 2002). Comparable reductions in soil nitrate following microbial inoculation have been reported in cereals and legumes, where increased microbial-assisted uptake reduces residual soil nitrate pools (Mahmood et al. 2014; Vessey 2003). Phosphorus depletion in soils receiving combined PGPR&AMF treatments highlights the strong synergistic effect between bacterial consortia and mycorrhizal fungi. AMF are particularly efficient in P uptake through their extraradical hyphal networks, while PGPR enhance P solubilization and mineralization, increasing P availability for plant absorption (Jha and Saraf 2015; Marschner 2011b). In *O. ficus-indica*, such synergistic interactions have previously been shown to improve plant growth and nutrient status under both optimal and stressful environmental conditions (Greco et al. 2025; Lahbouki et al. 2022, 2023). Similar responses have been observed in other arid and semi-arid plant species, emphasizing the ecological relevance of combined microbial inoculation strategies (Zarik et al. 2016). The significant decline in soil potassium levels under combined treatments, particularly under drought, reflects increased plant uptake driven by enhanced microbial activity. Potassium plays a central role in osmotic adjustment, stomatal regulation, and enzyme activation, all of which are critical for drought tolerance (Marschner 2011a). AMF associated plants often exhibit improved K nutrition under water stress, contributing to better water-use efficiency and stress resilience (Robert 2001; Ruiz-Lozano et al. 2012). The reduction in soil NH₄⁺, NO₃⁻, K, Mg, and P observed in inoculated treatments should therefore be interpreted as an indicator of enhanced plant nutrient acquisition rather than a decline in soil fertility, as similar patterns have been widely associated with increased nutrient accumulation in plant tissues following microbial inoculation (Singh et al. 2019; Tahiri et al. 2022). The limited variation in soil Ca and Mg concentrations among treatments under non-stressed conditions suggests that these elements were not limiting and that microbial inoculation did not substantially alter their soil availability. The pronounced reductions in soil nitrate, potassium, and phosphorus under water stress in inoculated treatments highlight the crucial role of microbial symbioses in sustaining nutrient acquisition under limiting water availability. AMF and combined PGPR&AMF treatments were particularly effective, supporting previous findings that mycorrhizal associations enhance drought tolerance by improving nutrient uptake, root hydraulic conductivity, and

soil exploration (Lahbouki et al. 2022, 2023; Raklami et al. 2019; Sbbar et al. 2025; Tahiri et al. 2022).

Table 3 summarizes soil pH (H₂O and KCl), electrical conductivity, and organic matter content under non-stressed and water-stressed conditions following different microbial treatments. Under non-stressed and stressed conditions, soil pH remained alkaline across all treatments but was consistently lower in PGPR and combined PGPR&AMF inoculations compared with the uninoculated control. This observation is consistent with previous research (Chen et al. 2023; Orhan et al. 2006) showing that PGPR and AMF can decrease soil pH through organic acid production, and enhanced root exudation, which improve nutrient solubility, particularly under alkaline conditions (Chen et al. 2023). The relatively small changes in pH KCl reflect moderate shifts in the soil acidity buffer rather than dramatic chemical alteration. In contrast, Soil pH KCl was highest in AMF-treated soil under stress, possibly reflecting altered ionic exchange in the presence of extended hyphal networks, which has been reported to influence soil acidity gradients and nutrient dynamics in other AMF studies (Yu et al. 2022). Under non-stressed conditions, electrical conductivity, which reflects the concentration of soluble ions, varied significantly among treatments. Inoculated soils exhibited higher EC values (Cs2&AMF showing the highest EC values +15.01% compared to the control) suggesting increased ionic mobilization. In similar inoculation studies, elevated EC has been interpreted not as salinity stress but as enhanced nutrient release and turnover driven by microbial activity and root exudation (Cancellier et al. 2018). However, EC was significantly higher in the stressed control than in any inoculated treatment, indicating that microbial inoculation stabilized soil ionic balance and reduced salt accumulation under drought. Similar trends have been reported in saline-alkaline soils where combined AMF and PGPR inoculation can lower EC by improving ion homeostasis and reducing the concentration of free salts through enhanced plant uptake and soil binding processes

(Yu et al. 2022). Organic matter content was systematically higher in inoculated soils than in the control, in both irrigation regimes (OM content exceeded 200% for the Cs2 and Cs2&AMF treatments under non-stressed conditions, and remained above 100% under water stress, relative to the non-inoculated control), indicating that microbial inoculation enhanced carbon inputs and OM stabilization (Mason et al. 2023). This likely reflects increased root exudation, microbial biomass accumulation, and enhanced decomposition of residues, processes documented in synergistic PGPR&AMF studies in other crops and linked to improved soil structure and nutrient cycling in drought and salt stress (Jing et al. 2025; Tahiri et al. 2022). These results demonstrate that microbial inoculation modifies soil properties in ways that could improve soil quality and nutrient retention. The slight acidification observed with PGPR and combined PGPR&AMF under non-stressed conditions may enhance nutrient availability in alkaline soils, offsetting limitations common in many arid environments (Chen et al. 2023). Under water stress, the reduced EC and increased OM associated with inoculation likely reflect a more balanced soil ionic composition and improved structural stability, both of which are vital for sustaining plant growth on soils that are low in mineral and organic compounds, with poor fertility and mineral nutrient scarcity (Weber et al. 2007).

