Phosphate-solubilizing inoculants induce stage-specific rhizosphere microbiome shifts and enhance chickpea symbiosis under low P availability

Hicham Chaouki, Said Khourchi, Wissal Elhaissoufi, Mohamed Idbella, Ammar Ibnyasser, Cherki Ghoulam, Abdellatif Barakat, Adnane Bargaz

Hicham Chaouki, Said Khourchi, Wissal Elhaissoufi, Mohamed Idbella, Ammar Ibnyasser, et al.. Phosphate-solubilizing inoculants induce stage-specific rhizosphere microbiome shifts and enhance chickpea symbiosis under low P availability. Biology and Fertility of Soils, In press, ⟨10.1007/s00374-026-01989-7⟩. ⟨hal-05589513⟩

Biology and Fertility of Soils
https://doi.org/10.1007/s00374-026-01989-7

RESEARCH

Phosphate-solubilizing inoculants induce stage-specific rhizosphere microbiome shifts and enhance chickpea symbiosis under low P availability

Hicham Chaouki[1] · **Said Khourchi**[1] · **Wissal Elhaissoufi**[1] · **Mohamed Idbella**[1] · **Ammar Ibnyasser**[1] · **Cherki Ghoulam**[1] · **Abdellatif Barakat**[1,2] · **Adnane Bargaz**[1]

Received: 6 November 2025 / Revised: 30 January 2026 / Accepted: 2 February 2026

Abstract

Understanding legume symbiotic efficiency in response to phosphate (P) solubilizing microbes (PSM) is crucial, given their ability to synergize with rhizobial strains and promote rhizosphere interactions that enhance soil P availability and legume nodulation. However, the mechanistic understanding deciphering how PSM likely to influence legume symbiotic efficiency, including rhizosphere microbial dynamics, remains not well known. This study assessed the ability of twenty-four PSM (bacteria "PSB" and fungi "PSF") to induce chickpea nodulation and identified key rhizosphere traits through which PSM may enhance chickpea symbiotic performance under low P availability. Results demonstrated that only five PSM inoculants enhanced symbiotic traits of *Mesorhizobium*-inoculated chickpea, including nodule biomass, leghemoglobin content, intra-tissular P (Pi), and acid phosphatase (APase) activity, as well as plant biomass and nutrient uptake at both flowering and reproductive growth stages. Notably, PSM consortia significantly outperformed single inoculants by strengthening the link between above- and below-ground plant traits. This was supported by strong positive correlations between nodule Pi content and both nodule APase activity ($R = 0.94^{**}$, $p \leq 0.01$) and leghemoglobin content ($R = 0.95^{**}$, $p \leq 0.01$). The rhizosphere P-related traits, including P availability, rhizosphere and root APase activity, root Pi content, and shoot P uptake were significantly higher than in uninoculated treatments. These responses were concomitant with specific modifications in rhizosphere microbial communities that were significantly correlated with chickpea symbiotic traits and overall growth parameters. At the flowering stage, bacterial community composition favored beneficial N_2-fixing taxa (*Acetobactereaceae*, *Beijerinckiaceae*, *Rhizobiales*), with *Mesorhizobium* showing strong correlations with plant growth traits. At the reproductive stage, the fungal community was enriched in disease-suppressive genera (*Trichoderma* and *Talaromyces*). Our findings reveal a stage-specific microbial cooperation that enhances symbiotic performance, plant growth, and nutrient uptake in chickpea under low P availability.

Keywords Bacteria · Fungi · Microbial communities · Chickpea · Nodulation · Rhizosphere · Root · Phosphate

Introduction

Chickpea (*Cicer arietinum*) accounts for approximately 18% of global legume production, ranking as the second most important legume crop in terms of production and consumption (Calia et al. 2024). Its agronomic importance is further reinforced by its ability to meet a substantial portion of its nitrogen (N) requirements through biological N fixation (BNF). The chickpea-*Mesorhizobium ciceri* symbiosis significantly contributes to N cycling and availability in agricultural systems (Ladha et al. 2022). Chickpea cropping can contribute up to approximately 60 kg N ha^{-1} which

✉ Hicham Chaouki
hicham.chaouki@um6p.ma

✉ Adnane Bargaz
adnane.bargaz@um6p.ma

[1] AgroBioSciences Program, College of Agriculture and Environmental Sciences, Mohammed VI Polytechnic University (UM6P), Ben Guerir, Morocco

[2] IATE, University of Montpellier, INRAE, Agro Institute, Montpellier 34060, France

correspond to 50%−78% of N derived from the atmospheric (Ndfa), although this contribution strongly depends on plant and pedo-climatic factors, including phosphorus (P) availability, soil physicochemical properties, and the efficiency of the nodulating rhizobial strains. These dependencies highlight the importance of optimizing this symbiosis to enhance N use efficiency while sustainably increasing soil N inputs (Jensen et al. 2010; Hossain et al. 2016; Kour et al. 2020; Kebede 2021; Shome et al. 2022). Low soil P availability negatively affects a wide range of structural, molecular, and physiological processes involved in root-rhizobia recognition, nodule formation, and nodule functioning. Consequently, P deficiency impairs symbiotic efficiency and plant growth, particularly under BNF-dependent conditions (Chaudhary et al. 2008; Itelima et al. 2018; Li et al. 2019). Therefore, improving P availability and acquisition is of paramount importance for enhancing BNF efficiency.

To address these challenges, several strategies have been adopted, including appropriate P fertilization and the selection of highly efficient *Mesorhizobium* cultivars with superior nodulation capacity (Abd-Alla et al. 2023). More recently, inoculation with functional microbial groups such as P solubilizing bacteria (PSB) and fungi (PSF) has emerged as a sustainable biological strategy to increase the bioavailable P pool. When combined with *Mesorhizobium*, these microorganisms can enhance nodulation and symbiotic N_2 fixation under P-limited conditions. Several studies have demonstrated that PSB and PSF, applied alone or co-inoculated with N-fixing microbes, improve P availability and plant P uptake, which in turn positively impacts BNF. The beneficial effects of combined rhizobia-PSM inoculation have been attributed to synergistic improvements in N and P cycling, stimulation of root development, and nodulation, ultimately leading to improved plant growth performance (Kaur et al. 2015; Benjelloun et al. 2021; Janati et al. 2021; Mansour et al. 2021). Despite the growing evidence supporting the benefits of dual *Mesorhizobium*-PSM inoculation, the underlying mechanisms driving enhanced BNF under P-limited conditions remain insufficiently explored. In particular, the role of PSF in shaping belowground interactions and influencing *Mesorhizobium*-mediated symbiosis has received limited attention. Moreover, the effects of microbial inoculation across different plant growth stages remain poorly understood, especially regarding the extent to which *Mesorhizobium*-PSF interactions shape the legume-associated microbiome to enhance nodulation and BNF efficiency. Microbial inoculants are known to significantly modulate rhizosphere microbial communities, particularly P-solubilizing and N_2-fixing taxa, thereby enhancing soil nutrient cycling (Tagore et al. 2013; Wei et al. 2019; Wang et al. 2020; Chen et al. 2021; Lagunas et al. 2023). In addition, rhizobial inoculation can alter nodule-associated microbiota by increasing the relative abundance of efficient N_2-fixing rhizobial species, which is the case of inoculated soybean with *Bradyrhizobium japonicum* and *Bradyrhizobium diazoefficiens* showed high relative abundance of *Bradyrhizobium* strains within the nodules, rather than in the rhizosphere soil.

However, most of the available studies have been conducted under sterilized soil conditions, which may overlook the contribution of native soil microbiota and alter soil physico-chemical properties. Consequently, such approach may fail to capture the true effects of microbial inoculants on the composition and functioning of soil- and nodule-associated microbial communities. Uncovering the belowground functional traits associated with *Mesorhizobium*-PSM inoculation in chickpea is therefore essential for refining microbial inoculation strategies, particularly the co-inoculation of rhizobia and PSM, in P-deficient soils, where microbial approach can enhance chickpea productivity and support sustainable legume-based cropping systems (Han et al. 2020; Lagunas et al. 2023; Cangioli et al. 2025; Liu et al. 2025). Collectively, available literature highlights the need for a comprehensive understanding of the plant- and microbe-driven mechanisms underlying chickpea responses to *Mesorhizobium*-PSM co-inoculation under nutrient-limited conditions, particularly low soil P availability. Therefore, the present study investigates impacts of PSM on chickpea symbiotic performance by first evaluating the functional diversity of PSM in promoting nodulation. Subsequently, the study examines plant traits, rhizosphere properties, and soil microbial community dynamics to decipher the key mechanisms contributing to enhance nodulation and overall plant growth. We hypothesize that the application of PSM in combination with chickpea-nodulating *Mesorhizobium* will directly enhance P use for both plant growth and BNF, while indirectly reshaping soil bacterial and fungal community composition in a plant growth stage-dependent manner, ultimately improving chickpea symbiotic efficiency under low P availability.

Materials and methods

In vitro biochemical and physiological characterization of P solubilizing bacteria and fungi

P solubilizing microbial inoculants using single-strains and consortia

Twenty-four microbial inoculants consisting of 18 PSB (15 individual strains and 3 bacterial consortia “BC”) and 6 PSF (4 individual strains and 2 fungal consortia “FC”) were tested for their plant growth promoting traits (Table 1 and Table S1). These microbial inoculants isolated from

Table 1 Biochemical characterization of phosphate solubilizing bacteria (PSB), fungi (PSF) and consortia

Bacteria and fungi		Molecular identification	Solubilized P (mg P_2O_5 L^{-1})		Dominant organic acid Produced		EPS (g/L)	Drought tolerance (PEG 6000)		Salinity [NaCl]		Acidity pH		Temperature (°C)	
			TCP	RP	TCP	RP		10%	20%	8 %	10 %	4	5	40	50
Single PSB	**B_{13}**	*Pseudomonas atacamensis*	193.83^{ab}	111.12^{a}	Citric acid	Citric acid	0.38^{f}	1.07^{b}	0.65^{c}	🟦	🟨	🟥	🟦	🟦	🟩
	B_{14}	*Arthrobacter pascens*	189.82^{ab}	88.42^{g}	Citric acid	Citric and gluconic acid	0.34^{fg}	1.04^{b}	0.77^{ab}	🟦	🟨	🟥	🟦	🟦	🟥
	B_{12}	*Bacillus siamensis*	186.27^{b}	94.13^{cd}	Malic and gluconic acid	α-Ketoglutaric acid	0.19^{hi}	0.96^{d}	0.33^{fg}	🟩	🟨	🟥	🟩	🟨	🟥
	B_{16}	*Acinetobacter lactucae*	185.68^{b}	99.38^{b}	Citric acid	Citric acid	0.37^{f}	0.61^{g}	0.36^{ef}	🟦	🟥	🟥	🟩	🟩	🟥
	B_{35}	*Bacillus paramycoides*	158.75^{c}	92.04^{de}	α-Ketoglutaric and glutamic acid	α-Ketoglutaric. citric and gluconic acid	0.69^{c}	1.06^{b}	0.77^{ab}	🟩	🟨	🟥	🟨	🟩	🟨
	B_{31}	*Rahnella aceris*	147.39^{c}	89.20^{de}	Malic and α-Ketoglutaric acid	Citric and gluconic acid	1.32^{a}	0.58^{g}	0.53^{d}	🟨	🟥	🟥	🟥	🟦	🟥
	B_{8}	*Bacillus cereus*	79.67^{d}	78.53^{f}	Citric and gluconic acid	Citric and gluconic acid	0.61^{cd}	0.97^{cd}	0.65^{c}	🟩	🟥	🟥	🟦	🟩	🟥
	B_{37}	*Pseudomonas sp*	71.84^{de}	20.65^{j}	Malic. citric and gluconic acid	Malic. α-Ketoglutaric and citric acid	0.20^{hi}	0.59^{g}	0.27^{fg}	🟦	🟨	🟨	🟥	🟩	🟥
	B_{33}	*Pantoea hericii*	69.91^{de}	46.18^{g}	Citric acid	Glutaric. α-Ketoglutaric. ctric and Gluconic acid	0.66^{cd}	1.07^{b}	0.55^{d}	🟦	🟨	🟨	🟨	🟦	🟦
	B_{25}	*Arthrobacter oryzae*	63.75^{de}	39.70^{h}	Glutamic and gluconic acid	Citric acid	0.18^{i}	0.36^{i}	0.26^{g}	🟩	🟨	🟥	🟥	🟩	🟥
	B_{60}	*Micrococcus luteus*	59.65^{e}	18.80^{jk}	Glutamic and citric acid	Glutaric. α-Ketoglutaric. shikimic. citric and Gluconic acid	0.29^{fg}	0.36^{i}	0.29^{fg}	🟦	🟥	🟥	🟥	🟩	🟥
	B_{49}	*Leuconostoc mesenteroides*	38.18^{f}	14.98^{k}	Citric and gluconic acid	Glutaric. α-Ketoglutaric. citric and Gluconic acid	0.52^{e}	0.47^{h}	0.34^{fg}	🟩	🟥	🟥	🟥	🟩	🟥
	B_{36}	*Pantoea brenneri*	36.99^{f}	34.15^{i}	Glutamic and citric acid	Glutaric. α-Ketoglutaric and citric and gluconic acid	0.16^{i}	0.31^{i}	0.25^{g}	🟦	🟨	🟨	🟨	🟥	🟥
	B_{53}	*Bacillus altitudinis*	30.42^{f}	6.57^{l}	Citric acid	Glutaric. α-Ketoglutaric and citric acid	0.08^{j}	0.76^{e}	0.54^{d}	🟩	🟥	🟥	🟥	🟦	🟥
	B_{28}	*Bacillus paranthracis*	23.56^{f}	46.06^{g}	Citric acid	Citric acid	0.27^{gh}	0.70^{f}	0.44^{e}	🟩	🟨	🟥	🟥	🟩	🟥
Consortia PSB	**BC_c**	B_{14}, B_{16}, B_{31} and B_{13}	203.15^{a}	115.44^{a}	Citric acid	Citric. gluconic and glutamic acid	0.91^{b}	1.19^{a}	0.86^{a}	🟩	🟨	🟥	🟨	🟦	🟩
	BC_a	B_{14}, B_{60}, B_{36} and B_{13}	182.59^{b}	99.51b^{b}	Citric acid	Citric acid	0.59^{de}	1.03^{bc}	0.72^{bc}	🟦	🟨	🟨	🟨	🟦	🟩
	BC_b	B_{16}, B_{36}, B_{37} and B_{28}	181.28^{b}	98.85^{bc}	glutamic acid	Citric acid	0.59^{de}	1.03^{bc}	0.72^{bc}	🟦	🟨	🟥	🟥	🟦	🟩
Single PSF	**F_{11}**	*Talaromyces. sp*	170.64^{b}	94.62^{c}	Gluconic and citric acid	Gluconic and citric acid	1.52^{b}	0.81^{c}	0.55^{d}	🟨	🟨	🟩	🟦	🟨	🟥
	F_{12}	*Rhizopus arrhizus*	167.49^{b}	107.96^{ab}	Malic and citric acid	Citric. malic and gluconic acid	0	1.00^{b}	0.81^{c}	🟨	🟥	🟩	🟦	🟥	🟥
	F_{2}	*Talaromyces oumae*	74.43^{d}	63.63^{d}	Citric and gluconic acid	Citric and gluconic acid	0	0.76^{c}	0.32^{e}	🟨	🟥	🟩	🟦	🟦	🟥
	F_{9}	*Talaromyces verruculosus*	115.35^{c}	91.34^{c}	Citric. malic and gluconic acid	Citric and gluconic acid	0.53^{c}	0.55^{d}	0.35^{e}	🟨	🟥	🟩	🟦	🟨	🟥
Consortia PSF	**FC_{17}**	F_9 and F_{11}	192.78^{a}	110.47^{a}	Citric and gluconic acid	Citric. malic and gluconic acid	2.05^{a}	1.17^{a}	0.87^{b}	🟨	🟨	🟩	🟦	🟨	🟥
	FC_{14}	F_{11} and F_{12}	188.18^{a}	103.94^{b}	Citric and gluconic acid	Citric. malic and gluconic acid	0.56^{c}	1.14^{a}	0.93^{a}	🟨	🟥	🟩	🟦	🟥	🟥

Different letters indicate mean values (n=3) that are statistically different at $p \leq 0.05$ according to Duncan's test. Abbreviations: B, Bacteria; BC, bacterial consortia; F, fungi; FC, Fungal consortia; TCP, tricalcium phosphate; RP, rock phosphate; EPS, exopolysaccharide and PEG, polyethylene glycol.

Stress tolerance 🟥 0 🟨 1 🟩 2 🟦 3

the rhizosphere and rhizoplane of several crops grown in Moroccan soils (wheat, maize, faba beans, and alfalfa (Table S1) were obtained from the microbial collection of Plant-Microbes Interaction Laboratory (AgroBioscience department, UM6P) and were previously tested by our research group for their P solubilizing ability using rock P (Elhaissoufi et al. 2020, 2024; Benbrik et al. 2025) and polyphosphate fertilizers (Khourchi et al. 2022a) as well as plant growth promotion.

The microbial inoculants were tested for their ability to solubilize P using rock P (Table S1) as a natural insoluble P and tricalcium P (TCP) as a synthetic insoluble P. The National Botanical Research Institute's Phosphate (NBRIP) broth media (glucose: 10 g; $MgCl_2$ 6 H_2O: 5 g; $MgSO_4$ 7 H_2O: 0.25 g; KCl: 0.2 g and $(NH4)_2SO4$: 0.1 g) was used to grow PSM. After 7 days of incubation under continuous agitation, microbial cultures were centrifuged at 10,000 × g for 15 min and the resulted supernatant was used to determine the available P following the ascorbic acid colorimetric method as described by Benmrid et al. (2025). Organic acids and pH were also determined in NBRIP medium to assess the role of acidification in enhancing TCP and RP solubilization. The supernatants were filtered using 0.22 µm and the cell-free filtrates were stored at

20 °C before the organic acids content was determined by LC-MS^2 (Shimadzu, Japan). These microbial isolates were also characterized for their ability to produce exopolysaccharides (EPS) following the phenol-sulfuric acid method described by Karadeniz et al. (2021).

Out of 24 inoculants, 3 bacterial and 2 fungal consortia were tested as described above. Consortia were constructed using a rational and trait-based approach. Before PSB consortia are constructed according to Elhaissoufi et al. (2024): individual PSB were first classified according to their P solubilization capacity (classified as high, moderate, or low) regardless of their ability to form halo of solubilization, tested for multiple PGP traits including their ability to induce plant growth at different growth stages. Multiple bacterial consortia were constructed, tested in-vitro and *in-planta* with each bacterial consortium containing, synergistic and PGP isolates exhibiting functional complementarity, with each isolate exhibiting at least one distinct PGP trait than others (Elhaissoufi et al. 2023). The two PSF consortia used in this study were chosen among 28 wheat rhizoplane fungal consortia constructed and tested *in-planta* using a niche-conserved consortium approach. These two PSF consortia (Table S1) emerged as top candidates among several constructed PSF consortia and were ultimately selected based on their P solubilizing ability and consistent performance based on *in-planta* experiments (Benbrik et al. 2025).