Soil enzymatic activities

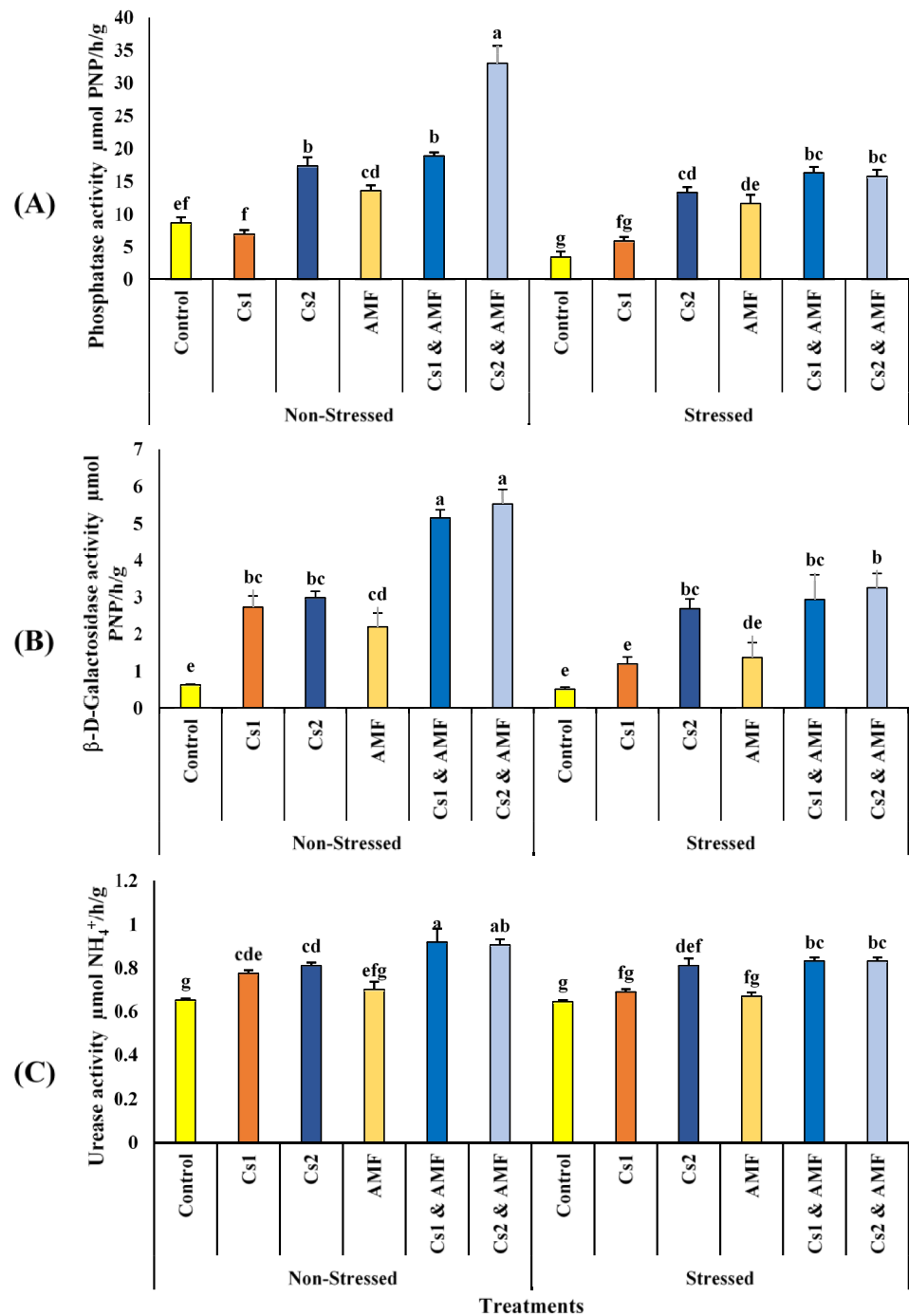
Figure 4A shows soil phosphatase activity ($\mu\text{g PNP g}^{-1} \text{h}^{-1}$) under non-stressed and water-stressed conditions for the non-inoculated control and microbial treatments. Under non-stressed conditions, phosphatase activity differed significantly among treatments. The control exhibited the lowest activity, while inoculated soils showed markedly higher values. Among treatments, Cs2&AMF (+282.27%) displayed the highest phosphatase activity, followed by Cs1&AMF (+118.42%) and Cs2 (+100.67%), indicating

Table 3 Effects of microbial inoculants on soil pH, electrical conductivity, and organic matter content under non-stressed and water-stressed conditions

	Treatments	pH _{H₂O}	pH _{KCl}	Conductivity ($\mu\text{S/cm}$)	Organic matter (%)
Non-Stressed	Control	8.82 $\pm$ 0.05 ^a	7.86 $\pm$ 0.05 ^{ab}	141.23 $\pm$ 4.87 ^c	1.64 $\pm$ 0.41 ^c
	Cs1	8.47 $\pm$ 0.04 ^{bc}	7.85 $\pm$ 0.03 ^b	156.23 $\pm$ 3.65 ^{bc}	3.52 $\pm$ 0.71 ^{bc}
	Cs2	8.37 $\pm$ 0.04 ^{cde}	7.76 $\pm$ 0.04 ^b	156.80 $\pm$ 2.42 ^{bc}	5.87 $\pm$ 0.41 ^a
	AMF	8.47 $\pm$ 0.02 ^{bcd}	7.89 $\pm$ 0.04 ^{ab}	140.20 $\pm$ 2.91 ^e	3.99 $\pm$ 1.08 ^{ab}
	Cs1 & AMF	8.32 $\pm$ 0.05 ^{de}	7.93 $\pm$ 0.05 ^{ab}	158.37 $\pm$ 1.63 ^{abc}	4.46 $\pm$ 0.81 ^{ab}
	Cs2 & AMF	8.35 $\pm$ 0.05 ^{cde}	7.88 $\pm$ 0.07 ^{ab}	162.43 $\pm$ 2.91 ^{ab}	5.64 $\pm$ 0.71 ^a
Stressed	Control	8.56 $\pm$ 0.12 ^b	7.76 $\pm$ 0.13 ^b	165.80 $\pm$ 2.17 ^a	1.88 $\pm$ 0.41 ^c
	Cs1	8.53 $\pm$ 0.02 ^b	7.86 $\pm$ 0.06 ^{ab}	151.03 $\pm$ 3.41 ^{cd}	3.29 $\pm$ 1.08 ^{bc}
	Cs2	8.36 $\pm$ 0.03 ^{cde}	7.82 $\pm$ 0.02 ^b	144.13 $\pm$ 1.74 ^{de}	4.23 $\pm$ 0.71 ^{ab}
	AMF	8.43 $\pm$ 0.01 ^{bcd}	8.03 $\pm$ 0.06 ^a	141.30 $\pm$ 0.92 ^e	3.52 $\pm$ 0.71 ^{bc}
	Cs1 & AMF	8.49 $\pm$ 0.07 ^{bc}	7.82 $\pm$ 0.05 ^b	151.40 $\pm$ 3.32 ^{cd}	3.99 $\pm$ 0.41 ^{ab}
	Cs2 & AMF	8.24 $\pm$ 0.03 ^e	7.90 $\pm$ 0.08 ^{ab}	127.73 $\pm$ 2.72 ^f	5.17 $\pm$ 0.40 ^{ab}

Distinct letters indicate significant differences among treatments at the same time point (one-way ANOVA followed by Tukey's test, $P \leq 0.05$)

Fig. 4 Effect of microbial inoculants on soil phosphatase (A), b-D-Galactosidase (B), and urease (C) activities under non-stressed and water-stressed conditions in *O. ficus-indica*. Distinct letters indicate significant differences among treatments at the same time point (one-way ANOVA followed by Tukey's test, $P \leq 0.05$)



a strong stimulation of phosphorus-related enzymatic processes. Under water-stressed conditions, phosphatase activity was generally reduced compared with non-stressed soils. However, inoculated treatments maintained significantly higher activity than the control. The combined treatments (Cs1&AMF and Cs2&AMF) again showed the highest values, corresponding to an increase of 728.31% and 789.25%, respectively, relative to the control, whereas single inoculations exhibited intermediate responses.

Soil β -D-galactosidase activity ($\mu\text{mol PNP g}^{-1} \text{h}^{-1}$) varied significantly among treatments under both water regimes (Fig. 4B). Under non-stressed conditions, microbial inoculation significantly enhanced β -galactosidase activity compared with the control. The very large relative increases are attributable to the low basal β -galactosidase activity measured in the non-inoculated control soil, resulting in high percentage differences despite moderate absolute increases in enzyme activity. The highest activities were recorded in

soils treated with Cs1&AMF and Cs2&AMF by 728.31% and 789.25%, respectively, followed by bacterial consortia and AMF alone. Under water stress, β -galactosidase activity decreased across all treatments; however, inoculated soils retained significantly higher activity than the non-inoculated control. Combined treatments showed superior performance compared with single inoculations, indicating greater maintenance of carbon-degrading enzymatic activity under stress conditions.

Urease activity ($\mu\text{mol NH}_4^+ \text{ g}^{-1} \text{ h}^{-1}$) also showed significant differences among treatments under both non-stressed and stressed conditions (Fig. 4C). Under non-stressed conditions, inoculated treatments exhibited higher urease activity than the control, with the highest values observed in Cs1&AMF and Cs2&AMF treatments (+40.68% and +38.86%, respectively). Single inoculations showed intermediate activities, while the control had the lowest value. Under water-stressed conditions, urease activity declined slightly compared with non-stressed soils. Nevertheless, microbial inoculation significantly mitigated this reduction. Combined PGPR&AMF treatments maintained the highest urease activity, followed by Cs2 alone, whereas the control again showed the lowest activity.

Soil enzymes are critical biochemical catalysts that drive nutrient mineralization and organic matter transformation, thereby regulating soil fertility and ecosystem function (Ferioun, et al. 2025). These enzymes mediate the depolymerization of complex organic substrates into bioavailable forms of carbon, phosphorus, and nitrogen (Moreira and Siqueira 2006). As sensitive functional indicators of soil biological activity, specific enzymes such as β -galactosidase (C cycling), phosphatase (P cycling), and urease (N cycling) are widely utilized in soil health assessments (Stone et al. 2014; Wallenstein and Burns 2011). In the present study, microbial inoculation markedly enhanced soil enzymatic activities compared with the non-inoculated control under both irrigation regimes. Under non-stressed conditions, phosphatase activity was strongly stimulated in inoculated soils, with the highest values observed in the combined treatments (Cs1&AMF and Cs2&AMF). This response is consistent with previous findings in *O. ficus-indica* rhizosphere soils, where elevated phosphatase activity was associated with higher microbial abundance, organic matter content, and phosphorus availability (Zouitane et al. 2025). Phosphatases are essential for organic phosphorus mineralization, and their activity often increases when phosphorus is limiting, as extracellular phosphatases hydrolyze phosphate esters to release plant-available inorganic phosphate (Jones and Oburger 2011). Similar stimulation of phosphatase activity following microbial inoculation has been widely reported, with some bacterial isolates increasing soil phosphatase activity compared with the uninoculated