Growth conditions and in-vitro biochemical and physiological characterization of microbial inoculants: P solubilization and stress tolerance

Before testing the PSB and PSF for their PGP traits and stress tolerance, individual PSF isolates were cultured in potato dextrose broth (PDB) and incubated at 30 °C for 5 days. Similarly, individual PSB isolates were grown in Luria-Bertani (LB) liquid medium at 28 °C for 48 h. Each consortium was prepared by mixing equal volumes of the individual isolates (10^8 CFU ml^{-1} ≈ OD = 1.2) previously centrifuged at 12,000 × g for 10 min and rinsed three times with sterile physiological water to remove all traces of the culture medium. Finally, pellets were suspended in 20 ml of sterile physiological water and the OD was adjusted to approximately 1.2 (Elhaissoufi et al. 2024; Benbrik et al. 2025).

The ability of PSM inoculants to tolerate drought and salinity stress was evaluated in TSB and PDB media supplemented with PEG-6000 (10% and 20%, simulating moderate and severe drought conditions) and with NaCl (8% and 10% NaCl, moderate and severe salt stress), respectively. The growth media were inoculated with PSM inoculants (10^8 CFU ml^{-1} ≈ OD = 1.2) and incubated at 28 °C under continuous agitation at 150 rpm. Microbial growth was quantified by measuring the OD at 600 nm after 48 h using a FLUOstar® Omega spectrophotometer.

Heat stress tolerance of PSB and PSF isolates and consortia was assessed by inoculating PSB onto TSA (Tryptic Soy Agar) and PSF onto PDA (Potato Dextrose Agar) plates, which were incubated at 40 °C and 50 °C for 48 h. Acidity tolerance was evaluated on solid media (TSA for PSB and PDA for PSF) adjusted to pH 4 and 5 and incubated at 28 °C. Microbial growth was compared to the controls at 28 °C for heat stress and pH 7 for acidity.

Microbial growth under abovementioned stress conditions (salinity, drought, heat and acidity) was evaluated using a semi-quantitative scoring system based on comparison with unstressed conditions (Hoffmann et al. 2025). Microbial growth responses (compared to corresponding controls) were grouped as follows: no growth (0, non-tolerance), weak growth (score = 1), moderate growth (score = 2) and strong growth (score = 3) (Table 1). These scores (0–3) were used to assess the tolerance of PSM isolates and consortia to salinity, drought, increasing temperatures and acidity stresses and were visualized using heatmaps with a color scale ranging from 0 (no tolerance) to 3 (strong tolerance).

Physiological evaluation of P solubilizing bacteria and fungi to enhance chickpea symbiotic efficiency and nodulation

The microbial inoculants were prepared as described above and co-cultured with *Mesorhizobium ciceri*, a chickpea-nodulating bacterium (Table S1), to co-inoculate the Moroccan winter chickpea variety namely Arifi (FLIP98-50 C, medium-seeded) (Table S1). All tested PSM exhibited synergistic growth with *Mesorhizobium ciceri*. The variety was evaluated under two greenhouse experiments; the first consists of studying the nodulation rate of 60-day old chickpea (flowering stage) in response to twenty-four PSB and PSF inoculants, which allowed to retain only five potential microbial treatments (based on nodulation and P-related plant parameters) that were tested in a second experiment on 80-day old chickpea plants (reproductive stage) under rock P supply.

Plant inoculation experiments and growth conditions

Chickpea (*Cicer arietinum*, Arifi variety) seeds (Table S1) were surface disinfected with sodium hypochlorite (6%, 3 min), followed by a thorough washing with sterile distilled water. Subsequently, the disinfected seeds were pregerminated in autoclaved sand for 7 days under dark conditions at 25 °C. Germinated seedlings were immersed separately in microbial inoculants before they were transplanted in pots filled with non-sterile P deficient (4.96 mg/kg of Olsen P, pH 8.2) sandy loamy clay soil, containing 15.29 mg/kg NH_4^+ and 2% organic matter collected from the experimental farm of Innovation and Technology Transfer Center for Agriculture (AITTC, 32° 13′ 11.5″ N 7° 53′29.9″) located at UM6P, Morocco. Detailed physicochemical properties of used soil were listed in Table S1.

The first inoculation experiment (60-day-old chickpea) was conducted in small sized pots filled with 750 g of a sterilized plant growth substrate consisting of a mixture of P-deficient soil, sand and peat (2 V:0.5 V:0.5 V) to avoid soil compaction and facilitate root-nodule development. Each pot was supplied with rock P at a rate equivalent to 172 kg RP ha^{-1}, ensuring a recommended P dose (21.85 kg P ha^{-1}) required for optimum chickpea growth and development. The inoculated seedlings were transplanted, and pots were placed in a greenhouse under controlled conditions (28 °C, 70% relative humidity, 16/8 h photoperiod, and an illumination intensity of 240 mmol m^{-2} s^{-1}). Besides microbial inoculant treatments described in Table 1, three uninoculated controls added with: RP at 21.85 kg P/ha (recommended level), OrthoP (available P form) at 21.85 kg P/ha (recommended level), and P_0 with no P added. For all treatments, N was added at a starter dose equivalent to 10 kg N ha^{-1}, and K was added at 33.20 kg K/ha. In addition, micronutrients (including boron, manganese, copper, zinc, molybdenum and iron) were supplied every 15 days using Hoagland solution. Pots (one seedling per pot) were arranged in a randomized complete block design with six replicates per treatment, with each pot considered as a replicate. Sixty days after transplantation coinciding with chickpea flowering stage, plants were harvested to evaluate the ability of PSM to enhance chickpea symbiotic performance and growth under low P conditions. Based on the results of nodulation and P-related plant parameters, five potential microbial treatments (B_8, B_{35}, BC_c, F_{11} and FC_{17}) were selected to be tested in a second experiment which was conducted in large-sized plastic pots (diameter 25 cm, height 21 cm) filled with 8 kg of the same plant growth substrate mentioned above. Two inoculated seedlings were transplanted per pot with three replicates per treatment. The same controls used at flowering stage were also used in this experiment. Macro- and micronutrients were supplied as described above. Eighty days after sowing, corresponding to the reproductive stage, the chickpea plants were harvested, and agro-physiological parameters were measured particularly yield-related parameters including pods number, pods dry weight and pods nutrients content.

Physiological evaluation of above- and below-ground plant parameters

Nodulation and root morphological traits

Sixty and eighty days after sowing, above- and below-ground plant components were separated. Roots were carefully collected and separated from the rhizosphere soils that were 2 mm sieved and stored at −20 °C for enzymatic and microbial sequencing analyses. Roots were carefully washed to remove adhered soil particles to facilitate collecting nodules that were gently detached from roots and immediately frozen at −20 °C. Before root biomass was determined, root morphological traits; root length (RL), surface area (RSA), diameter (RD) and volume (RV) were quantified using software WinRHIZO™ (Regent Instruments Inc., Quebec City, Canada). The Epson Perfection LA2400 scanner was used to scan roots at a resolution of 300 dpi (Ammar et al. 2024; Nkir et al. 2024).

Nodule physiological traits, notably leghemoglobin (Leg), nodule Pi content and nodule APase activity were measured. Leghemoglobin content was determined in fresh nodules using the cyanmethemoglobin method (Eylenbosch et al. 2018). Aliquots of fresh ground nodules (100 mg) in liquid nitrogen were added to 0.6 mL of Drabkin's solution. The homogenate was centrifuged at 500 × g for 15 min at 4 °C, the supernatant was collected, and the solid phase was re-extracted and centrifuged twice following the same steps. The supernatants from different centrifugations were combined and adjusted to a final volume of 2 mL using Drabkin's solution. The total volume was centrifuged again at 20,000 × g for 30 min at 4 °C. The Leg content was measured spectrophotometrically at 540 nm using Drabkin's solution as a blank.

For nodule Pi measurement, aliquots of 100 mg fresh weight were finely ground in 500 µL of cold sodium acetate buffer (0.2 M, pH 5.6) at 4 °C. the extract was centrifuged (12000 × g for 10 min at 4 °C) and supernatant was used to determine Pi spectrophotometrically at 880 nm using a microplate reader (FLUOstar® Omega) (Bargaz et al. 2012). The nodules APase activity was measured using the *p*-nitrophenyl P (*p-NPP*) method according to Bargaz et al. (2017). Aliquots of the supernatants (125µL) were added to cold acetate buffer (pH 5.6, 0.2 M) and *p-NPP* (10 mM) as a substrate. After 1 h of incubation at 37 °C, the enzymatic reaction was stopped by adding NaOH (0.5 M) and $CaCl_2$ (0.5 M). The APase activity, expressed as the amount of APase required to release 1 nmol of *p*NP g^{-1} h^{-1}, was determined by measuring the absorbance at 405 nm using a UV–Vis spectrophotometer.

Measurement of plant biomass and nutrients (N, P, K, S, Mg and Zn) content in shoots and pods

Before plants biomass were determined, shoot samples were collected for biochemical analysis. Shoot dry weight (SDW) and pods dry weight (PDW) were determined following oven-dried of shoots and pods.

Shoots and pods were oven-dried for (3 days at 60 °C) and finely ground to serve for the quantification of the total nutrient (N, P, K, S, Mg, and Zn) content using the ICP-OES (Agilent 5110 Inductively Coupled Plasma Optical Emission Spectrometry, USA). Total N content was determined using the Kjeldahl method (KjelMaster K-375, Netherlands). In addition, inorganic P (Pi) contents in shoots and roots along with root APase activity were determined as described above for nodule Pi content and nodule APase activity.

Determination of available P, microbial biomass P and acid phosphatases activities in rhizosphere soil

Available P content in rhizosphere soil was measured using molybdenum blue method (Fernández et al. 2007). A volume of 20 mL of 0,5 M sodium bicarbonate was added to 1 g of dried rhizosphere soil. After 30 min of shaking at 150 rpm, the mixture was filtered and 1 mL of filtrate was used to measure the available P fraction spectrophotometrically at 880 nm (Fernández et al. 2007). In addition, measurement of rhizosphere soil APase activity was determined using the *p*-NPP as described above for nodule APase activity.

The soil microbial biomass P (MBP) was determined using the chloroform fumigation-extraction method as described by Corstanj et al. (2007). Fresh rhizosphere soil was divided into two subsamples (2 g each) from the same sample. The first sub-sample was fumigated with chloroform ($CHCl_3$) under vacuum at 200 mbar for 18 h, while the second sub-sample was treated in the same way (200 mbar for 18 h) but without chloroform. Both fumigated and non-fumigated samples were placed in centrifuge tubes where 40 mL of $NaHCO_3$ (0.5 M, pH 8.5) was added (soil: extractant ratio 1:20). The suspensions were shaken for 30 min, followed by centrifugation at 2683 × g for 20 min at 25 °C. The supernatants were filtered (0.45 µm) using a syringe and collected in clean tubes. MBP was then measured spectrophotometrically at 880 nm using the ascorbic acid method as described above (Ibnyasser et al. 2024). MBP concentration was calculated as the difference between $NaHCO_3$-extractable P in fumigated and unfumigated soils, divided by the extraction efficiency factor (Kep = 0.40), according to the following equation:

$$MBP\ (ppm) = \frac{\mathrm{PF} - PNF}{K_{ep}}$$

where MBP (ppm): soil microbial biomass P, PF (ppm): NaHCO-extractable P from fumigated soil, PNF (ppm): NaHCO-extractable from unfumigated soil and Kep = 0.40 the extraction efficiency factor, according to previous studies (Brookes et al. 1982; Fan et al. 2021; Su et al. 2025).

Rhizosphere microbial community analysis: DNA extraction, amplicon sequencing

Rhizosphere soil (~0.25 g) DNA was extracted using DNeasy PowerSoil Pro DNA extraction kit (Qiagen, Hilden, Germany) with the QIAcube Connect automated DNA extractor, following the manufacturer's instructions. DNA concentration and purity were assessed using a Nanodrop spectrophotometer (VWR® mySPEC). PCR was conducted to amplify the bacterial 16S rRNA (V3–V4) and fungal ITS2 rRNA regions with the bacterial (5‘CCTAYGGGRB-GCASCAG-3’ and 5‘-GGACTACNGGGTATCTAAT-3’) and fungal (5‘-GCATCGATGAAGAACGCAGC-3’ and 5‘-TCCTCCGCTTATTGATATGC-3’) primer sets, using a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Canada) with the following program : 95 °C for 3 min, followed by 25 cycles of 30 s at 95 °C for denaturation, 30 s at 55 °C for annealing, and 30 s at 72 °C for elongation, and a final extension step was performed at 72 °C for 5 min (Benmrid et al. 2024). Amplicons were sequenced on the Illumina MiSeq platform using 250 bp paired end reads. Demultiplexed FASTQ sequences were filtered and analyzed using the DADA2 pipeline (version 1.16.0, Callahan et al. (2016). Raw sequences were trimmed, stripped of primers, and then filtered using maxN = 0, maxEE = 2, and truncQ = 2 where reads shorter than 250 bp, with more than one primer mismatch and with an average quality score lower than 25 were discarded. The sequences were then clustered into amplicon sequence variants (ASVs) based on the error rates estimation using the DADA2 algorithm. For the fungal pipeline, adapters and low-quality ends were initially trimmed using Cutadapt software. Taxonomy assignments were then performed using the SILVA (V132) database for bacterial identification and UNITE database for fungi (Quast et al. 2013). *Chloroplast* and *Streptophyta* contamination, as well as singleton ASVs, were removed and the relative abundance recalculated. To avoid biases due to the different sequencing depths, ASVs tables were rarefied to the lowest number of sequences per sample.

Statistical analysis and data processing

Statistical data analysis was performed using R studio software (version 4.1.1). The significance effects of microbial treatment on morpho-, agro-physiological, and biochemical parameters were assessed using one-way analysis of variance (ANOVA) at significant levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$. Duncan's post hoc test was subsequently applied to determine significant differences between means at significance levels of $p < 0.05$.

Microbial data analysis involved calculating alpha diversity metrics, specifically the Shannon index, to assess within-sample diversity. Heatmaps were generated using PRIMER 7 software (Primer-E Ltd., Plymouth, United Kingdom) to visualize variations in microbial community composition at the finest taxonomic resolution. These heatmaps displayed the 80 most abundant bacterial and fungal taxa, with clustering based on an association similarity index. To evaluate community composition, a resemblance matrix was constructed using Bray-Curtis dissimilarity, followed by non-metric multidimensional scaling (nMDS) performed with the *meta.mds* function from the *vegan* package in R. Vector fitting was applied using the *envfit* function in *vegan* to examine the influence of plant, nodule, and soil traits on microbial community composition. Differences in bacterial and fungal community composition were statistically assessed using PERMANOVA (999 permutations), with P type and inoculation as fixed factors. Relationships between variables (plant, nodule, and soil properties) and microbial diversity and composition were further explored using Mantel tests. Pearson correlation coefficients and Mantel test results were visualized using the *ggcor* R package. In addition, co-occurrence network analyses were performed for both bacterial and fungal communities, considering individual and combined applications (consortia) of bacterial and fungal inoculants. Pairwise associations among ASVs were evaluated using Spearman's rank correlation in R with the Hmisc package. To maintain analytical rigor, only correlations with a coefficient greater than 0.6 or less than −0.6 and a significance level of $P < 0.05$ were retained. The resulting networks were visualized in Gephi (version 0.9.2; Bastian et al. 2009), with nodes representing ASVs and edges representing significant associations. Network modules or subcommunities were highlighted through node and edge color schemes. Various topological indices were calculated and compared to characterize the structure and complexity of the networks.

Results

Plant growth promoting traits and abiotic stress tolerance of phosphate solubilizing microorganisms

Bacterial inoculants exhibited contrasting abilities to solubilize RP and TCP, ranging from 23 mg/L (B_{53} and B_{28}) to 193 mg/L (B_{13} and B_{14}), with the consortia BC_c showed the highest solubilization rate of RP (115.44 mg/L) and TCP (203.15 mg/L) (Table 1). Fungal inoculants showed higher RP and TCP solubilization (63 mg/L − 170 mg/L) compared to single-strain bacterial inoculants, with FC_{17} showed the highest TCP (192.78 mg/L) and RP (110.47 mg/L) solubilization. In addition, only four inoculants; B_{31}, $B_{33,}$ F_{11} and FC_{17} showed the ability to produce EPS. Moreover, the tested microbial inoculants exhibited stress-resilience, showing tolerance to various abiotic stresses, notably drought (B_{13}, B_{35}, BC_c and FC_{17}), salinity (B_{13}, B_{14}, and BC_a) and heat stress (B_{33}, BC_C, BC_a and BC_b).

Ability of PSM inoculants to enhance chickpea's symbiotic performance and plant growth under low P conditions

Chickpea nodulation and nodule physiological traits

At the flowering stage, co-inoculation with *Mesorhizobium* and PSM enhanced nodulation (biomass and number) under limited P availability (RP supply). Specifically, co-inoculation with B_{53}, B_{60}, B_{13}, B_{14}, B_{16}, B_8, B_{33}, B_{35}, BC_a and BC_c strongly induced chickpea nodulation, increasing nodule biomass (from 62% to 284%) and nodule number (from 44% to 190%) compared to OrthoP treatment.

Single-strain PSF, notably F_{11} and F_{12}, enhanced nodule biomass (294% and 126%) and number (40% and 20%) compared to OrthoP treatment (Fig. 1a, b). Fungal consortia FC_{14} and FC_{17} enhanced nodule biomass (73% and 128%) and numbers (50% and 51%) compared to OrthoP treatment.

Inorganic P (Pi), APase activity and leghemoglobin in nodules of chickpea increased in response to co-inoculation with PSM (Fig. 2a, c). Regarding nodule Pi content, B_{31}, B_{35}, and B_8 isolates enhanced nodule Pi by 237%, 233% and 215%, respectively, compared to RP treatment (Fig. 2a). Similarly, BC_c induced the highest nodule Pi content (more than 3 times higher than RP treatment). Fungal isolates, particularly F_{11} and FC_{17}, significantly enhanced nodule Pi content by 237% and 250%, respectively, compared to RP treatment (Fig. 2a). Inoculation with B_{35}, B_8 and BC_c stimulated nodule APase by 73%, 53% and 53%, respectively, compared to RP treatment. Similarly, F_{11} and FC_{17} enhanced nodule APase by 73% and 95%, respectively, compared to RP treatments (Fig. 2b). Additionally, the nodule leghemoglobin content was significantly improved by 149% and 152% in response to B_8 and BC_c, respectively, compared to RP treatment. Likewise, F_{11} and F_{12} increased significantly the leghemoglobin content by 138% and 158%, respectively, with FC_{17} showed nodule leghemoglobin content more than 3 times higher as compared to RP treatment (Fig. 2c).