control (Tirry et al. 2024). β -D-galactosidase activity, a key indicator of soil carbon cycling and microbial metabolism of labile carbon substrates, was significantly elevated in inoculated treatments, with the most pronounced increases observed under combined PGPR&AMF inoculation. This pattern aligns with findings from *Opuntia* rhizosphere soils in sub-humid and semi-arid ecosystems, where heightened β -galactosidase activity correlated positively with increased soil organic matter content and enhanced microbial functional diversity (Gianfreda and Ruggiero 2006; Zouitane et al. 2025). Comparable inoculation studies in wheat have reported substantial boosts in β -galactosidase activity following bacterial treatments with *Flavobacterium* (Mawdsley and Burns 1994). Urease activity showed a similar response pattern, with significantly higher activity in inoculated soils compared with the control, and maximum values recorded under combined PGPR&AMF treatments. Urease catalyzes the hydrolysis of urea into ammonium, a key step in nitrogen mineralization and availability. In *O. ficus-indica* rhizosphere soils, high urease activity has previously been linked to elevated ammonium content and higher microbial abundance, particularly in soils from more favorable climatic regions (Zouitane et al. 2025). The increased urease activity observed in this study suggests enhanced nitrogen cycling and stimulation of overall soil microbial activity following microbial inoculation production (Calvo et al. 2019). Although urease production by the inoculated bacterial strains was not specifically evaluated, the higher soil urease activity may result from the synergistic interactions between PGPR, AMF, and the indigenous soil microbial community, which collectively promote nitrogen transformation and nutrient availability (Wang et al. 2025). Under water-stressed conditions, all enzymatic activities declined relative to non-stressed soils, reflecting the well-known inhibitory effect of drought on microbial metabolism and enzyme activity (Bergstrom et al. 1998). Soil moisture strongly regulates enzyme diffusion, substrate availability, and microbial proliferation, making enzymatic activities highly sensitive to water deficit. This pattern has also been documented in *O. ficus-indica* rhizosphere soils across arid and semi-arid regions, where enzymatic activities decrease with increasing climatic aridity (Zouitane et al. 2025). Nevertheless, despite this general decline, inoculated treatments, particularly the combined PGPR&AMF inoculations, maintained significantly higher enzymatic activities than the non-inoculated control. The present findings, together with previous work on *O. ficus-indica* rhizosphere soils (Zouitane et al. 2025) and inoculation studies in other plants (Ferioun et al. 2025; Tirry et al. 2024), demonstrate that microbial inoculation significantly stimulates soil enzymatic activities associated with carbon, phosphorus, and nitrogen cycling. This stimulation most likely reflects

the combined response of the inoculated microorganisms, arbuscular mycorrhizal fungi, and the indigenous soil microbial community rather than direct evidence of enzyme production by the inoculated bacterial strains.

Soil microbial functional diversity

Soil microbial metabolic activity, measured via AWCD, increased progressively over time in all treatments, though water stress globally reduced its magnitude (Fig. 5A). Under non-stressed conditions, Cs1 (+352.61%) and Cs1&AMF (+304.82%) reached the highest metabolic levels by the end of incubation. In contrast, under water stress, the combined Cs2&AMF (+129.04%) and AMF (+127.34%) treatments were the most effective in maintaining significantly higher functional activity than the control. These results show a progressive increase in microbial metabolic activity over incubation time, following a typical sigmoid pattern of AWCD, which reflects successive phases of microbial acclimation, growth, and stabilization of substrate utilization capacity. Such sigmoid kinetic trends have been widely reported in community-level physiological profiling studies and are considered indicative of the gradual activation of soil microbial communities in response to available carbon sources (Grzadziel et al. 2019; Xu and Ge 2015). Similar AWCD patterns were previously observed in rhizospheric soils associated with *O. ficus-indica* across different regions, where variations in metabolic kinetics were strongly influenced by biogeoclimatic conditions and soil properties (De Lyra et al. 2021; Karray et al. 2020; Zouitane et al. 2025). Under non-stressed conditions, the higher AWCD values recorded in inoculated soils, particularly in treatments combining bacterial consortia with arbuscular mycorrhizal fungi (Fig. 5), indicates enhanced microbial metabolic activity and functional diversity compared with the non-inoculated control. This stimulation of microbial metabolism is consistent with previous studies on barley soils, which demonstrated that microbial communities rapidly respond to changes in soil chemical and biological conditions and play a central role in ecosystem functioning (Ferioun et al. 2025). As a CAM species adapted to semi-arid environments, *O. ficus-indica* can significantly modify its rhizospheric microbial community, promoting the development of functionally active microorganisms capable of efficient substrate utilization under favorable conditions (Collins et al. 2008; De Lyra et al. 2021). Under water-stressed conditions, the overall reduction in AWCD values confirms the inhibitory effect of moisture limitation on microbial metabolism, as water stress restricts substrate diffusion and enzymatic activity (Schimel 2018; Taketani et al. 2014). However, inoculated treatments maintained significantly higher AWCD levels than the stressed control, highlighting the functional resilience of

microbial communities supported by bacterial consortia and AMF. This resilience is characteristic of microorganisms inhabiting arid and semi-arid soils, where repeated cycles of drying and rewetting exert strong selective pressure on microbial communities (Placella et al. 2012; Schimel et al. 2007). The ability of these microorganisms to persist under stress has been linked to the presence of genes involved in survival mechanisms such as biofilm formation, extracellular polysaccharide production, and osmolyte synthesis (Ude et al. 2006; Ward et al. 2009; Yancey 2005), as well as the upregulation of carbohydrate metabolism genes that contribute to water retention and cellular protection (McNeil et al. 1999; Nocker et al. 2012).

Inoculation also expanded the functional breadth of the soil microbial communities compared to the control (Fig. 5B). While non-inoculated soils preferred carbohydrates and polymers, inoculated treatments, especially the Cs&AMF combinations, exhibited a more versatile profile, with dramatic increases in amino acid and carboxylic acid consumption (up to 492% and 557%, respectively). Under stress, the Cs2&AMF combination maintained the highest utilization of amines, amides (+101.24%), and polymers (+32.29%). This metabolic flexibility highlights the synergy between PGPR and AMF in enhancing resource partitioning and community resilience. Such shifts in substrate utilization patterns reflect enhanced functional diversity and ecological efficiency and have been associated with changes in the bacteria-to-fungi ratio and soil physicochemical properties, particularly soil pH (Aciego Pietri & Brookes 2008; Janusz et al. 2010). Previous studies demonstrated that soils with pH values closer to neutrality support higher bacterial abundance and metabolic activity, whereas acidic soils tend to favor fungal dominance and reduced overall microbial activity (Grzadziel et al. 2019; Janusz et al. 2010). The reduced metabolism of amino acids observed in less biologically active soils can be explained by the strong influence of pH on enzyme activity, as most redox enzymes involved in amino acid degradation have optimal activity at neutral pH values (Grzadziel et al. 2019; Januszek et al. 2015). Similarly, higher carboxylic acid utilization has been associated with soils exhibiting greater microbial activity and favorable physicochemical conditions, although this relationship may vary depending on substrate type and soil characteristics (Adams et al. 2017; Gałazka et al. 2017). Under water-stressed conditions, substrate utilization decreased across all treatments; however, inoculated soils retained higher metabolic activity than the stressed control. Combined treatments, particularly Cs2&AMF, showed enhanced utilization of amines, amides, and polymers, suggesting an adaptive metabolic response to stress. The preferential metabolism of nitrogen-rich compounds such as amines and amides may reflect microbial strategies aimed at maintaining osmotic

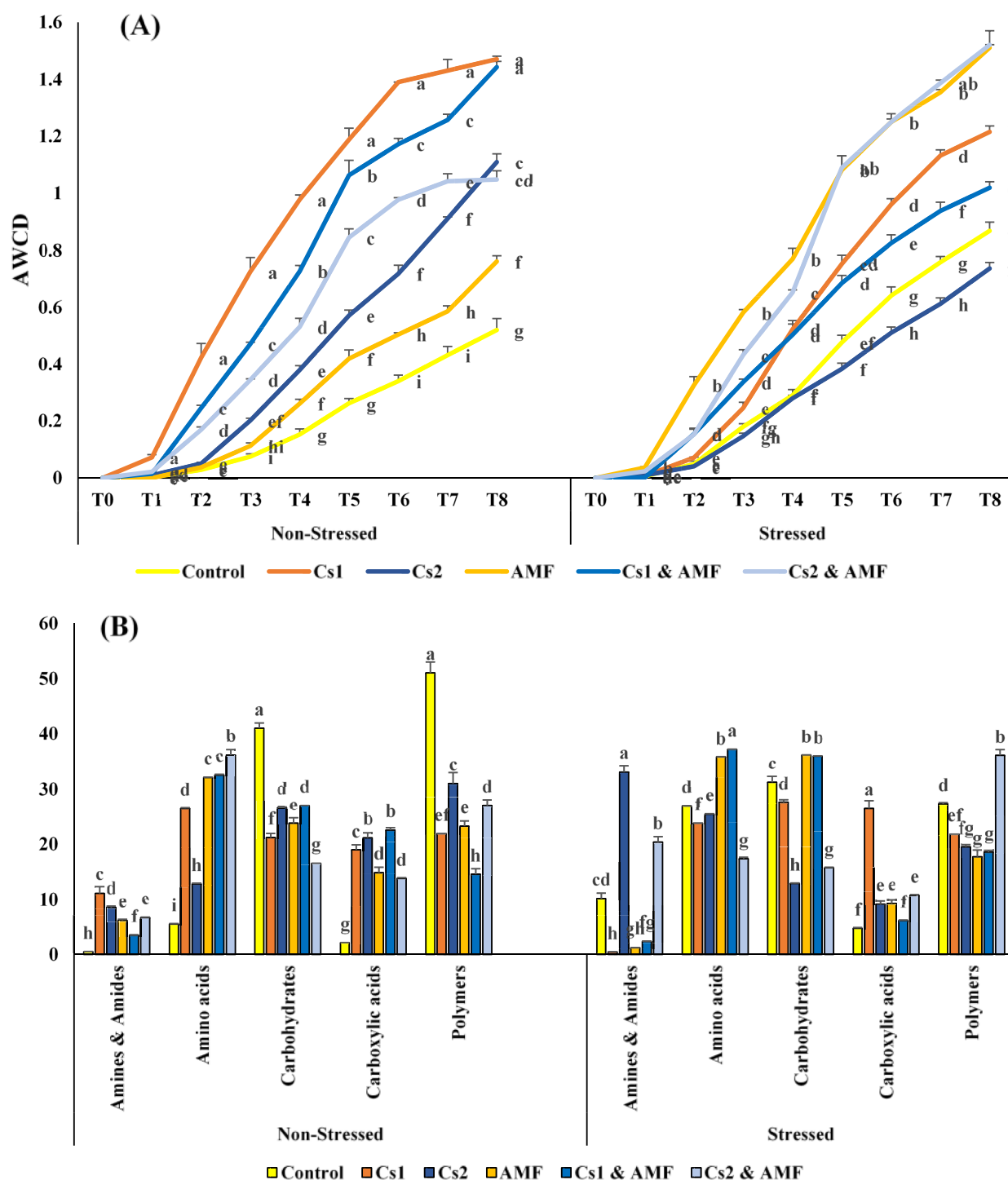


Fig. 5 Microbial metabolic activity kinetics (A) and substrate utilization patterns (B) of soil microbial communities under non-stressed and water-stressed conditions assessed by Biolog EcoPlates. Distinct let-

ters indicate significant differences among treatments at the same time point (one-way ANOVA followed by Tukey's test, $P \leq 0.05$)

balance and stress tolerance through the synthesis and turnover of organic osmolytes (Grzyb et al. 2021; Yancey 2005). Such metabolic flexibility is a key trait of microorganisms adapted to arid environments and contributes to sustained

organic matter cycling under limiting conditions (Schimel et al. 2007).

Microbial diversity indices significantly improved following inoculation, regardless of the water regime (Table 4).

Under non-stressed conditions, the control exhibited the lowest Shannon diversity ($H' = 2.21$) and richness ($S = 6.33$), whereas bacterial treatments, led by Cs1 and Cs1&AMF, achieved the highest values ($H' = 2.90$ – 2.93 ; $S = 18$ – 23.67). While diversity increased, evenness slightly decreased in these treatments, reflecting the dominance of functionally active taxa. Under water stress, diversity patterns shifted; the AMF treatment reached the highest diversity ($H' = 2.97$) and richness ($S = 21$). Combined inoculations, notably Cs2&AMF, maintained robust functional diversity, demonstrating their ability to sustain the functional potential of the soil microbial community under water-stressed conditions.