Both PSF and single PSB exhibited significant positive correlations between inorganic P, leghemoglobin and APase activity in nodules. Specifically, nodule Pi content

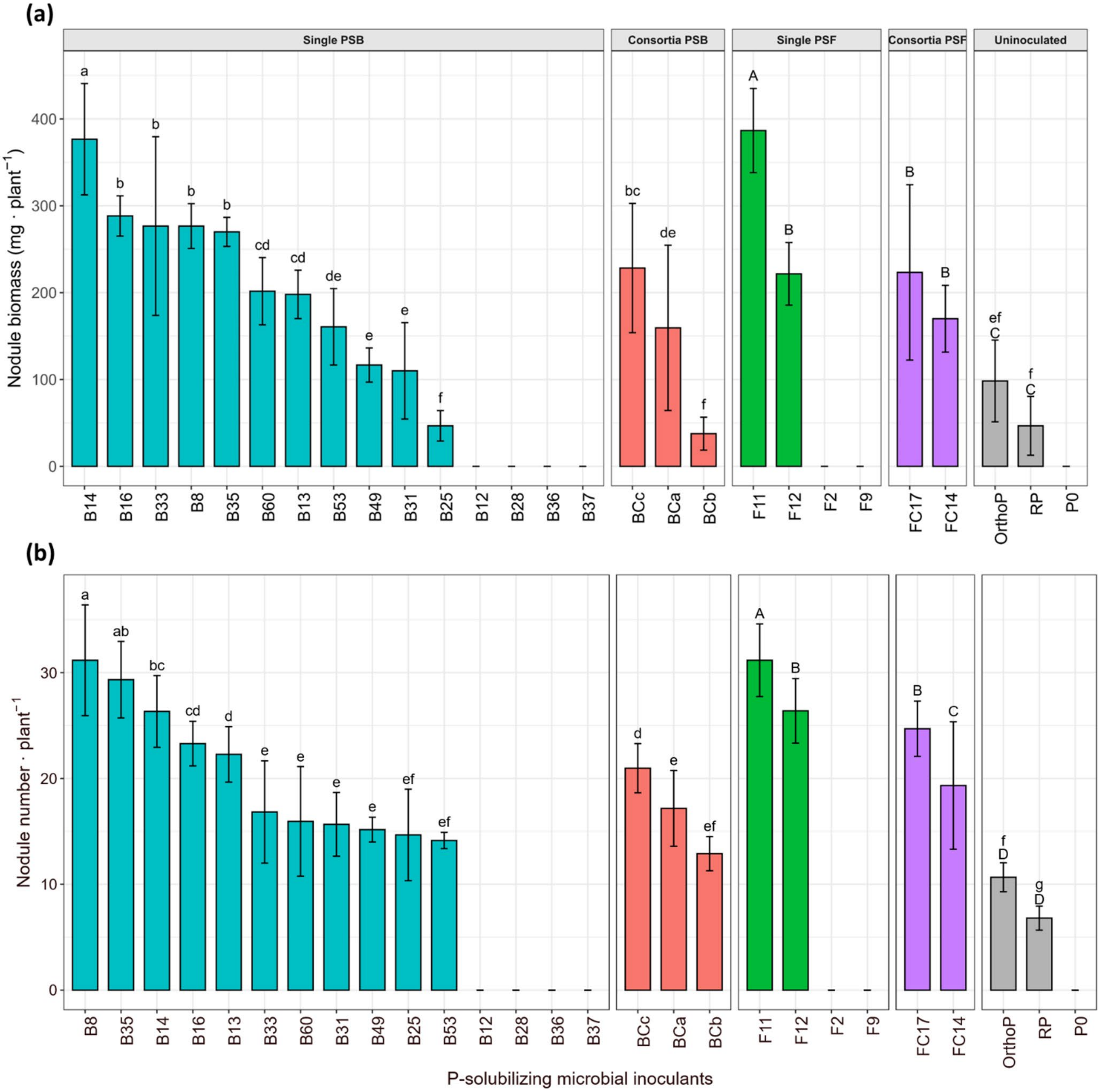


Fig. 1 Biomass (**a**) and number (**b**) of nodules of 60-days old chickpea (Arifi variety) in response to co-inoculation of *Mesorhizobium ciceri* with P solubilizing bacteria (PSB), fungi (PSF) and consortia under RP supply. The results are means ± SD of six replicates. Statistical significance indicated by different lowercase (PSB) and uppercase (PSF) letters at $p \leq 0.05$ according to Duncan's test. Abbreviation: BC, bacterial consortia; FC, fungal consortia; P_0, unfertilized; RP, rock phosphate and OrthoP, orthophosphate

significantly correlated with both nodule APase activity ($R = 0.94^{**}$, $p \leq 0.01$) and leghemoglobin content ($R = 0.95^{**}$, $p \leq 0.01$) in response to inoculation with PSF consortia (Fig. 3a). Similarly, single-strain PSF exhibited the highest significant correlations between leghemoglobin content and nodule APase activity ($R = 0.99^{**}$, $p \leq 0.01$) (Fig. 3b). These correlations provide strong evidence linking the intracellular P use efficiency and chickpea-symbiotic efficiency.

Chickpea growth parameters, root traits and nutrient uptake

At the flowering stage, PSM significantly enhanced SDW and RDW under limited P availability as compared to uninoculated plants. Specifically, B_{33}, B_{35} and BC_c were the best promoters for chickpea biomass, showing enhanced SDW (22%, 11% and 33%) and RDW (17%, 4% and 26%)

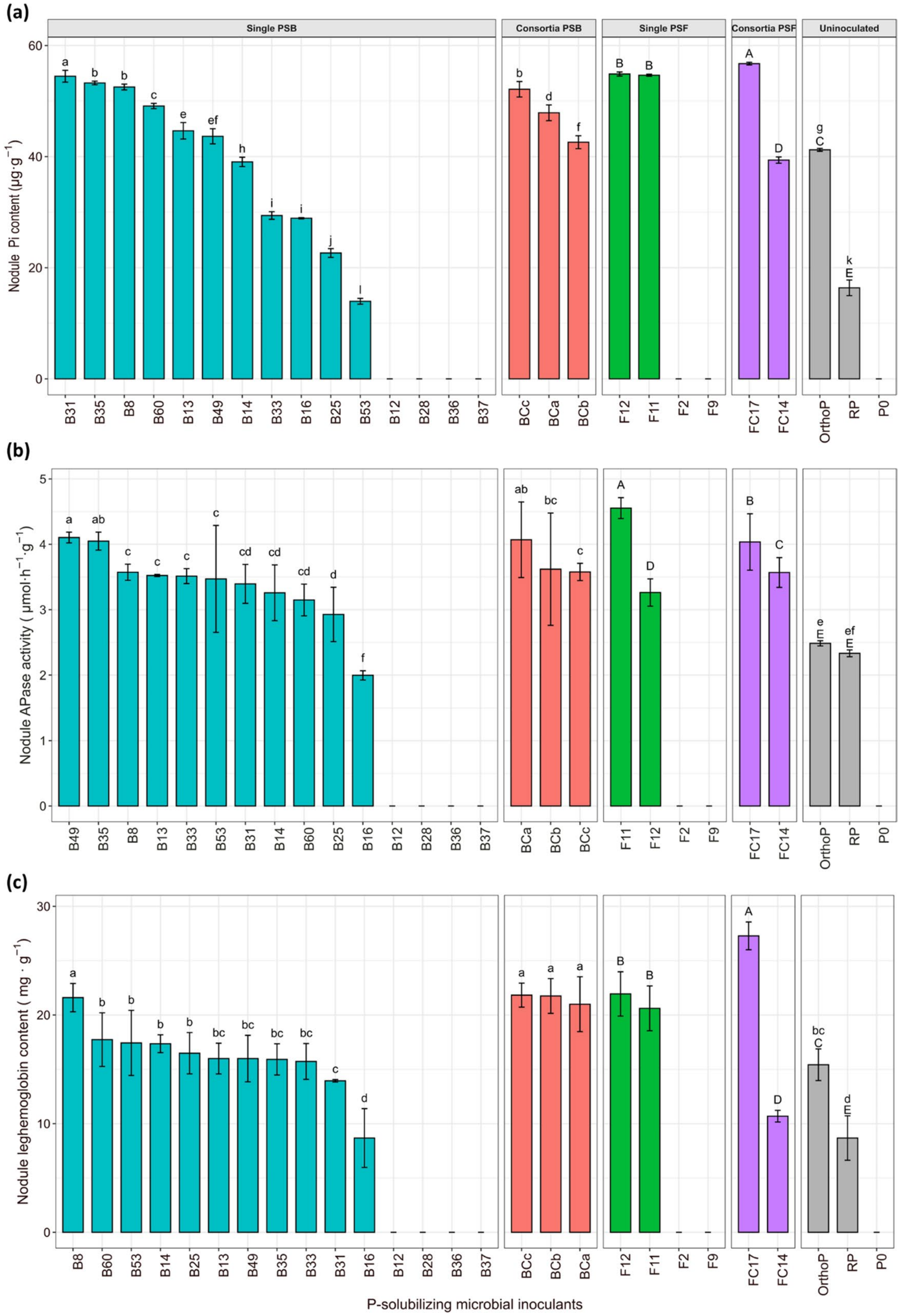
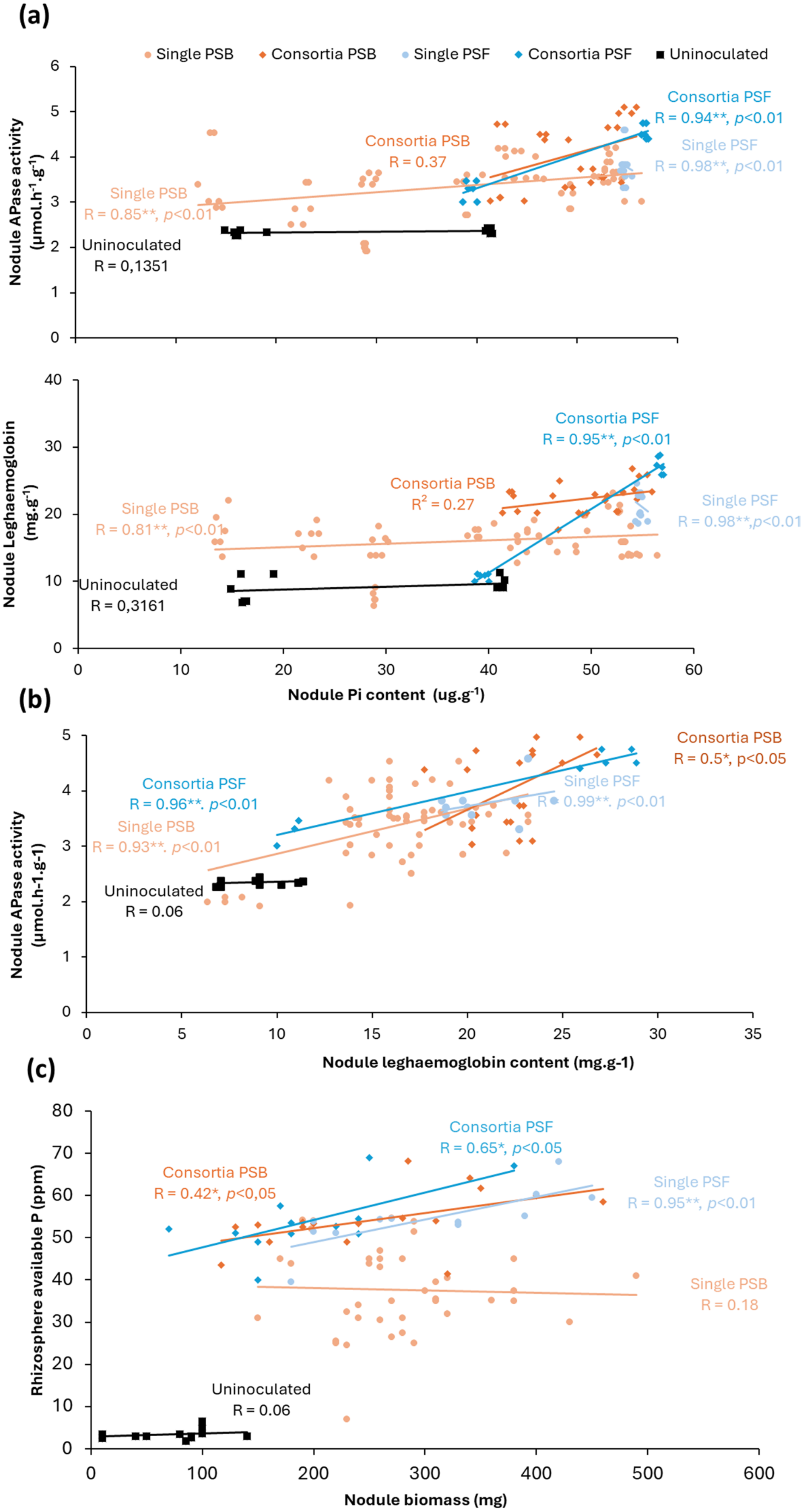


Fig. 2 Nodule Pi content (**a**), nodule APase activity (**b**) and nodule leghemoglobin content (**c**) of 60-days old chickpea (Arifi variety) in response to co-inoculation of Mesorhizobium ciceri with P solubilizing bacteria (PSB), fungi (PSF) and consortia under RP supply. The results are means ± SD of six replicates. Statistical significance indicated by different lowercase (PSB) and uppercase (PSF) letters at $p \leq 0.05$ according to Duncan's test. Abbreviation: BC, bacterial consortia; FC, fungal consortia; P_0, unfertilized; RP, rock phosphate and OrthoP, orthophosphate

Fig. 3 Correlation between nodule Pi content and nodule APase Activity and nodule leghemoglobin content (**a**), between nodule APase activity and nodule leghemoglobin content (**b**) and between nodule biomass and rhizosphere available P (**c**) in response to co-inoculation of *Mesorhizobium ciceri* with P solubilizing bacteria (PSB), fungi (PSF) and consortia under RP supply



compared to OrthoP treatment. PSF inoculants, notably F_{11}, F_{12}, FC_{14} and FC_{17} significantly enhanced SDW (22%, 11%, 11% and 11%) and RDW (16%, 8%, 10% and 11%), respectively, compared to OrthoP treatment (Fig. 4). Co-inoculation with PSM significantly improved RL, RSA, RV and RD, that were improved by at least 50% in response to B_{13}, B_{33}, B_{35} and BC_c compared to RP treatment. Similarly, F_{11}, F_{12}, FC_{14} and FC_{17} enhanced RL by 67%, 23%, 69%, and 35%, respectively, compared to RP treatment (Table 2).

Co-inoculation with B_{13}, B_8, B_{35} and BC_c significantly enhanced root Pi (35%, 29%, 39% and 93%) and soil MBP (233%, 230%, 257% and 282%), respectively, compared to P_0 treatment. The same inoculants also enhanced rhizosphere available P by 227%, 146%, 224%, and 307%, respectively, compared to RP treatment. Regarding PSF inoculants, F_{11} and FC_{17} enhanced root Pi (89% and 100%) and soil MBP (260% and 267%), respectively, compared to P_0 treatment, while rhizosphere available P increased by 287% and 289%, respectively, compared to RP treatment (Table 3). However, rhizosphere soil available P was positively and significantly correlated with nodule biomass, particularly in response to single PSB inoculants ($R = 0.95^{**}$, $p \leq 0.01$) (Fig. 3c).

Regarding shoot nutrients (N, P, K and S) content, co-inoculation with B_8 and BC_c enhanced shoot N (230% and 200%), P (247% and 206%), K (189% and 71%), and S content (250% and 210%), respectively, compared to P_0 treatment. PSF inoculants, notably F_{11} and FC_{17} significantly stimulated shoot N (226% and 208%), P (219% and 211%), K (180% and 173%) and S contents (262% and 288%), respectively compared to P_0 treatment (Table 4).

Based on PSM inoculants performance in terms of nodulation and P-related parameters at the flowering stage, five microbial inoculants including B_8, B_{35}, BC_c, F_{11} and FC_{17} were sorted out to further evaluate their ability to enhance plant growth and P-use efficiency at the reproductive stage under RP supply (Fig. 5).

Physiological performance of PSM inoculants to enhance growth and yield of chickpea under low P conditions

Chickpea growth parameters, root traits and nutrient uptake

At the reproductive stage, shoot, root and pods dry weights significantly enhanced in response to co-inoculation with *Mesorhizobium* and PSM inoculants under rock P supply. Specifically, BC_c, F_{11} and FC_{17} enhanced pods number (987%, 1056% and 1017%), PDW (987%, 1056% and 1017%), SDW (67%, 88% and 80%) and RDW (76%, 80% and 104%), respectively, compared to P_0 treatment (Fig. 6a, b). Moreover, these treatments significantly increased RL by 121%, 94% and 100%, respectively, compared to RP treatment.