Similar increases in microbial diversity, assessed through next-generation sequencing approaches, have been reported in *O. ficus-indica* soils across different bioclimatic zones, where both aridity gradients and plant compartment were identified as key drivers of microbial community differentiation (Karray et al. 2020). Studies conducted on cactus-associated soils in Brazil also showed that environmental and soil conditions strongly influence microbial diversity patterns, reinforcing the role of *O. ficus-indica* as a driver of rhizospheric microbial structuring (De Lyra et al. 2021). Under water stress, the shift in diversity patterns, with higher Shannon diversity and richness observed in AMF treated soils, suggests a stabilizing role of mycorrhizal fungi in maintaining microbial community complexity under adverse conditions (Chourasiya et al. 2021). This protective effect could be attributed to improved soil structure, enhanced carbon allocation to the rhizosphere, and better plant–microbe interactions under stress (Estrada-González et al. 2023; Kavamura et al. 2018). In arid ecosystems, microbial diversity and community composition are closely linked to the capacity of microorganisms to endure water limitation and fluctuating moisture availability (Schimel 2018).

The heatmap (Fig. 6) illustrates the carbon substrate utilization profiles obtained from the Biolog EcoPlate assay,

reflecting the metabolic activity of viable microorganisms present in the soil samples under non-stressed and water-stressed conditions following the different microbial inoculation treatments. Under non-stressed conditions, microbial communities associated with inoculated treatments exhibited higher and more diversified substrate utilization profiles compared with the non-inoculated control. The combined treatments (Cs1&AMF and Cs2&AMF) formed distinct clusters characterized by elevated utilization of a wide range of substrates, including carbohydrates (e.g., β -methyl-D-glucoside, D-mannitol), amino acids (e.g., L-asparagine, L-serine, L-phenylalanine), and organic acids (e.g., α -ketobutyric acid). In contrast, the control showed generally lower metabolic activity across most substrates. Under water-stressed conditions, the overall intensity of substrate utilization decreased relative to non-stressed soils. However, inoculated treatments, particularly AMF and the combined bacterial consortia, maintained higher metabolic activity and clustered separately from the control. Notably, the Cs2 and Cs2&AMF treatments exhibited high utilization of putrescine and glycyl-L-glutamic acid, suggesting an enhanced capacity to metabolize nitrogen-rich and stress-related compounds. In addition, the combined treatments Cs1&AMF and Cs2&AMF showed pronounced utilization of L-asparagine and pyruvic acid methyl ester, indicating an active engagement in amino acid and central carbon metabolism under stress conditions. In contrast, the control and some single bacterial inoculations displayed weaker and less diverse metabolic responses.

The heatmap and cluster analyses further demonstrated that microbial inoculation substantially altered the functional organization of soil microbial communities. Comparable results have been reported in long-term studies of soils with contrasting biological quality, where higher enzymatic activity and functional redundancy were associated with greater buffering capacity of the soil microbiome and persistence of physiological profiles over time (Ferioun et

Table 4 Effects of microbial inoculations on soil microbial diversity indices (Shannon diversity, Shannon evenness, and richness) under non-stressed and stressed conditions

	Treatments	Shannon diversity H'	Shannon Evenness E	Richness S
Non-stressed	Control	2.21 ± 0.02 g	1.24 ± 0.02^a	6.33 ± 0.58 g
	Cs1	2.90 ± 0.01^{abc}	0.90 ± 0.09^d	23.67 ± 1.53^a
	Cs2	2.79 ± 0.1 cd	0.99 ± 0.09^{bcd}	16.00 ± 1.00^{bc}
	AMF	2.68 ± 0.01^d	1.22 ± 0.02^a	9.00 ± 1.00 fg
	Cs1 & AMF	2.93 ± 0.02^{ab}	1.00 ± 0.02^{bcd}	18.00 ± 1.00^b
	Cs2 & AMF	2.67 ± 0.07^{de}	0.96 ± 0.03 cd	16.33 ± 0.58^{bc}
Stressed	Control	2.66 ± 0.01^{de}	1.07 ± 0.01^{bc}	12.00 ± 0.00^{de}
	Cs1	2.79 ± 0.05 cd	1.03 ± 0.03^{bcd}	14.00 ± 1.00 cd
	Cs2	2.54 ± 0.04^{ef}	1.11 ± 0.05^{ab}	9.67 ± 0.58^{ef}
	AMF	2.97 ± 0.02^a	0.98 ± 0.04^{bcd}	21.00 ± 0.00^a
	Cs1 & AMF	2.45 ± 0.04^f	0.99 ± 0.03^{bcd}	11.33 ± 0.58^{def}
	Cs2 & AMF	2.83 ± 0.05^{bc}	1.03 ± 0.06^{bcd}	17.33 ± 1.53^b

Distinct letters indicate significant differences among treatments at the same time point (one-way ANOVA followed by Tukey's test, $P \leq 0.05$)