In addition, co-inoculation with $BC_{c,}$ F_{11} and FC_{17} significantly stimulated root Pi and APase activity by at least 40% compared to RP treatment. These inoculants also

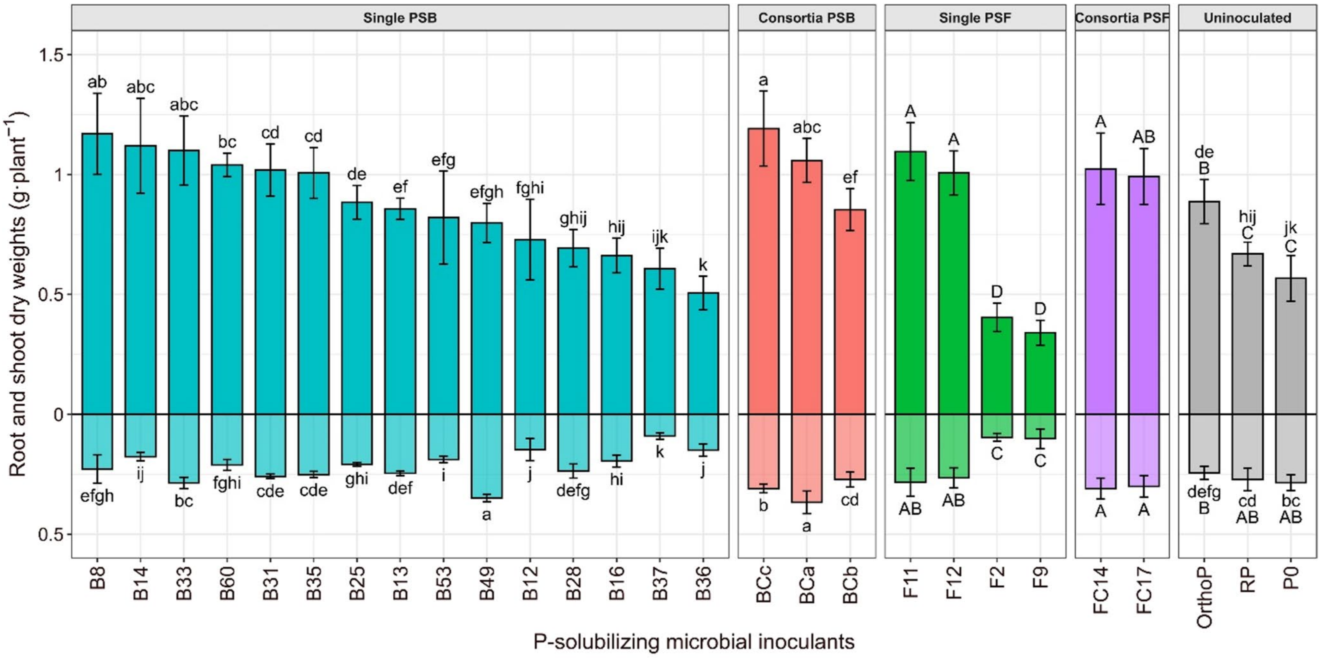


Fig. 4 Shoot and root dry weights of 60-days old chickpea (Arifi variety) in response to co-inoculation of *Mesorhizobium ciceri* with P solubilizing bacteria (PSB), fungi (PSF) and consortia under RP supply. The results are means±SD of six replicates. Statistical significance indicated by different lowercase (PSB) and uppercase (PSF) letters at $p \leq 0.05$ according to Duncan's test. Abbreviation: BC, bacterial consortia; FC, fungal consortia; P_0, unfertilized; RP, rock phosphate and OrthoP, orthophosphate

Table 2 Morphological root traits of chickpea plant in response to co-inoculation with *Mesorhizobium ciceri* and P solubilizing bacteria (PSB), fungi (PSF) and consortia under RP supply

	P-solubilizing microbial inoculants	RL (cm)	RSA (cm^2)	RD (mm)	RV (cm^3)	SRL ($m\ g^{-1}$)	RLD ($cm\ cm^{-3}$)	SRA ($cm^3\ g^{-1}$)
Single PSB	$\mathbf{B_{13}}$	109.23^{a}	20.33^{ab}	0.60^{c-f}	2.63^{cd}	4.44^{ab}	1.81^{a}	829.68^{ab}
	$\mathbf{B_{33}}$	105.67^{a}	20.82^{a}	0.64^{a-d}	3.35^{b}	3.72^{cde}	1.63^{abc}	730.89^{bcd}
	$\mathbf{B_{35}}$	105.83^{a}	20.93^{a}	0.63^{b-e}	3.33^{b}	4.25^{bc}	1.70^{ab}	838.85^{ab}
	$\mathbf{B_{49}}$	92.79^{b}	19.05^{abc}	0.71^{a}	3.95^{a}	2.66^{h-k}	1.31^{efg}	547.48^{e-h}
	$\mathbf{B_{25}}$	85.04^{bc}	15.15^{ef}	0.53^{fgh}	2.08^{e}	4.10^{bcd}	1.58^{bcd}	729.07^{bcd}
	$\mathbf{B_{14}}$	85.50^{bc}	17.06^{cde}	0.57^{d-h}	2.68^{cd}	4.87^{a}	1.5^{b-e}	971.04^{a}
	$\mathbf{B_{60}}$	75.11^{f-k}	14.33^{f}	0.56^{e-h}	2.12^{e}	3.60^{de}	1.34^{efg}	685.94^{b-e}
	$\mathbf{B_{8}}$	72.64^{d}	18.15^{bc}	0.63^{b-e}	2.80^{cd}	3.34^{efg}	1.15^{g-j}	858.90^{ab}
	$\mathbf{B_{53}}$	71.83^{de}	15.75^{def}	0.60^{c-f}	2.53^{cd}	3.83^{cde}	1.18^{fgh}	847.19^{ab}
	$\mathbf{B_{28}}$	63.72^{ef}	8.32^{g}	0.54^{fgh}	1.13^{f}	2.73^{g-k}	1.17^{ghi}	361.64^{ij}
	$\mathbf{B_{31}}$	57.2^{f}	9.84^{g}	0.59^{d-g}	2.08^{e}	2.21^{kl}	0.96^{ij}	381.21^{ij}
	$\mathbf{B_{36}}$	55.3^{f}	9.47^{g}	0.58^{d-h}	1.37^{f}	3.81^{cde}	0.95^{j}	646.66^{c-f}
	$\mathbf{B_{16}}$	55.3^{f}	8.66^{g}	0.55^{fgh}	1.13^{f}	2.86^{f-g}	1.02^{hij}	446.56^{ghi}
	$\mathbf{B_{12}}$	25.3^{g}	4.62^{h}	0.58^{d-h}	0.67^{g}	1.90^{l}	0.44^{k}	343.50^{ij}
	$\mathbf{B_{37}}$	9.92^{h}	2^{i}	0.67^{abc}	0.34^{g}	1.12^{m}	0.15^{l}	229.58^{j}
	$\mathbf{BC_{c}}$	92.17^{bc}	17.89^{bcd}	0.64^{a-d}	2.82^{cd}	2.98^{f-i}	1.42^{cde}	580.55^{d-g}
Consortia PSB	$\mathbf{BC_{a}}$	84.19^{bc}	18.06^{bcd}	0.70^{ab}	2.79^{cd}	2.3^{jkl}	1.21^{def}	495.99^{f-i}
	$\mathbf{BC_{b}}$	70.84^{jde}	10.63^{g}	0.51^{h}	1.27^{f}	2.64^{ijk}	1.40^{fgh}	398.77^{hi}
	$\mathbf{F_{12}}$	112.09^{A}	18.15^{AB}	0.54^{B}	2.48^{A}	4.33^{A}	2.08^{A}	698.45^{ABC}
Single PSF	$\mathbf{F_{11}}$	102.95^{B}	14.44^{C}	0.60^{A}	2.8^{A}	3.78^{AB}	1.72^{B}	516.17^{D}
	$\mathbf{F_{2}}$	17.79^{F}	3.08^{D}	0.59^{A}	0.68^{B}	1.93^{C}	0.30^{D}	333.60^{E}
	$\mathbf{F_{9}}$	4.20^{G}	1.19^{D}	0.60^{A}	0.29^{B}	0.47^{D}	0.07^{E}	133.27^{F}
Consortia PSF	$\mathbf{FC_{14}}$	103.00^{B}	17.9^{AB}	0.62^{A}	2.77^{A}	3.37^{B}	1.65^{B}	597.11^{BCD}
	$\mathbf{FC_{17}}$	96.67^{BC}	16.27^{BC}	0.60^{A}	2.71^{A}	3.26^{B}	1.62^{B}	546.70^{CD}
	OrthoP	83.72^{cE}	18.82^{abcAB}	0.63^{b-eA}	2.84^{cA}	3.45^{efB}	1.31^{efgC}	783.08^{bcA}
Uninoculated	**RP**	86.75^{bcDE}	19.64^{abA}	0.51^{ghB}	2.69^{cdA}	3.27^{e-hB}	1.69^{abB}	744.85^{bcdAB}
	$\mathbf{P_{0}}$	91.47^{bcCD}	19.27^{abcA}	0.59^{d-gA}	2.43^{deA}	3.25^{e-hB}	1.56^{bcdB}	$686.09^{b-e, ABC}$

Different letters indicate mean values that are statistically different at $p \leq 0.05$ according to Duncan's test. ($n = 6$). Abbreviation: RL, root length; RSA, root surface area; RD, root diameter; RV, root volume; SRL, specific root length; RLD, root length density; SRA, specific root area; BC, bacterial consortia; FC, fungal consortia; P0, unfertilized; RP, rock phosphate and OrthoP, orthophosphate

enhanced rhizosphere available P (692–749%), soil MBP (296–340%) and rhizosphere APase activity (125–169%), respectively, compared to P_0 treatment (Table 5).

PSM significantly stimulated shoot and pods nutrients (N, P, K, S, Mg, and Zn) content under RP supply (Table 6). Particularly, FC_{17}, F_{11}, and BC_c enhanced shoot N (46%, 124%, and 143%) and K contents (95%, 67%, and 66%), respectively, compared to P_0 treatment. While FC_{17} and BC_c strongly improved pods N content by 974% and 769%, respectively, compared to P_0 treatments. BC_c also enhanced pods P content, by 67%, 122%, and 497% compared to OrthoP, RP, and P_0 treatments, respectively. Moreover, FC_{17} substantially improved pod Mg (102% and 1422%) and Zn content (73% and 1275%) compared to RP and P_0 treatments, respectively (Table 6).

Principal component analysis (PCA) of chickpea above- and below-ground parameters under five microbial inoculants, explained 86% of the total variation (PC1: 78.5%, PC2: 8%; Fig. S1). Most yield-related traits, including pods biomass, pods number and pods nutrients content were positively correlated with PC1. Treatments such as F_{11}, BC_c, and FC_{17} clustered on the positive side of PC1, indicating their strong influence on productivity- traits.

Effect of PSM inoculants on the rhizosphere soil microbial communities of chickpea

Beta-diversity revealed distinct clustering of the microbial composition in terms of the growth stage. Microbial communities at the reproductive stage formed distinct clusters, clearly separated from those observed at the flowering stage, particularly in the fungal treatments, highlighting a marked shift in community composition across the growth stage (Fig. S2a, b). The Shannon index exhibited that microbial Alpha-diversity varied with the PSM inoculants and the growth stage. For instance, at the reproductive stage for bacterial community, the PSB consortia (BC_c) showed more diversity effect on bacteria compared to the PSF consortia

Table 3 Shoot and root Pi, root APase activity, rhizosphere available P and APase activity and soil microbial biomass P ofchickpea plant in response to co-inoculation with *Mesorhizobium ciceri* and P solubilizing bacteria (PSB), fungi (PSF) and consortia under RP supply

	P-solubilizing microbial inoculants	Shoot Pi (ug g^{-1})	Root Pi (ug g^{-1})	Root APase activity (μmol h^{-1} g^{-1})	Rhizosphere APase activity (nmol g^{-1} h^{-1})	Rhizosphere available P (ppm)	Soil microbial biomass P (ppm)
Single PSB	$\mathbf{B_{13}}$	101.23^{ab}	74.25^{d-g}	3.25^{cde}	970^{bc}	49^{b}	20.00^{bc}
	$\mathbf{B_{35}}$	100.43^{abc}	76.27^{c-g}	3.47^{bcd}	860^{c}	48.61^{b}	21.46^{ab}
	$\mathbf{B_{8}}$	99.68^{abc}	71.08^{e-h}	3.09^{def}	1000^{cd}	37.33^{de}	19.79^{bcd}
	$\mathbf{B_{33}}$	85.25^{b-g}	78.79^{c-f}	3.75^{b}	990^{bc}	31.93^{e-h}	17.08^{e-h}
	$\mathbf{B_{60}}$	84.46^{b-g}	60.91	2.24^{g}	550^{de}	25.41^{hij}	16.04^{ghi}
	$\mathbf{B_{49}}$	83.42^{c-g}	70.85^{e-h}	2.96^{ef}	930^{bc}	35.84^{def}	17.92^{c-g}
	$\mathbf{B_{16}}$	82.41^{d-g}	50.29^{k-l}	1.94^{gh}	410^{def}	29.50^{fgh}	16.25^{f-i}
	$\mathbf{B_{53}}$	77.41^{d-h}	41.13^{l}	1.66^{hi}	420^{def}	31.47^{e-h}	17.71^{c-h}
	$\mathbf{B_{12}}$	76.88^{e-h}	61.33^{ghi}	1.54^{hij}	400^{def}	33.50^{efg}	16.67^{e-i}
	$\mathbf{B_{25}}$	76.46^{e-h}	65.54^{fgh}	1.83^{h}	360^{f}	25.92^{ghi}	16.25^{f-i}
	$\mathbf{B_{14}}$	75.58^{fgh}	89.98^{bc}	3.09^{def}	580^{d}	39.00^{cde}	20.00^{bc}
	$\mathbf{B_{31}}$	71.73^{ghi}	79.34^{cde}	3.55^{bc}	970^{bc}	45.77^{bc}	18.54^{cde}
	$\mathbf{B_{36}}$	62.21^{hij}	22.18^{l}	2.26^{g}	410^{def}	18.43^{jk}	14.79^{i}
	$\mathbf{B_{37}}$	49.00^{jk}	26.60^{l}	1.80^{h}	410^{def}	19.47^{ijk}	15.63^{hi}
	$\mathbf{B_{28}}$	44.47^{k}	57.71^{hij}	1.87^{h}	390^{ef}	26.25^{ghi}	16.04^{ghi}
	$\mathbf{BC_{c}}$	109.86^{a}	106.74^{a}	4.68^{a}	1500^{a}	61.08^{a}	22.92^{a}
ConsortiaPSB	$\mathbf{BC_{a}}$	92.91^{b-e}	103.14^{a}	2.92^{ef}	1420^{a}	50.45^{b}	21.04^{ab}
	$\mathbf{BC_{b}}$	91.23^{b-f}	100.26^{a}	3.04^{ef}	1090^{b}	51.28^{b}	20.00^{bc}
	F11	115.57^{A}	104.63^{A}	3.22^{A}	1020^{A}	58.26^{A}	21.60^{A}
Single PSF	$\mathbf{F_{12}}$	90.89^{B}	65.48^{C}	2.87^{B}	940^{C}	50.78^{B}	19.38^{B}
	$\mathbf{F_{9}}$	59.98^{C}	60.32^{D}	1.25^{C}	440^{D}	20.52^{D}	17.10^{CD}
	$\mathbf{F_{2}}$	53.77^{C}	62.73^{D}	1.50^{C}	360^{DE}	18.58^{D}	16.04^{D}
Consortia PSF	$\mathbf{FC_{17}}$	117.15^{A}	110.29^{A}	3.47^{A}	1530^{A}	58.11^{A}	22.02^{A}
	$\mathbf{FC_{14}}$	113.24^{A}	78.52^{B}	3.21^{A}	1290^{B}	49.94^{B}	19.58^{B}
	OrthoP	94.12^{a-dB}	84.56^{bcdB}	1.22^{jC}	420^{defD}	41.24^{cdC}	18.33^{c-fBC}
Uninoculated	RP	68.60^{ghiC}	68.65^{fghBC}	2.34^{fB}	310^{fEF}	14.92^{kD}	11.04^{jE}
	$\mathbf{P_{0}}$	56.05^{ijkC}	55.30^{ijC}	1.47^{ijC}	250^{fF}	3.08^{iE}	6.04^{kF}

Different letters indicate mean values ($n=6$) that are statistically different at $p \leq 0.05$ according to Duncan's test. Abbreviation: P, phosphorus; APase, phosphatase acid; BC, bacterial consortia; FC, fungal consortia; P0, unfertilized; RP, rock phosphate and OrthoP, orthophosphate

(FC_{17}), while this difference was less evident at flowering stage. In contrast, for the fungal community, PSF inoculants (F_{11} and FC_{17}) led to a slightly increased fungal diversity compared to PSB inoculants (B_8, B_{35} and BC_c) (Fig. S2c, d).

Chickpea rhizosphere microbial composition shifted significantly with PSM inoculants across plant growth stages. *Proteobacteria* and *Chloroflexi* were the dominant bacterial phyla ranging from 30% in P_0 at the reproductive stage to 45% in BC_c at flowering stage. BC_c enhanced *Proteobacteria* more than PSF inoculants (F_{11} and FC_{17}) at flowering stage while at the reproductive stage PSB consortia had a stronger influence. Overall, consortium treatments generally induced greater shifts in both diversity and phylum relative abundance than single inoculants, with BC_c and FC_{17} had more effects than B_{35} or F_{11} (Fig. S3a). In fungal communities, *Ascomycota* was clearly dominant across all treatments and growth stages ranging from 60% to over 80%. The flowering stage showed higher fungal diversity, with notable presence of *Mortierellomycota* and *Basidiomycota*, particularly in FC_{17}, whereas diversity declined during the reproductive stage with *Ascomycota* becoming even more dominant. PSF consortia (FC_{17}) promoted greater diversity at flowering compared to single inoculants (F_{11}), but this effect faded at the reproductive stage. Bacterial consortia (BCc) had limited effect on fungal diversity. Uninoculated treatments (P_0, RP) had the lowest diversity, confirming the role of PSM inoculants. Interestingly, RP treatment had a slightly better impact than P_0 at flowering (Fig. S3b).