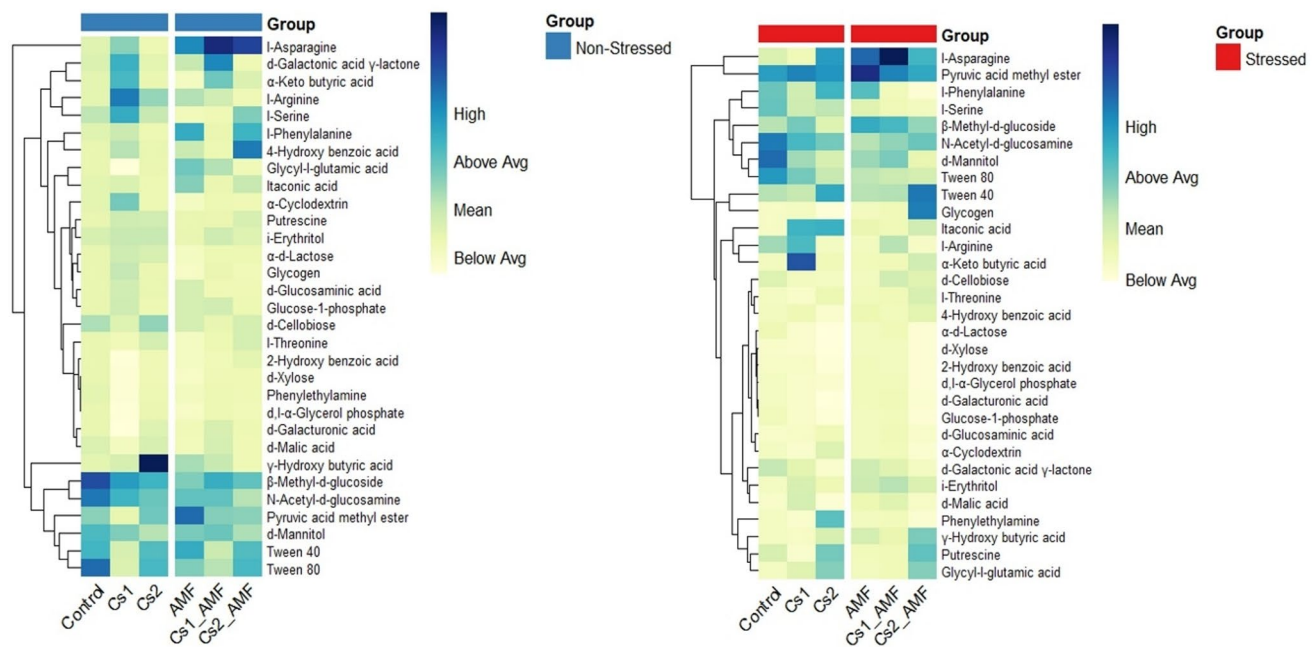


Fig. 6 Heatmap of carbon substrate utilization patterns of soil microbial communities associated with *O. ficus-indica* under non-stressed and water-stressed conditions. Color intensity reflects the relative level

of substrate utilization, ranging from below-average to high activity, while hierarchical clustering highlights similarities among treatments and substrates

al. 2025; Grzadziel et al. 2019). Overall, these results demonstrate that microbial inoculation, particularly the combination of bacterial consortia with arbuscular mycorrhizal fungi, enhances microbial metabolic activity, functional diversity, and substrate utilization efficiency in soils associated with *O. ficus-indica*. These effects are especially pronounced under water stress, highlighting the crucial role of beneficial soil microbiota in sustaining soil biological functioning, nutrient cycling, and ecosystem resilience in arid and semi-arid environments (Collins et al. 2008; Karray et al. 2020; Kavamura et al. 2018; Schimel et al. 2007).

Relationships among microbial treatments under non-stressed and water-stressed conditions

A principal component analysis (PCA) (Fig. 7) was performed to explore the relationships among microbial treatments (Control, Cs1, Cs2, AMF, Cs1&AMF, and Cs2&AMF) under non-stressed (NS) and water-stressed (S) conditions, taking into account soil properties, microbial activity, and growth traits of *O. ficus-indica*. The first two principal components (F1 and F2) explained 58% of the total variance, with F1 accounting for 44% and F2 for 14%.

Figure 7A illustrates the relationships between soil and plant traits. Soil mineral contents (NH_4^+ , NO_3^- , Ca, Mg, Na, and soil P), pH H_2O , and carbohydrate metabolism are distributed on the negative side of F1, showing strong positive correlations among these variables. The area defined

by the positive sides of F1 and F2 highlights strong positive associations among AWCD, S, H' , pH KCl, and amino acid and carboxylic acid metabolism. In contrast, the region located on the positive side of F1 and the negative side of F2 groups plant growth traits (plant height and cladode area), plant mineral contents (N, P, and K), soil enzyme activities (phosphatase, urease, and β -galactosidase), and soil organic matter content, with strong positive correlations observed among these traits.

Figure 7B illustrates the distribution of treatment plots on the F1 and F2 axes. The F1 axis showed a clear discrimination between uninoculated plants, inoculated by consortia or AMF separately, and plants inoculated with both consortia and AMF. Uninoculated plants are discriminated on the extreme negative side of F1, characterized by high levels of available soil minerals (NH_4^+ , NO_3^- , Ca, Mg, Na, and soil P). In contrast, plants inoculated with both Consortium and AMF are distributed on the extreme positive side of F1, generally exhibiting high scores of AWCD, S, H' , pH KCl, amino acid metabolism, carboxylic acid metabolism, plant growth traits (plant height and cladode area), plant mineral contents (N, P, and K), soil enzyme activities (phosphatase, urease, and β -galactosidase), and soil organic matter content. This distribution highlights a functional synergy between PGPR and AMF, promoting improved structuring of soil microbial communities and optimized plant growth under both favorable and drought stress conditions. Plants inoculated with Cs or AMF alone are positioned in the