At the lowest taxonomic level, the frequencies of bacterial community range from 0 to 1000 and those of the fungal community range from 0 to 10,000. Both bacterial and fungal communities show distinct composition at the flowering and reproductive stages. For example, at the reproductive stage, bacterial taxa such as *Mesorhizobial*, *Beijennckiaceae*, *and Rhizobial* were more abundant. For fungi, *Trichoderma* and *Talaromyces* were dominant at the same stage. On the other hand, at the flowering stage bacterial taxa like *Alphaproteobacteria*, *Pseulabrys*, *Acidimicrobiia*,

Table 4 Shoot nutrients (N, P, K and S) content of chickpea plant in response to co-inoculation with *Mesorhizobium ciceri* and P solubilizing bacteria (PSB), fungi (PSF) and consortia under RP supply

	P-solubilizing microbial inoculants	Shoot nutrients content (mg plant^{-1})			
		N	*P*	K	S
	B_8	25.39^{a}	1.25^{ab}	19.78^{a}	5.01^{a}
	B_{35}	24.35^{ab}	1.14^{abc}	18.85^{a}	3.99^{c}
	B_{14}	24.16^{ab}	1.14^{a}	18.80^{a}	3.15^{d}
	B_{33}	22.06^{ab}	1.04^{a-e}	16.02^{b}	2.54^{e}
	B_{31}	21.87^{ab}	1.04^{b-f}	15.93^{b}	1.97^{fg}
	B_{36}	17.05^{c}	0.84^{b-f}	15.54^{a}	2.28^{ef}
Single PSB	**B_{13}**	16.49^{c}	0.80^{b-f}	14.52^{bc}	1.97^{fg}
	B_{25}	16.00^{cd}	0.80^{a-e}	12.74^{cd}	1.94^{fgh}
	B_{53}	15.03^{cd}	0.65^{d-g}	11.91^{def}	1.94^{fgh}
	B_{12}	14.98^{cd}	0.63^{c-g}	10.99^{ef}	1.70^{ghi}
	B_{60}	14.65^{cd}	0.61^{c-g}	10.23^{de}	1.82^{f-i}
	B_{16}	14.42^{cde}	0.61^{d-g}	10.21^{ef}	1.82^{f-i}
	B_{28}	12.63^{de}	0.61^{d-g}	9.57^{ef}	1.69^{jhi}
	B_{49}	11.07^{ef}	0.56^{efg}	9.33^{f}	1.5^{hij}
	B_{37}	8.35^{fg}	0.43^{g}	6.94^{g}	1.15^{j}
	BC_c	23.02^{ab}	1.10^{abc}	18.61^{b}	4.44^{b}
Consortia PSF	**BC_b**	22.11^{ab}	1.05^{b-f}	16.41^{b}	3.76^{c}
	BC_a	20.85^{b}	0.95^{a-d}	15.74^{b}	2.58^{e}
	F_{11}	25.07^{A}	1.15^{A}	19.20^{A}	5.18^{B}
Single PSF	**F_{12}**	22.13^{B}	1.09^{BC}	18.52^{A}	6.31^{A}
	F_2	7.25^{E}	0.35^{E}	6.42^{D}	1.07^{E}
	F_9	6.72^{E}	0.25^{E}	4.68^{D}	0.84^{E}
Consortia PSF	**FC_{17}**	23.68^{AB}	1.12^{A}	18.71^{A}	5.55^{B}
	FC_{14}	21.46^{B}	0.96^{AB}	15.91^{B}	2.84^{C}
	OrthoP	17.15^{cC}	0.90^{a-eC}	15.73^{bB}	3.12^{dC}
Uninoculated	**RP**	12.57^{deD}	0.56^{d-gC}	9.55^{efC}	1.98^{fgD}
	P_0	7.68^{gE}	0.36^{fgED}	6.85^{gD}	1.43^{ijDE}

Different letters indicate mean values ($n=6$) that are statistically different at $p \leq 0.05$ according to Duncan's test. Abbreviation: BC, bacterial consortia; FC, fungal consortia; P0, unfertilized; RP, rock phosphate; OrthoP, orthophosphate; N, nitrogen; P, phosphorus; K, potassium and S, sulfur

Microverga and *Microtrichales* corresponding to bacterial community and the groups belonging to *Iodophanus*, *Didymellaceae*, *Eurotiomycetes*, *Ascobolus*, and *Ascomycota* corresponding to fungal community were higher in the rhizosphere soil of plants (Fig. S3a, d).

The figure presents PCA biplot illustrating multivariate relationships between dominant bacterial and fungal groups, studied treatments, plant, nodule and soil properties across both growth stages. For bacterial communities, PC1 and PC2 explained 64.7% and 76.4% of the total variation at flowering stage and reproductive stage, respectively. For fungal communities, PC1 and PC2 of the flowering stage explained 66.7% and 74.3% of the total variation at the same stages (Fig. 7). The NMDS ordination showed that plant growth and symbiotic traits were closely associated with inoculated treatments (bacterial and fungal), either single-strain or consortia, while uninoculated treatments were clearly separated along the main ordination axes. In bacterial NMDS plots, inoculation treatments were tightly associated with plant growth traits. *Mesorhizobium* stands out as a key driver of variation between treatments, as confirmed by the SIMPER analysis (Fig. 7a, b). In fungal NMDS, the F_{11} treatment showed strong alignment with multiple plant traits, notably influenced by *Trichoderma*, which, although not inoculated, appears in high relative abundance and correlates positively with growth (Fig. 7c, d).

Network analyses illustrated the relationships between plant and soil parameters with microbial diversity metrics (species richness, diversity and composition) at flowering (Fig. 8a) and reproductive (Fig. 8b) stages. For both plant growth stages, Pearson's correlation analysis revealed positive and significative correlations between most of the plant and soil properties ranging from $0.25 < r < 1$. For instance, at the flowering stage, strong and positive correlations were observed between nodular traits (biomass, number, Pi, APase and leghemoglobin contents) and most aboveground variables (SDW, shoot Pi, shoot Pt, shoot Nt, shoot Kt) as well as soil rhizosphere properties (e.g., rhizo P and SMBP) ($r > 0.75$) (Fig. 8a). In contrast, most root and nodule traits showed low correlations ($r < 0.5$; Fig. 8a). At the reproductive stage, significant positive correlations were observed between pods nutrient content (N, P, K, Mg and Zn) and plant physiological traits (e.g. root and shoot biomass, root Pi and APase, and shoot NPK contents) and soil properties (e.g. soil available, APase and MBP) ($r > 0.6$). However, root traits are low correlated with pods nutrients contents ($r < 0.5$) and slightly correlated with plant and soil properties ($0.25 < r < 0.6$; Fig. 8b). On the other hand, the Mantel test revealed more significant correlations between microbial communities and plant traits at the flowering stage than at the reproductive stage.

At the flowering stage, fungal diversity was positively correlated with nodule traits (number, P inorganic, APase activity and leghemoglobin), aboveground traits (SDW, shoot Pi, Nt, Pt and Kt) and rhizosphere properties (available P and MBP). In contrast, bacterial richness was significantly correlated only with SMBP, whereas bacterial community composition was positively correlated with nodule leghemoglobin and shoot Pi content. Correlations coefficients ranged from $0.2 \leq r \leq 0.4$ with significance levels of $0.01 < p < 0.05$.

Co-occurrence network analysis revealed substantial changes in microbial connectivity, modularity, and community stability across single and consortia application of inoculants for both bacterial and fungal ones (Fig. 9). For bacterial communities, the network under the single

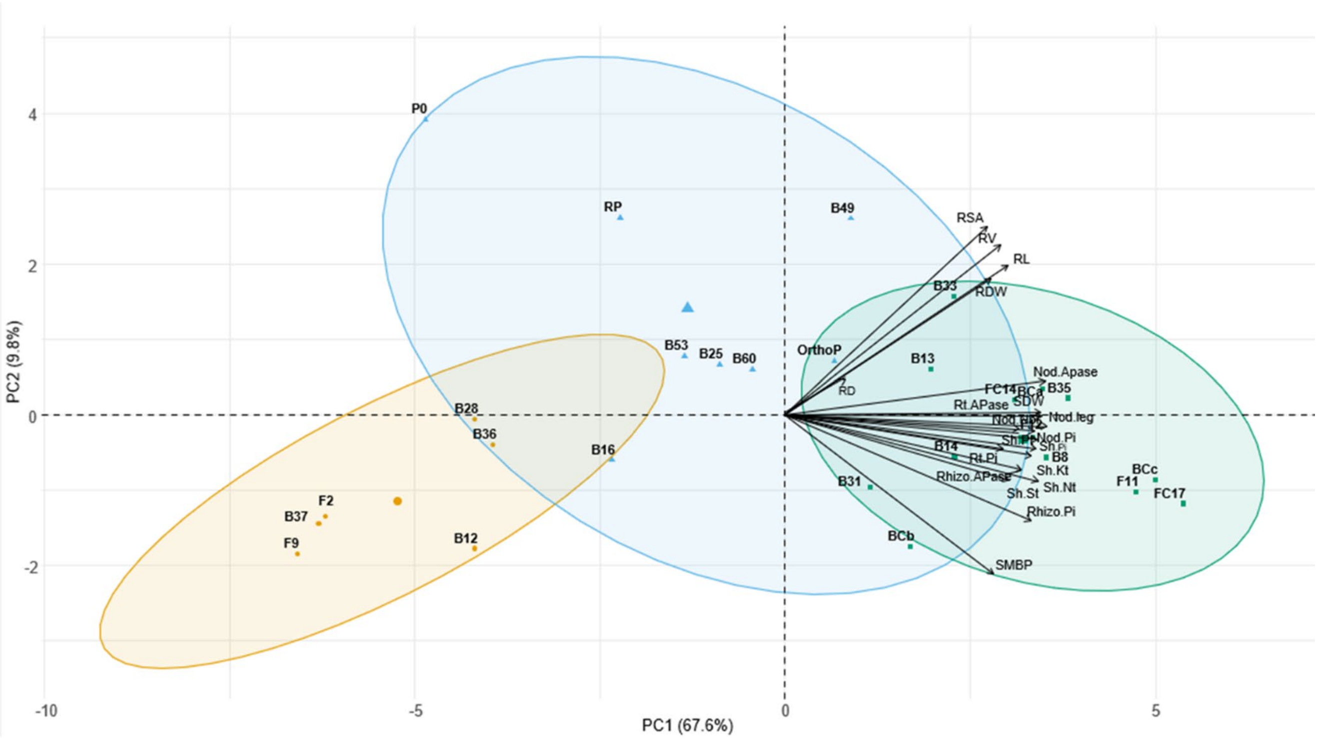


Fig. 5 Principal component analysis based on above and belowground parameters of chickpea plant (Arifi variety) in response to co-inoculation of *Mesorhizobium ciceri* with P solubilizing bacteria (PSB), fungi (PSF) and consortia under RP supply. Values represent the mean of six replicates. Abbreviation: Nod. Biomass, Nodule biomass; Nod. Number, nodule number; Nod Pi, nodule inorganic phosphorus content; Nod. APase, nodule APase activity; Nod. Leg, nodule leghaemoglobin content; Root. P,root inorganic phosphorus content; SDW, shoot dry weight; Sh.Pi, shoot inorganic phosphorus; Sh.Pt, shoot total phosphorus; Sh.Nt, shoot total nitrogen; Sh.Kt, shoot total potassium; Sh.St, shoot total sulfur content; PDW, pods dry weight; Pd.Nt, pods total nitrogen; Pods.P, pods total phosphorus; Pd.Kt, pods total potassium; Pd.Mgt, pods total magnesium; Pd.Znt, pods total Zinc; Pd.St,pods total sulfur content; Rhizo.P, rhizosphere available P; Rhizo.APase, rhizosphere APase activity; SMB.P, soil microbial biomass phosphorus; FC, fungal consortia; BC, bacterial consortia; RP, rock phosphate; OrthoP, orthophosphate and P_0, unfertilized

inoculant treatment showed a higher number of nodes (626) and edges (16,226) compared to the consortia treatment (408 nodes, 8,401 edges). The average degree, indicating the number of connections per node, is also higher in the single inoculant network (51.84) than in the consortia one (41.18). In contrast, the bacterial consortia network displays greater modularity (0.816 vs. 0.663) and a higher average clustering coefficient (0.777 vs. 0.663), suggesting more tightly knit and structured subcommunities. The average path length is longer in the consortia network (3.647 vs. 2.644), indicating a more complex network topology. Regarding fungal communities, the single inoculant network includes 500 nodes and 12,805 edges, while the consortia network contains fewer nodes (439) and edges (9,071). In addition, the average degree is higher in the single inoculant treatment (51.22) compared to the consortia treatment (41.33). However, the fungal consortia network again exhibits slightly higher modularity (0.814 vs. 0.787) and a comparable clustering coefficient (0.76 vs. 0.769), along with a longer average path length (3.469 vs. 3.037), indicating a compartmentalized structure.

Discussion

The present study demonstrated the functional physiological potential of PSM inoculants, whether applied individually or as consortia, to enhance chickpea-*Mesorhizobium* symbiotic efficiency under low P availability conditions. Through inducing nodulation, enhancing rhizosphere P availability, P acquisition, and modulating the rhizosphere microbiome, PSM consortia significantly outperformed single-strain inoculants in driving chickpea growth and yield performance across both flowering and reproductive stages. These findings suggest that combining PSM with *Mesorhizobium* signficanlty improves symbiotic efficiency leading to enhanced BNF and chickpea productivity in P-deficient soils.

Symbiotic and growth traits of chickpea were induced in response to P solubilizing inoculants under low P availability

PSM inoculants enhanced chickpea-*Mesorhizobium* symbiotic efficiency at the flowering stage and improved overall

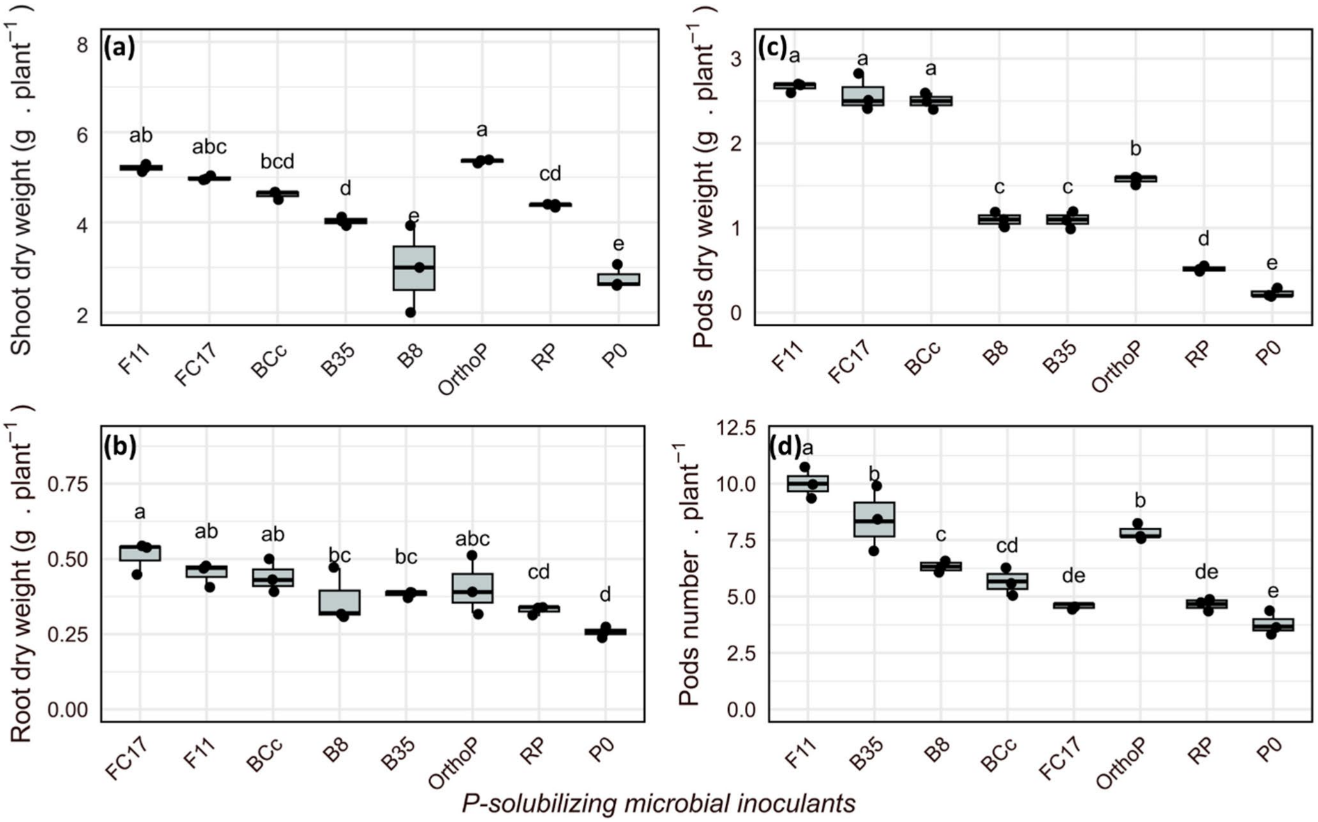


Fig. 6 Shoot (**a**), root (**b**) and pods dry weights, (**c**) and pods number (**d**) of 80-days old chickpea (Arifi variety) in response to co-inoculation of *Mesorhizobium ciceri* with P solubilizing microbe (PSM) under RP supply. The results are means ± SD of six replicates. Different letters indicate mean values that are statistically different at $p \leq 0.05$ according to Duncan's test. Abbreviation: BC, bacterial consortia; FC, fungal consortia; P_0, unfertilized; RP, rock phosphate and OrthoP, orthophosphate

Table 5 Morphysiological traits of roots and rhizosphere of 80-days old chickpea plant in response to co-inoculation with *Mesorhizobium ciceri* and P solubilizing microorganisms (PSM) under RP supply

P-solubilizing microbial inoculants	Root morphological traits			Root physiological traits		Rhizosphere traits		
	RL (cm plant^{-1})	RSA (cm^2 plant^{-1})	RD (mm plant^{-1})	Root Pi (µg g^{-1})	Root APase activity (μmol g^{-1} h^{-1})	Rhizosphere APase activity (μmol g^{-1} h^{-1})	Rhizosphere available *P* (ppm)	Soil microbial biomass *P* (ppm)
B_{35}	685.38^a	123.27^a	0.62^a	17.23^c	0.88^c	0.30^d	23.46^d	19.70^c
B_8	468.69^{ab}	120.61^a	0.60^a	16.08^c	0.71^d	0.29^d	22.8^d	20.37^{bc}
BC_c	722.89^a	131.35^a	0.63^a	22.12^a	1.11^b	0.43^a	28.93^b	25.33^a
F_{11}	636.94^a	123.30^a	0.59^a	21.28^a	1.05^b	0.36^c	31.06^a	22.79^{ab}
FC_{17}	627.17^a	121.39^a	0.62^a	21.37^a	1.17^a	0.38^b	30.13^{ab}	22.83^{ab}
OrthoP	637.34^a	143.97^a	0.59^a	19.75^b	0.64^e	0.22^f	27.53^c	0.59^c
RP	544.32^{ab}	106.71^a	0.58^a	14.3^d	0.74^d	0.26^e	20.93^e	0.58^d
P_0	327.52^b	56.47^b	0.50^b	11.68^e	0.54^f	0.16^g	3.66^f	0.50^e

Different letters indicate mean values ($n = 6$) that are statistically different at $p \leq 0.05$ according to Duncan's test. Abbreviation: RL, root length; RSA, root surface area; RD, root diameter; BC, bacterial consortia; FC, fungal consortia; P0,: unfertilized; RP, rock phosphate; OrthoP, orthophosphate

plant physiological performance at both flowering and reproductive stages. At the flowering stage, they induced nodulation and stimulated key P-related physiological traits within nodules, as evidenced by increased nodule Pi, APase activity, and leghemoglobin contents. This is consistent with the findings of Alamzeb et al. (2023), who reported that co-inoculation with *Pseudomonas striata* and rhizobium enhances chickpea nodulation through increasing P availability from RP. In addition, the present study provides deeper insight into the mechanisms likely explaining the improved nodulation. In this context, PSM inoculation can improve BNF efficiency not only through optimizing P acquisition but also by enhancing

Table 6 Nutrients content in shoots and pods (N. P. K. S, Mg and Zn) of 80-days old chickpea plant (Arifi variety) in response to co-inoculation with *Mesorhizobium ciceri* and P solubilizing microbe (PSM) under RP supply

P-solubilizing microbial inoculants	Shoot nutrients content (mg plant^{-1})				Pods nutrients content (mg pot^{-1})					
	N	*P*	K	S	*N*	*P*	K	S	Mg	Zn
B_8	98.16^b	10.63^c	69.80^c	7.4^c	45.28^d	3.11^d	20.99^d	1.53^c	2.27^d	0.043^c
B_{35}	134.34^{ab}	9.70^c	90.58^b	10.02^b	49.20^{cd}	3.04^d	22.19^d	1.47^c	2.53^d	0.045^c
BC_c	194.63^a	11.35^b	113.29^a	13.39^a	141.92^b	7.58^b	65.53^a	4.85^a	6.25^{ab}	0.10^a
F_{11}	179.45^a	11.46^b	124.23^a	12.03^a	140.97^b	8.26^a	54.56^b	4.59^a	6.04^b	0.11^a
FC_{17}	197.36^a	13.38^a	125.46^a	12.16^a	175.31^a	7.37^b	65.79^a	4.78^a	6.69^a	0.11^a
OrthoP	184.26^a	12.28^{ab}	127.92^a	13.36^a	77.26^c	4.54^c	35.3^c	2.69^b	3.70^c	0.064^b
RP	133.56^{ab}	6.94^e	93.71^b	10.09^b	20.63^{de}	1.25^e	9.69^e	0.68^d	1.01^e	0.018^d
P_0	80.15^b	6.85^d	57.03^c	6.5^c	8.60^e	0.56^f	4.06^f	0.28^d	0.44^f	0.008^e

Different letters indicate mean values ($n=6$) that are statistically different at $p \leq 0.05$ according to Duncan's test. Abbreviation: BC, bacterial consortia; FC, fungal consortia; P0, unfertilized; RP, rock phosphate; OrthoP, orthophosphate; N, nitrogen; P, phosphorus; K, potassium; S, sulfur; Mg, magnesium and Zn, zinc

intra-nodule P use efficiency, which likely supports more effective nitrogenase functioning. Consequently, more efficient P mobilization will activate key nodule physiological pathways related to nodule formation, metabolic activity, and overall plant growth. Furthermore, nodule leghemoglobin content is a crucial regulator that ensures nitrogenase compartmentalization is markedly induced in response to PSM inoculation. The strong positive correlation between leghemoglobin and nodule Pi contents suggests that enhanced nodulation in response to PSM inoculation may be driven by enhanced intra-nodular P use efficiency, which support an optimal energy supply and redox balance for optimized nitrogenase activity (Zaidi et al. 2010). This is consistent with findings by Shome et al. (2022), who demonstrated that co-inoculation with *Rhizobium japonicum* and *Pseudomonas striata* in soybean can lead to more efficient N_2 fixation, as reflected by higher leghemoglobin content in nodules.

At both plant growth stages, PSM inoculation promoted root development owing to the ability of different PSM to produce phytohormones and other microbial signals that contributed to better nutrient acquisition with positive consequences on nodulation and overall symbiotic traits. This is consistent with findings by Shome et al. (2022), that demonstrated enhance P acquisition as a result of developed rooting system in response to combined *Rhizobium japonicum* - *Pseudomonas striata* inoculation. Khourchi et al. (2022b) reported that inoculation with individual PSB strains and PSB consortia enhanced wheat P acquisition efficiency through promoting root hair proliferation and improving root morpho-physiological traits.

Furthermore, PSM inoculants can positively influence chickpea growth by modulating rhizosphere properties and enhancing P availability and nutrient cycling. By promoting a well-developed and functional root system, PSM indirectly support plant productivity, highlighting the critical role of root-mediated nutrient uptake in sustaining crop performance under limited P conditions, thus supporting the significant correlations between rhizosphere P related parameters and plant/root traits (Fig. 7, S1). Our findings corroborate the findings of previous studies reporting the agronomic advantage of co-inoculating *Mesorhizobium* with PSB strains (*Pseudomonas sp.* (Benjelloun et al. 2021) and *Pseudomonas jessenii PS06* (Valverde et al. 2006) on root system development and improved nutrient uptake along with a higher chickpea seed yield. In addition, Rafique et al. (2021) highlighted the importance of adequate P availability for optimal pods formation and grain filling, which supports the increased pods biomass observed in response to PSM inoculation in the present study (Fig. 6c). Overall, these results clearly indicate that PSM inoculants enhance chickpea growth and symbiotic performance through enhancing root

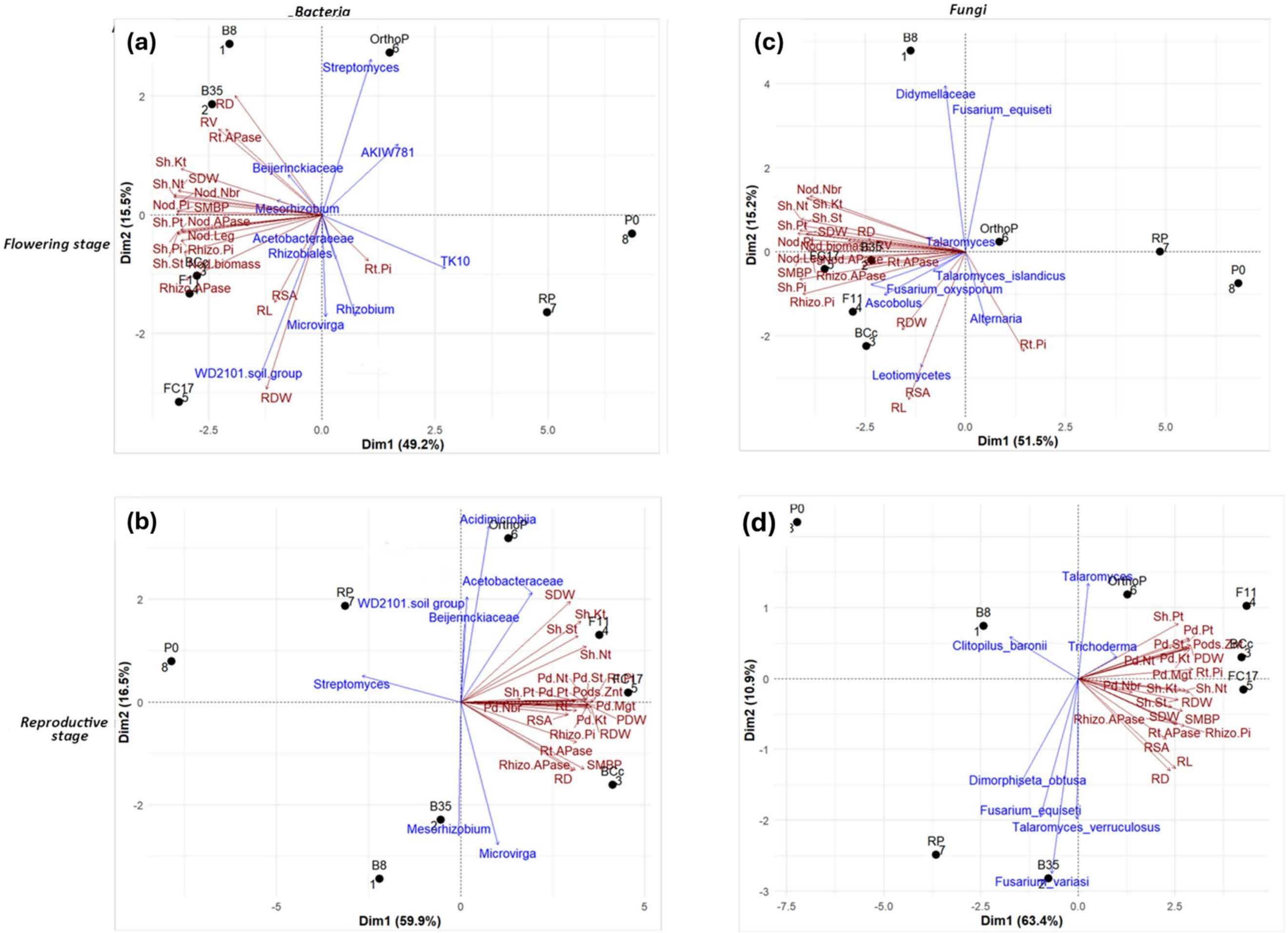


Fig. 7 SIMPER test performed on Non-metric multidimensional Scaling (NMDS), presenting multiple comparison among the predominant bacterial (**a**, **c**) and fugal groups (TAXA) (**b**, **d**) in the rhizosphere soil and growth traits at flowering (**a**, **b**) and reproductive (**c**, **d**) stages of chickpea fertilized with rock phosphate and co-inoculated with *Mesorhizobium ciceri* and P solubilizing bacteria (PSB), fungi (PSF) and consortia. Each data point represents dominant bacterial and fungal communities. The red points represent plant growth traits. Abbreviation: Nod. Biomass, nodule biomass; Nod. Number, nodule number; Nod Pi, nodule inorganic phosphorus content; Nod. APase, nodule APase activity; Nod. Leg, nodule leghaemoglobin content; Root. P, root inorganic phosphorus content; SDW, shoot dry weight; Sh.Pi, shoot inorganic phosphorus; Sh.Pt, shoot total phosphorus; Sh.Nt, shoot total nitrogen; Sh.Kt, shoot total potassium; Sh.St, shoot total sulfur content; PDW, pods dry weight; Pd.Nt, pods total nitrogen; Pods.P, pods total phosphorus; Pd.Kt, pods total potassium; Pd.Mgt, pods total magnesium; Pd.Znt, pods total Zinc; Pd.St, pods total sulfur content; Rhizo.P, rhizosphere available P; Rhizo.APase, rhizosphere APase activity; SMB.P, soil microbial biomass phosphorus; FC, fungal consortia; BC, bacterial consortia; RP, rock phosphate; OrthoP, orthophosphate and P_0, unfertilized

functional traits, which enabled rhizosphere optimization and plant P acquisition, thereby promoting nodulation efficiency and BNF.

Symbiotic and growth traits of chickpea were mostly induced by consortia than single-strain inoculants

At the flowering stage, PSF consortia (FC_{17} and FC_{14}) significantly enhanced chickpea nodulation and nodule physiological parameters (e.g., nodule Pi, leghemoglobin and APase activity contents) and such improvement can be attributed to the significant enhancement of intra-nodular P use efficiency and the subsequent positive impact on symbiotic N_2 fixation. These observations are consistent with previous nodule-P metabolism focused studies (Araújo et al. 2008; Bargaz et al. 2012) and on the key role of APase activity, nodule P content and P availability on the *P. vulgaris* rhizobial symbiosis efficiency under P-limited conditions. Furthermore, the present study added a new knowledge that PSM consortia (particularly BC_c and FC_{17}) inoculation can advantageously influence nodule P metabolism while enhancing morphological and symbiotic traits (Figs. 1a and b and 2a and c), which is likely due to synergistic interactions within microbial strains as compared to single PSM inoculants. This is consistent with Mansotra et al. (2015), who reported that chickpea co-inoculated with *Mesorhizobium* and mixture of *Piriformospora indica* and different *Pseudomonas spp* improved nutrient acquisition, nodule biomass and P uptake.

At the reproductive stage, PSM consortia significantly enhanced chickpea growth and yield compared to single PSM inoculants, increasing shoot, root, and pod biomass as well as nutrients content (Fig. 6; Table 6). These results are consistent with Pawar (2025), who demonstrated that co-inoculation of PSB consortia with *Rhizobium spp.* can enhance biomass, nutrient uptake and yield in chickpea under field conditions more effectively than single inoculation. These results suggest that microbial consortia provide multiple plant-growth-promoting functions such as P- solubilization, phytohormone production and enhanced root physio-morphological traits that interact synergistically to enhance nutrient uptake and allocation to pods, leading to superior growth and productivity.

PSM inoculants enhanced rhizosphere P and MBP at both growth stages under RP supply, which coincided with improved nodulation. This soil microbial trait brings another indicator that support the promoting effect of PSM consortia (bacterial and fungal), on P use efficiency in chickpea nodules and consequently maintains an efficient nodule functioning for BNF under P-limited conditions. The soil MBP is key parameter allowing to understand the contribution of microbial immobilized P to understand belowground P cycling and how it affects chickpea symbiotic performance, however care should be taking in interpreting MBP variation across treatment which was calculated based on a constant Kep (0.40) value (Fan et al. 2021; Su et al. 2025) that provides robust and comparable estimates of MBP across soils when $NaHCO_3$ is used as extractant.

Rhizosphere microbial communities significantly influenced chickpea symbiotic performance with distinct microbiome patterns along plant growth stages

Our findings offer valuable insights into how PSM inoculants influence indigenous rhizosphere microbial communities and induce chickpea nodulation. This study showed that co-inoculation with *Mesorhizobium* and PSM differentially influences microbial diversity and community composition, positively impacting chickpea growth at both flowering and reproductive stages.

This study demonstrated that PSM inoculation induced significant shifts in soil microbial community composition across growth stages, with dominance of functionally important bacterial phyla such as *Proteobacteria* and *Chloroflexi* (Fig. S3a, b). Members of these phyla were reported to play an key roles in N cycling, including BNF and nitrification, suggesting that our bacterial inoculants may enhance chickpea nodulation and growth performance by promoting beneficial microbial taxa associated with N fixation (Rutten and Poole 2019; Rahimlou et al. 2021). For instance, Solanki et al. (2020) demonstrated that NFB (e.g., *Bradyrhizobium* and *Pseudacidovorax)* and their symbionts can stimulate *Proteobacteria* growth in legumes, consistent with the increased *Proteobacteria* relative abundance observed during the reproductive stage under PSB inoculation. The persistence of *Proteobacteria* dominance at flowering stage, under this inoculation align with Sugiyama et al. (2014), who reported that *Proteobacteria* are frequently dominant in the legume rhizosphere during growth. The increased *Chloroflexi* relative abundance under PSF inoculation at the reproductive stage is consistent with Liu et al. (2024), who reported that members of this phyla are actively involved in nutrient mobilization, especially under P and N limited conditions. *Chloroflexi* are also known for their role in N cycling through organic matter decomposition and ammonification, which could contribute to increased N availability for the plant (Mukherjee et al. 2022; Zhang et al. 2023). *Actinobacteria* constitute a key phylum of beneficial bacteria known for promoting plant growth and stimulating root nodulation (Kucho et al. 2014; Põlme et al. 2014; Ghodhbane-Gtari et al. 2019). Our results demonstrated that PSB inoculation increased the relative abundance of *Actinobacteria*, where they comprised over 15% of total microbiota at reproductive stage. These findings indicates that PSM inoculation may indirectly enhance chickpea nodulation by fostering synergies with indigenous NFB in the rhizosphere of chickpea (Boukhatem et al. 2022). Additionally, co-inoculation with PSM increased the relative abundance of *Ascomycota* and *Basidiomycota* at the flowering stages, while *Basidiomycota* relative abundance reduced at reproductive stage. *Ascomycota* include many plant-beneficial fungi previously reported as playing an important role in nutrient cycling and potentially support root nodulation, especially under nutrient-deficient conditions (Challacombe et al. 2019; Li et al. 2022; Yao et al. 2023).

Indeed, PSF inoculants favored the relative abundance of *Trichoderma* and *Talaromyces* genera, known as disease-suppressive. This positively impacted plant agro-morpho-physiological traits at the reproductive stage. This is consistent with Zhang et al. (2023), who reported that these genera support legume root development and N_2 fixation. Moreover, PSF inoculation also increased *Basidiomycota* relative abundance during the flowering stage, potentially inducing chickpea nodulation through improved nutrient cycling. At this stage, fungal diversity and composition were clearly associated with nodulation, highlighting the crucial role of PSF inoculants in inducing nodulation. These observations align with Hartman and Tringe (2019), who reported that microbial inoculation at early growth

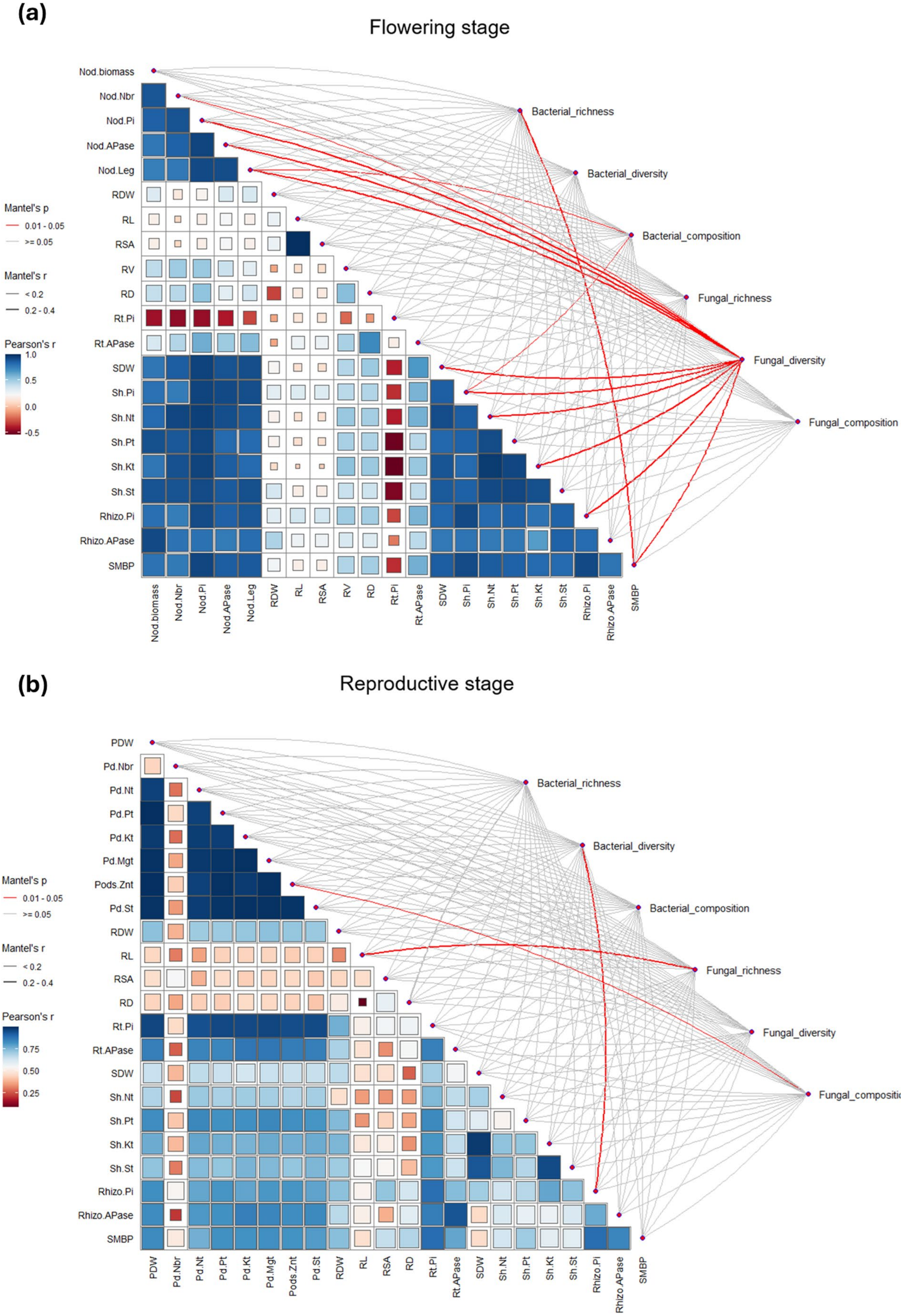

(a)
Flowering stage
Nod.biomass
Nod.Nbr
Nod.Pi
Nod.APase
Nod.Leg
RDW
RL
RSA
RV
RD
Rt.Pi
Rt.APase
SDW
Sh.Pi
Sh.Nt
Sh.Pt
Sh.Kt
Sh.St
Rhizo.Pi
Rhizo.APase
SMBP
Bacterial_richness
Bacterial_diversity
Bacterial_composition
Fungal_richness
Fungal_diversity
Fungal_composition
Mantel's p
0.01 - 0.05
>= 0.05
Mantel's r
< 0.2
0.2 - 0.4
Pearson's r
1.0
0.5
0.0
-0.5
(b)
Reproductive stage
PDW
Pd.Nbr
Pd.Nt
Pd.Pt
Pd.Kt
Pd.Mgt
Pods.Znt
Pd.St
RDW
RL
RSA
RD
Rt.Pi
Rt.APase
SDW
Sh.Nt
Sh.Pt
Sh.Kt
Sh.St
Rhizo.Pi
Rhizo.APase
SMBP
Bacterial_richness
Bacterial_diversity
Bacterial_composition
Fungal_richness
Fungal_diversity
Fungal_compositio
Mantel's p
0.01 - 0.05
>= 0.05
Mantel's r
< 0.2
0.2 - 0.4
Pearson's r
0.75
0.50
0.25