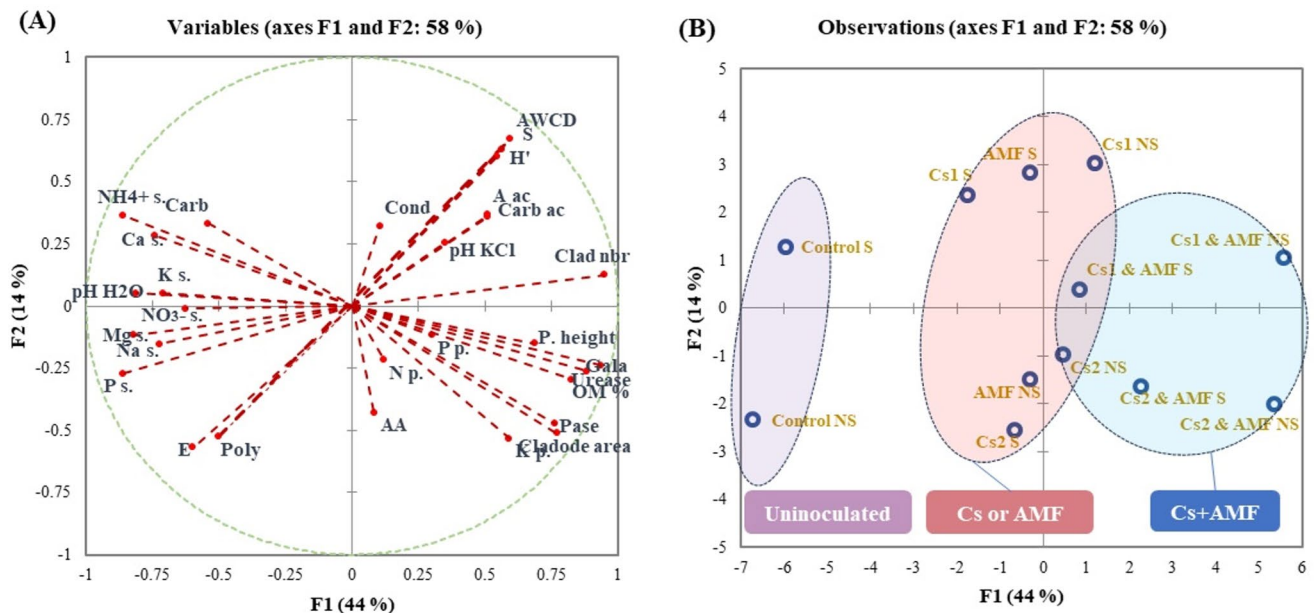


Fig. 7 Principal component analysis (PCA) of soil properties, microbial activity, and growth traits of *O. ficus-indica* as affected by microbial treatments under non-stressed and stressed conditions. NS: Non-stressed conditions; S: Water-stressed conditions; Control: Non-inoculated soil; Cs1, Cs2: Bacterial consortia 1 and 2; AMF: Arbuscular mycorrhizal fungi; Cs1&AMF, Cs2&AMF: Combined inoculation of bacterial consortia with AMF; AWCD: Average Well Color Development; H': Shannon diversity index; E: Shannon evenness index; S: Substrate richness; AA: Amino acids; Carb: Carbohydrates; Carb ac:

Carboxylic acids; Poly: Polymers; Pase: Phosphatase activity; Urease: Urease activity; Gala: β -D-galactosidase activity; pH H₂O: Soil pH measured in water; pH KCl: Soil pH measured in KCl; Cond: Soil electrical conductivity; OM %: Soil organic matter content; N.p, P.p, K.p: Nitrogen, phosphorus, and potassium content in plant tissues; NH₄⁺.s, NO₃⁻.s: Ammonium and nitrate content in soil; Ca.s, Mg.s, Na.s, K.s, P.s: Calcium, magnesium, sodium, potassium, and phosphorus content in soil; Clad nbr: Number of cladodes; P. height: Plant height; Clad area: Cladode area

intermediate zone, reflecting a moderate enhancement of microbial activity and soil functionality.

Overall, the PCA highlights a shift in soil–plant system functioning induced by microbial inoculation. Non-inoculated treatments were characterized by higher mineral availability in the soil, whereas inoculated treatments, particularly PGPR&AMF combinations, were associated with intensified biological, enzymatic, and physiological processes. Under water stress, this structuring became even more pronounced, confirming that the PGPR&AMF association enhances nutrient acquisition, stimulates microbial metabolism, and improves stress tolerance in *O. ficus-indica*.

These multivariate trends are statistically validated by the multifactorial ANOVA (Fig. 1), which confirmed significant interactions between microbial treatments and water regimes. Specifically, the results revealed that most soil traits primarily depend on the inoculation factor, highlighting the crucial role of PGPR and AMF in enhancing soil properties. Consistent with our findings, several studies have reported significant improvements in soil quality following inoculation with PGPR (Prasad et al. 2015; Sharma et al. 2017) and AMF (Gujre et al. 2021). The improvement of soil quality mediated by microorganisms may be attributed to several mechanisms: (i) increased nutrient availability through the solubilization of insoluble compounds, atmospheric nitrogen

fixation, and the secretion of extracellular enzymes; (ii) the release of organic acids, which improves soil structure and mineral retention; (iii) the production of siderophores that chelate ferric iron (Fe³⁺); and (iv) the secretion of lytic enzymes that accelerate biogeochemical cycles (Hnini et al. 2024; Sharma et al. 2017). Inoculation factor showed high significant responsibility in plant traits variability, which might be explained by soil quality amelioration as well as by root system amelioration via phytohormones production, where bacterial auxins showed a significant increasing effect on root biomass and architecture in several plants (Hnini et al. 2024; Pathak et al. 2017). The water regime appears to be the primary source of variability for most plant traits, underscoring the strong dependence of plant growth on soil water availability. In contrast, the variability of rhizosphere microbial diversity indices, including Shannon evenness (E) and the Shannon diversity index (H'), is mainly driven by the interaction between water regime and inoculation, highlighting the complexity underlying the regulation of these traits. In this context, water regime influences microbial survival and competitiveness, while inoculation introduces new functional groups, and their combined effects generate pronounced variability in microbial community structure (Jha and Saraf 2015; Pandya et al. 2014). These findings emphasize the key role of combined microbial inoculations

as a sustainable strategy to enhance agroecosystem resilience in arid and semi-arid environments.

Conclusion

This study demonstrates that the combined application of plant growth-promoting rhizobacteria (PGPR) consortia and arbuscular mycorrhizal fungi (AMF) represents an effective biological strategy for improving the performance of *Opuntia ficus-indica* under contrasting water regimes. Beyond enhancing plant growth, microbial co-inoculation promoted the biological functioning of the soil by stimulating nutrient cycling and microbial metabolic activity, highlighting the importance of exploiting synergistic interactions between beneficial microorganisms within the plant-soil system.

The present work provides new evidence that integrating bacterial consortia with AMF contributes not only to improved plant nutrition but also to the maintenance of soil biological quality under water-limited conditions. These findings reinforce the potential of microbial-based biofertilization as a sustainable alternative to conventional agricultural practices for cactus cultivation in arid and semi-arid environments.

Future studies should evaluate the long-term performance of these microbial consortia under field conditions, investigate their persistence and interactions with native microbial communities, and assess their effectiveness across different soil types and climatic conditions before large-scale agricultural application.

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Data Availability The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no known competing interests.

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