Fig. 8 Network analysis of Mantel correlations among bacterial and fungal community richness, bacterial and fungal diversity, bacterial and fungal composition (Shannon index), and agro-morphophysiological parameters (morphological, biomass, and biochemical parameters); and Pearson correlation analysis among agro-morphophysiological parameters at flowering (**a**) and reproductive (**b**) stages of chickpea fertilized with rock phosphate and co-inoculated with *Mesorhizobium ciceri* and P solubilizing bacteria (PSB), fungi (PSF) and consortia (Arifi variety). The width of edges represents the size of the correlation coefficient (Mantel's r), while edge color represents the statistical significance based on Mantel's p. Abbreviation: Nod. Biomass, nodule biomass; Nod. Number, nodule number; Nod Pi, nodule inorganic phosphorus content; Nod. APase, nodule APase activity; Nod. Leg, nodule leghaemoglobin content; Root. P, root inorganic phosphorus content; SDW, shoot dry weight; Sh.Pi, shoot inorganic phosphorus; Sh.Pt, shoot total phosphorus; Sh.Nt, shoot total nitrogen; Sh.Kt, shoot total potassium; Sh.St, shoot total sulfur content; PDW, pods dry weight; Pd.Nt, pods total nitrogen; Pods.P, pods total phosphorus; Pd.Kt, pods total potassium; Pd.Mgt, pods total magnesium; Pd.Znt, pods total Zinc; Pd.St, pods total sulfur content; Rhizo.P, rhizosphere available P; Rhizo.APase, rhizosphere APase activity; SMB.P, soil microbial biomass phosphorus; FC, fungal consortia; BC, bacterial consortia; RP, rock phosphate; OrthoP, orthophosphate and P_0, unfertilized

stages enhances symbiotic interactions, nutrient uptake and overall plant performance. Moreover, the observed relationships between bacterial community composition and nodule leghemoglobin content at flowering suggest that microbial diversity can directly influence N_2 fixation efficiency. These findings are consistent with Lagunas et al. (2023), who demonstrated that rhizobial diversity directly influences N_2 fixation efficiency in *Medicago truncatula*, by promoting the formation of more effective nodules with higher leghemoglobin and improving oxygen regulation within nodules and supporting optimal nitrogenase activity. Our findings on the significant correlation between fungal diversity and nodule traits are consistent with previous studies highlighting the role of fungal communities in nutrient mobilization and symbiotic performance (Arrieta et al. 2015; Li et al. 2016; Zhang et al. 2024b).

At the reproductive stage (where nodule senescence likely begins) the relative abundance of this phylum declined. This trend aligns with recent study reported that inoculation with *Rhodotorula mucilaginosa JGTA-S1* (a *Basidiomycota* member) improved N uptake through upregulating the genes involved in N metabolism, N transporters, and nodule inception-like transcription factors (Paul et al. 2020). At the same stage, bacterial diversity, fungal species richness, and specific fungal compositions were significantly correlated with pods Zn content, RL, and rhizosphere available P, respectively. This is in agreement with reports demonstrating that microbial community shifts across different growth stages and affect micronutrients uptake (Richardson and Simpson 2011; Wang et al. 2017). Notably, co-inoculation with PSM and rhizobia enhances P availability improving root traits and overall plant growth (Wani et al. 2007; Alori et al. 2017). In line with this, our results show that co-inoculation with *Mesorhizobium* and PSM under RP supply significantly increased P use efficiency, leading to enhanced physiological performance.

Bacterial diversity was enhanced by PSB inoculation at the reproductive stage, whereas fungal diversity was higher at the flowering stage in plants inoculated with PSF, highlighting stage-specific effects of microbial inoculants on soil microbial diversity (Fig S2c, d). These results are consistent with previous research indicating that certain microbial treatments can boost microbial diversity, particularly under P-limited conditions (Rousk et al. 2010; Ambrosini et al. 2016; Zhang et al. 2021). Interestingly, the plant growth stage strongly influences shifts in microbial community composition (Fig. S2a, b). Notably, fungal community exhibited distinct microbial clustering at the reproductive stage under PSM inoculation, highlighting a stage-dependent orchestration of rhizosphere microbial communities under PSM inoculation. This reclustering can be attributed to nodule senescence which usually began during the reproductive stage, potentially altering microbial interactions and nutrients cycling. These results are supported by recent study of Lahijanian et al. (2025), who demonstrated that microbial inoculants and co-inoculation strategies significantly reshape soil bacterial and arbuscular mycorrhizal fungal diversity. The findings of the latter study highlight that microbial community responses to inoculation are strongly influenced by environmental context (cropping season and geographical site). PSM inoculation not only reshapes the rhizosphere microbial community but also appears to orchestrate functional shifts that align with the plant's developmental stage, effectively supporting symbiotic functions and nutrient acquisition according to changing physiological needs. These results are in line with those of Nuccio et al. (2020) and Xu et al. (2018), who found that the rhizosphere microbial communities are mostly shaped by plant growth stages. Furthermore, Hannula et al. (2017) found that fungal species are highly responsive to plant phenology and root exudate dynamics, and that these changes in rhizosphere diversity are often transitory, which is consistent with the temporary shifts observed in the fungal community across different chickpea growth stage. This in line with Lan et al. (2024) highlighting that plants dynamically adjust belowground strategies by modulating root traits, rhizosphere microbial communities, and enzymatic activities to mobilize organic and inorganic P. This study demonstrated a temporal shift from inorganic P mobilization driven by root morphological traits and organic acid exudation at early stages to a stronger reliance on microbial-mediated organic P mineralization and mycorrhizal associations at later growing season. These findings highlight the combined effect of plant growth stage and microbial inoculants in shaping rhizosphere microbial communities, with plant phenology appears to be the dominant factor driving microbial differentiation.

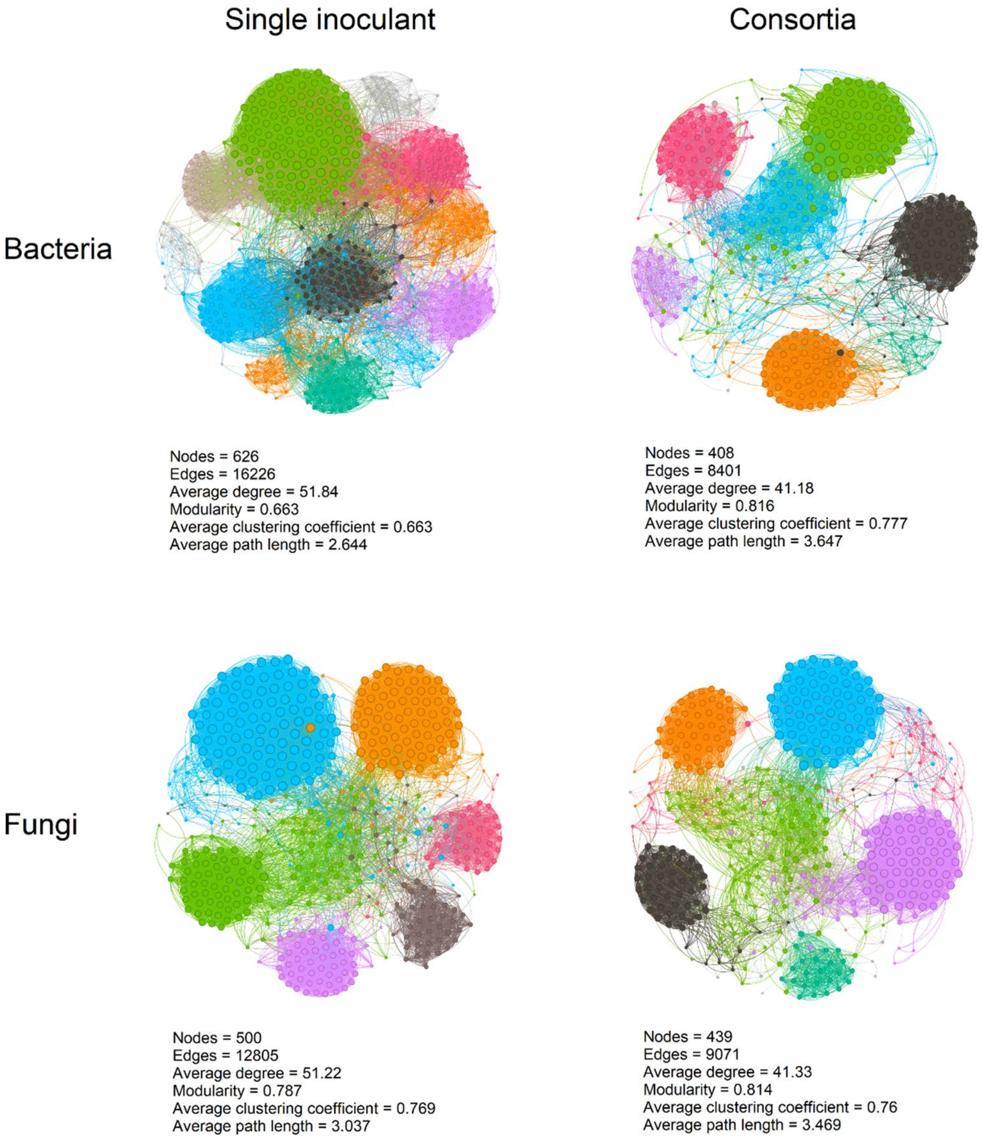


Fig. 9 Comparison of microbial co-occurrence networks in chickpea (Arifi variety) fertilized with rock phosphate and co-inoculated with *Mesorhizobium ciceri*, phosphate-solubilizing bacteria (PSB), fungi (PSF) and consortia

Co-occurrence network analysis revealed clear structural differences in microbial community organization between single and consortia inoculation treatments. Networks derived from single inoculant applications exhibited greater node and edge densities, along with increased average degrees, indicating more extensive and uniform microbial interaction among ASVs. This indicates that single inoculants could stimulate more extensive, less compartmentalized microbial interaction. Similar network expansion was noted by Liu et al. (2021) in soybean rhizospheres inoculated with individual strains of *Bradyrhizobium*, leading to more uniformly connected bacterial communities. These patterns are consistent with their findings. Furthermore, core rhizosphere taxa like *Bradyrhizobium* and other functionally significant genera tend to maintain robust network centrality in single inoculations, facilitating stable microbe-plant interactions in field settings, according to recent research conducted in established soybean fields by Sarao et al. (2024). In contrast, consortia treatments, especially in bacterial communities, produced networks with higher modularity and clustering coefficients but fewer nodes and connections. These features reflect a shift toward more compartmentalized and structured networks potentially indicating the formation of tightly interacting subcommunities. A more intricate and maybe specialized microbial architecture is further suggested by the increased average path lengths observed in consortia networks. Zhang et al. (2024a, 2024b) showed how arbuscular mycorrhizal (AM) fungus preferentially recruit various bacterial communities, such as *Devosia* sp. ZB163, into tightly connected modules around the hyphal zone, demonstrating how these network topologies are consistent with the concept of modular microbiota. These modules enhanced nitrogen uptake, regulated bacterial-fungus interactions, and increased plant resilience.

Conclusion

The present study demonstrates that co-inoculation of chickpea with *Mesorhizobium* and PSM significantly improves symbiotic efficiency and overall plant growth, which were significantly influenced by the rhizosphere-associated microbiota. The positive effects of PSM inoculants are closely related to the dynamics of soil microbiome across plant growth stages, leading to improved nodulation, nutrient uptake and productivity. At the flowering stage, bacterial diversity, especially different taxonomic levels of N_2-fixing bacteria (e.g., *Mesorhizobium*, *Beijerinckiaceae*, *Rhizobiales*, and *Microvirga* exhibited a strong correlation with nodulation, shoot nutrient content, and rhizosphere properties. At the reproductive stage, the fungal taxa with disease-suppressive functions (*Trichoderma*, *Talaromyces*, and *Dimorphiseta*) played a more prominent role, alongside a continued contribution of key bacterial taxa. Evaluating more chickpea genotypes under different agro-ecological conditions is needed to optimize microbial inoculation for sustainable legume production. Furthermore, the determination of N-fixed derived from the BNF is a paramount parameter needed to better understand the functional contribution of PSM inoculants under both controlled and field conditions. In parallel, particular focus should be paid to the metabolomic aspect and relationships with the nodule- soil-associated microbiome likely exhibiting a strong influence on chickpea symbiotic efficiency.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s00374-026-01989-7.

Acknowledgements This work was funded by the flagship Program P-driven funded by OCP Nutricrops and the Associate Professorship research grant N°112-25-8 at Mohammed VI Polytechnic University. We thank UM6P technical staff for their technical and intellectual assistance.

Author contributions **H.C, S.K, ** and **A.B** : conceived and designed the experiments and wrote the first version of the manuscript. **H.C** and **S.K** : conducted the experiments, collected and analyzed the data; **A.B** supervised, investigated and validated the research; **M.I** analyzed metagenomic data; **A.IY** and **K.S** provided technical assistance and performed the plant, soil and microbiological analyses. **S.K, W.E, M.I, C.G,** and **A.B** : discussed the data, contributed to the writing and reviewing of manuscript drafts. All authors reviewed and approved the submitted version.

Data availability The datasets supporting the findings of this article are included within the article and its supplementary files. All the raw sequences obtained in this study were deposited to the NCBI BioProject database under accession number PRJNA1262977.

Declarations

Competing interests The authors declare no competing interests.

References

Abd-Alla MH, Al-Amri SM, El-Enany AWE (2023) Enhancing Rhizobium-legume symbiosis and reducing nitrogen fertilizer use are potential options for mitigating climate change. Agriculture 13:2092. https://doi.org/10.3390/agriculture13112092

Alamzeb M, Inamullah (2023) Management of phosphorus sources in combination with *Rhizobium* and phosphate-solubilizing bacteria improve nodulation, yield and phosphorus uptake in Chickpea. Gesunde Pflanz 75:549–564. https://doi.org/10.1007/s10343-022-00722-2

Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol 8:1–8. https://doi.org/10.3389/fmicb.2017.00971

Ambrosini A, de Souza R, Passaglia LMP (2016) Ecological role of bacterial inoculants and their potential impact on soil microbial diversity. Plant Soil 400:193–207. https://doi.org/10.1007/s11104-015-2727-7

Ammar I, Wissal E, Meryem H, Rym S, Said K, Youssef Z, Abdellatif, Bargaz A (2024) Phosphate bio-solubilization and cadmium tolerance interplay in the root-microbe interface and consequences on root P absorption in wheat. Environ Exp Bot 222:105738. https://doi.org/10.1016/j.envexpbot.2024.105738

Araújo AP, Plassard C, Drevon JJ (2008) Phosphatase and phytase activities in nodules of common bean genotypes at different levels of phosphorus supply. Plant Soil 312:129–138. https://doi.org/10.1007/s11104-008-9595-3

Arrieta AM, Iannone LJ, Scervino JM, Vignale MV, Novas MV (2015) A foliar endophyte increases the diversity of phosphorus-solubilizing rhizospheric fungi and mycorrhizal colonization in the wild grass *Bromus auleticus*. Fungal Ecol 17:146–154. https://doi.org/10.1016/j.funeco.2015.07.001

Bargaz A, Faghire M, Abdi N, Farissi M, Sifi B, Drevon JJ, Cherkaoui Ikbal M, Ghoulam C (2012) Low soil phosphorus availability increases acid phosphatases activities and affects P partitioning in nodules, seeds and rhizosphere of *Phaseolus vulgaris*. Agriculture 2:139–153. https://doi.org/10.3390/agriculture2020139

Bargaz A, Noyce GL, Fulthorpe R, Carlsson G, Furze JR, Jensen ES, Driss D, Isaac ME (2017) Species interactions enhance root allocation, microbial diversity and P acquisition in intercropped wheat and soybean under P deficiency. Appl Soil Ecol 120:179–188. https://doi.org/10.1016/j.apsoil.2017.08.011

Bastian M, Heymann S, Jacomy M (2009) Gephi: an open source software for exploring and manipulating networks visualization and exploration of large graphs. Proc Third Int ICWSM Conf 361–362. https://doi.org/10.1609/icwsm.v3i1.13937

Benbrik B, Reid TE, Nkir D, Chaouki H, Aallam Y, Clark IM, Mauchline TH, Harris J, Pawlett M, Barakat M, Rchiad Z, Bargaz A

(2025) Unlocking the agro-physiological potential of wheat rhizoplane fungi under low P conditions using a niche-conserved consortium approach. Exp Bot 2–18. https://doi.org/10.1093/jxb/eraf042

Benjelloun I, Alami IT, Khadir ME, Douira A, Udupa SM (2021) Co-inoculation of *Mesorhizobium ciceri* with either *Bacillus* sp. or *Enterobacter aerogenes* on chickpea improves growth and productivity in phosphate-deficient soils in dry areas of a Mediterranean region. Plants 10:1–15. https://doi.org/10.3390/plants10030571

Benmrid B, Bargaz A, Oukfi H, Ibnyasser A, Saidi R, Meryem H, Imane C, Said C, Giuliano B, Mohamed I, Ghoulam C (2024) Species interactions and bacterial inoculation enhance plant growth and shape rhizosphere bacterial community structure in faba bean–wheat intercropping under water and P limitations. Environ Exp Bot 225:105858. https://doi.org/10.1016/j.envexpbot.2024.105858

Benmrid B, Idbella M, Bonanomi G, Khourchi S, Gherardelli M, Bargaz A (2025) Drought-tolerant rhizobacterial consortia with diverse plant growth promoting traits enhance wheat and faba bean growth under water and low-P availability. BMC Microbiol 25:771. https://doi.org/10.1186/s12866-025-04492-5

Boukhatem ZF, Merabet C, Tsaki H (2022) Plant growth promoting *Actinobacteria*, the most promising candidates as bioinoculants? Front Agron 4:1–19. https://doi.org/10.3389/fagro.2022.849911

Brookes PC, Powlson DS, Jenkinson DS (1982) Measurement of microbial biomass phosphorus in soil. Soil Biol Biochem 14:319–329. https://doi.org/10.1016/0038-0717(82)90001-3

Calia C, Pulvento C, Sellami MH, Tedone L, Ruta C, De Mastro G (2024) A bibliometric analysis of chickpea agronomic practices in the world during 45 years of scientific research. Legume Sci 6:1–12. https://doi.org/10.1002/leg3.219

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869

Cangioli L, Fagorzi C, Vaccaro F, Varriale S, Laura M, Mengoni A, Bianco C (2025) Soil bacteria and symbiotic rhizobia synergistically promote nitrogen fixation and biomass production of alfalfa (*Medicago sativa* L.) even under water shortage without altering the native rhizospheric microbiota. Appl Soil Ecol 213:106283. https://doi.org/10.1016/j.apsoil.2025.106283

Challacombe JF, Hesse CN, Bramer LM, McCue LA, Lipton M, Purvine S, Nicora C, Gallegos-Graves L, Porras-Alfaro A, Kuske CR (2019) Genomes and secretomes of *Ascomycota* fungi reveal diverse functions in plant biomass decomposition and pathogenesis. BMC Genomics 20:1–27. https://doi.org/10.1186/s12864-019-6358-x

Chaudhary MI, Adu-Gyamfi JJ, Saneoka H, Nguyen NT, Suwa R, Kanai S, El-Shemy HA, Lightfoot DA, Fujita K (2008) The effect of phosphorus deficiency on nutrient uptake, nitrogen fixation and photosynthetic rate in mashbean, Mungbean and soybean. Acta Physiol Plant 30:537–544. https://doi.org/10.1007/s11738-008-0152-8

Chen Y, Li S, Liu N, He H, Cao X, Lv C, Zhang K, Dai J (2021) Effects of different types of microbial inoculants on available nitrogen and phosphorus, soil microbial community, and wheat growth in high-P soil. Environ Sci Pollut Res 28:23036–23047. https://doi.org/10.1007/s11356-020-12203-y

Corstanje R, Reddy KR, Prenger JP, Newman S, Ogram AV (2007) Soil microbial eco-physiological response to nutrient enrichment in a subtropical wetland. Ecol Indic 7:277–289. https://doi.org/10.1016/j.ecolind.2006.02.002

Elhaissoufi W, Ibnyasser A, Haddine M, Zeroual Y, Ghani R, Barakat A, Rchiad Z, Ghoulam C, Bargaz A (2023) Screening of potential phosphate solubilizing bacteria inoculants should consider the contrast in phosphorus bio-solubilization rate along with plant growth promotion and phosphorus use efficiency. J Appl Microbiol 134:1–14. https://doi.org/10.1093/jambio/lxac077

Elhaissoufi W, Khourchi S, Ibnyasser A, Ghoulam C, Rchiad Z, Zeroual Y, Lyamlouli K, Bargaz A (2020) Phosphate solubilizing rhizobacteria could have a stronger influence on wheat root traits and aboveground physiology than rhizosphere P solubilization. Front Plant Sci 11:1–15. https://doi.org/10.3389/fpls.2020.00979

Elhaissoufi W, Khourchi S, Saidi R, Ibnyasser A, Haddine M, Ghani R, Zeroual Y, Rchiad Z, Ghoulam C, Bargaz A (2024) Inoculation with phosphate solubilizing bacterial consortia enhanced rock P agronomic efficiency and yield of wheat under low P conditions. J Plant Growth Regul 44:6895–6910. https://doi.org/10.1007/s00344-024-11350-7

Eylenbosch D, Dumont B, Baeten V, Bodson B, Delaplace P, Pierna J (2018) Quantification of leghaemoglobin content in pea nodules based on near infrared hyperspectral imaging spectroscopy and chemometrics. J Spectr Imaging 7:1–10. https://doi.org/10.1255/jsi.2018.a9

Fan Y, Lu S, He M, Yang L, Hu W, Yang Z, Huang S, Zhou J (2021) Long-term throughfall exclusion decreases soil organic phosphorus associated with reduced plant roots and soil microbial biomass in a subtropical forest. Geoderma 404:115309. https://doi.org/10.1016/j.geoderma.2021.115309

Fernández LA, Zalba P, Gómez MA, Sagardoy MA (2007) Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. Biol Fertil Soils 43:805–809. https://doi.org/10.1007/s00374-007-0172-3

Ghodhbane-Gtari F, Nouioui I, Hezbri K, Lundstedt E, D'Angelo T, McNutt Z, Laplaze L, Gherbi H, Vaissayre V, Svistoonoff S, Ben Ahmed H, Boudabous A, Tisa LS (2019) The plant-growth-promoting *Actinobacteria* of the genus *Nocardia* induces root nodule formation in *Casuarina glauca*. Antonie Van Leeuwenhoek 112:75–90. https://doi.org/10.1007/s10482-018-1147-0

Hannula SE, Morriën E, De Hollander M, Van Der Putten WH, Van Veen JA, De Boer W (2017) Shifts in rhizosphere fungal community during secondary succession following abandonment from agriculture. ISME J 11:2294–2304. https://doi.org/10.1038/ismej.2017.90

Han Q, Ma Q, Chen Y, Tian B, Xu L, Bai Y, Chen W, Li X (2020) Variation in rhizosphere microbial communities and its association with the symbiotic efficiency of rhizobia in soybean. ISME J 14:1915–19218. https://doi.org/10.1038/s41396-020-0648-9

Hartman K, Tringe SG (2019) Interactions between plants and soil shaping the root microbiome under abiotic stress. Biochem J 476:2705–2724. https://doi.org/10.1042/BCJ20180615

Hoffmann A, Zenelt W, Krawczyk K (2025) Bacteria isolated from dried tobacco to enhance drought tolerance in wheat. Appl Microbiol Biotechnol 109:1–14. https://doi.org/10.1007/s00253-025-13645-5

Hossain Z, Wang X, Hamel C, Knight JD, Morrison MJ, Gan Y (2016) Biological nitrogen fixation by pulse crops on semiarid Canadian prairies. Can J Plant Sci 97:119–131. https://doi.org/10.1139/cjps-2016-0185

Ibnyasser A, Saidi R, Elhaissoufi W, Khourchi S, Haddine M, Ghani R, Elghali A, Oukarroum A, Barakat A, Bargaz A (2024) Root acid phosphatases and microbial biomass phosphorus induced Cd tolerance and P acquisition in wheat inoculated with P solubilizing bacteria. Ecotoxicol Environ Saf 288:117402. https://doi.org/10.1016/j.ecoenv.2024.117402

Itelima JU, Bang WJ, Onyimba IA, Sila MD, Egbere OJ (2018) Biofertilizers as key player in enhancing soil fertility and crop productivity: a review. J Microbiol 2:74–83

Janati W, Benmrid B, Elhaissoufi W, Zeroual Y, Nasielski J, Bargaz A (2021) Will phosphate bio-solubilization stimulate biological nitrogen fixation in grain legumes? Front Agron 3:1–12. https://doi.org/10.3389/fagro.2021.637196

Jensen ES, Peoples MB, Hauggaard-Nielsen H (2010) Faba bean in cropping systems. Field Crops Res 115:203–216. https://doi.org/10.1016/j.fcr.2009.10.008

Karadeniz DG, Kaskatepe B, Kiymaci ME, Tok KC, Gumustas M, Karaaslan C (2021) Microbial exopolysaccharide production of *Streptococcus thermophilus* and its antiquorum sensing activity. Arch Microbiol 203:3331–3339. https://doi.org/10.1007/s00203-021-02313-7

Kaur N, Sharma P, Sharma S (2015) Co-inoculation of *Mesorhizobium* sp. and plant growth promoting rhizobacteria *Pseudomonas* sp. as bio-enhancer and bio-fertilizer in chickpea (*Cicer arietinum* L.). Legume Research - An International Journal 38:367–374. https://doi.org/10.5958/0976-0571.2015.00099.5

Kebede E (2021) Contribution, utilization, and improvement of legumes-driven biological nitrogen fixation in agricultural systems. Front Sustain Food Syst 5:1–18. https://doi.org/10.3389/fsufs.2021.767998

Khourchi S, Elhaissoufi W, Loum M, Ibnyasser A, Haddine M, Ghani R, Barakat A, Zeroual Y, Rchiad Z, Delaplace P, Bargaz A (2022) Phosphate solubilizing bacteria can significantly contribute to enhance P availability from polyphosphates and their use efficiency in wheat. Microbiol Res 262:127094. https://doi.org/10.1016/j.micres.2022.127094

Kour D, Rana KL, Yadav AN, Yadav N, Kumar M, Kumar V, Vyas P, Dhaliwal HS, Saxena AK (2020) Microbial biofertilizers: bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatal Agric Biotechnol 23:101487. https://doi.org/10.1016/j.bcab.2019.101487

Kucho K, Yamanaka T, Sasakawa H, Mansour SR, Uchiumi T (2014) Different dynamics of genome content shuffling among host-specificity groups of the symbiotic actinobacterium *Frankia*. BMC Genomics 15:1–12

Ladha JK, Peoples MB, Reddy PM, Biswas JC, Bennett A, Jat ML, Krupnik TJ (2022) Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. Field Crops Res 283:108541. https://doi.org/10.1016/j.fcr.2022.108541

Lagunas B, Richards L, Sergaki C, Burgess J, Pardal AJ, Hussain RMF, Richmond BL, Baxter L, Roy P, Pakidi A, Stovold G, Vázquez S, Ott S, Schäfer P, Gifford ML (2023) Rhizobial nitrogen fixation efficiency shapes endosphere bacterial communities and *Medicago truncatula* host growth. Microbiome 11:1–22. https://doi.org/10.1186/s40168-023-01592-0

Lahijanian S, Schmidt J, Feuerstein U, Polle A (2025) Effects of cover crops and microbial inoculants in different farming systems on soil microbial communities and yield of maize. Biol Fertil Soils 61:1165–1182. https://doi.org/10.1007/s00374-025-01929-x

Lan K, Li Y, Shuai Y, Zhai J, Ma Q, Kuzyakov Y, Liu M (2024) Phosphorus (P) mobilisation from inorganic and organic P sources depends on P acquisition strategies in *Populus euphratica*. Biol Fertil Soils 60:393–406. https://doi.org/10.1007/s00374-024-01799-9

Li F, Jin Z, Wang Z, Liao Y, Yu L, Li X (2022) Host plant selection imprints structure and assembly of fungal community along the soil-root continuum. MSystems 4:1–7. https://doi.org/10.1128/msystems.00361-22

Li J, Marschner P (2019) Phosphorus pools and plant uptake in manure-amended soil. J Soil Sci Plant Nutr 19:175–186. https://doi.org/10.1007/s42729-019-00025-y

Liu C, He Z, Chen Y, Xu Y, Tang W, Chen L (2024) Effects of nitrogen deposition on the rhizosphere nitrogen-fixing bacterial community structure and assembly mechanisms in *Camellia oleifera* plantations. Front Microbiol 15:1–11. https://doi.org/10.3389/fmicb.2024.1414724

Liu S, Zhao Z, Ji Y, Zhu H, Sun Y, Li M, Yu Q (2025) Synthetic bacterium-facilitated colonization of nitrogen-fixing bacteria for remodeling the rhizosphere microbiome and improving plant yield. Microbiome 14:7. https://doi.org/10.1186/s40168-025-02189-5

Liu Y, Ma B, Chen W, Schlaeppi K, Erb M, Stirling E, Hu L, Wang E, Zhang Y, Zhao K, Lu Z, Ye S, Xu J (2021) *Rhizobium* symbiotic capacity shapes root-associated microbiomes in soybean. Front Microbiol 12:1–17. https://doi.org/10.3389/fmicb.2021.709012

Li Z, Bai T, Dai L, Wang F, Tao J, Meng S, Hu Y, Wang S, Hu S (2016) A study of organic acid production in contrasts between two phosphate solubilizing fungi: *Penicillium oxalicum* and *Aspergillus Niger*. Sci Rep 6:1–8. https://doi.org/10.1038/srep25313

Mansotra P, Sharma P, Sharma S (2015) Bioaugmentation of mesorhizobium cicer, Pseudomonas spp. and Piriformospora indica for sustainable chickpea production. Physiol Mol Biol Plants 21:385–393. https://doi.org/10.1007/s12298-015-0296-0

Mansour E, Mahgoub HAM, Mahgoub SA, El-Sobky EEA, Abdul-Hamid MI, Kamara MM, AbuQamar SF, El-Tarabily KA, Desoky ESM (2021) Enhancement of drought tolerance in diverse *Vicia Faba* cultivars by inoculation with plant growth-promoting rhizobacteria under newly reclaimed soil conditions. Sci Rep 11:1–20. https://doi.org/10.1038/s41598-021-02847-2

Mukherjee A, Singh S, Gaurav AK, Chouhan GK, Jaiswal DK, de Araujo Pereira AP, Passari AK, Abdel-Azeem AM, Verma JP (2022) Harnessing of phytomicrobiome for developing potential biostimulant consortium for enhancing the productivity of chickpea and soil health under sustainable agriculture. Sci Total Environ 836:155550. https://doi.org/10.1016/j.scitotenv.2022.155550

Nkir D, Aallam Y, Ibnyasser A, Haddine M, Benbrik B, Barakat A, Reid TE, Lund G, Mauchline TH, Clark IM, Bargaz A, Rchiad Z (2024) Geographic bioprospection of maize rhizoplane-associated bacteria for consortia construction and impact on plant growth and nutrient uptake under low P availability. Environ Exp Bot 226:105939. https://doi.org/10.1016/j.envexpbot.2024.105939

Nuccio EE, Starr E, Karaoz U, Brodie EL, Zhou J, Tringe SG, Malmstrom RR, Woyke T, Banfield JF, Firestone MK, Pett-Ridge J (2020) Niche differentiation is spatially and temporally regulated in the rhizosphere. ISME J 14:999–10114. https://doi.org/10.1038/s41396-019-0582-x

Paul K, Saha C, Nag M, Mandal D, Naiya H, Sen D, Mitra S, Kumar M, Bose D, Mukherjee G, Naskar N, Lahiri S, Das Ghosh U, Tripathi S, Poddar Sarkar M, Banerjee M, Kleinert A, Valentine AJ, Tripathy S, Sinharoy S, Sea A (2020) A tripartite interaction among the basidiomycete *Rhodotorula mucilaginosa*, N2-fixing endobacteria, and rice improves plant nitrogen nutrition. Plant Cell 32:486–507. https://doi.org/10.1016/0038-0717(82)90001-3

Pawar KW (2025) Enhancing chickpea (*Cicer arietinum L.*) productivity through nitrogen-fixing and phosphorus-solubilizing bacteria: effects on nodulation, growth, *and yield*. J Adv Microbiol Res 6(2):09–13. https://doi.org/10.22271/micro.2025.v6.i2a.230

Põlme S, Bahram M, Kõljalg U, Tedersoo L (2014) Global biogeography of *Alnus*-associated *Frankia* actinobacteria. New Phytol 204:979–988. https://doi.org/10.1111/nph.12962

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. https://doi.org/10.1093/nar/gks1219

Rafique M, Naveed M, Mustafa A, Akhtar S, Munawar M, Kaukab S, Ali HM, Siddiqui MH, Salem MZM (2021) The combined effects of gibberellic acid and *Rhizobium* on growth, yield and nutritional status in chickpea (*Cicer arietinum* L.). Agronomy 11:105. https://doi.org/10.3390/agronomy11010105

Rahimlou S, Bahram M, Tedersoo L (2021) Phylogenomics reveals the evolution of root nodulating *alpha*- and *beta-Proteobacteria* (rhizobia). Microbiol Res 250:126788. https://doi.org/10.1016/j.micres.2021.126788

Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability. Plant Physiol 156:989–996. https://doi.org/10.1104/pp.111.175448

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:1340–1351. https://doi.org/10.1038/ismej.2010.58

Rutten PJ, Poole PS (2019) Oxygen regulatory mechanisms of nitrogen fixation in rhizobia. Adv Microb Physiol 75:325–389. https://doi.org/10.1016/bs.ampbs.2019.08.001

Sarao SK, Boothe V, Das BK, Gonzalez-Hernandez JL, Brözel VS (2024) *Bradyrhizobium* and the soybean rhizosphere: species level bacterial population dynamics in established soybean fields, rhizosphere and nodules. Plant Soil 580:515–530. https://doi.org/10.1007/s11104-024-06814-4

Shome S, Barman A, Solaiman ZM (2022) *Rhizobium* and phosphate-solubilizing bacteria influence soil nutrient availability, growth, yield, and quality of soybean. Agric (Switzerland) 12:1136. https://doi.org/10.3390/agriculture12081136

Solanki MK, Wang Z, Wang FY, Li CN, Gupta CL, Singh RK, Malviya MK, Singh P, Yang LT, Li YR (2020) Assessment of diazotrophic *Proteobacteria* in sugarcane rhizosphere when intercropped with legumes (*Peanut* and *Soybean*) in the field. Front Microbiol 11:1–12. https://doi.org/10.3389/fmicb.2020.01814

Sugiyama A, Ueda Y, Zushi T, Takase H, Yazaki K (2014) Changes in the bacterial community of soybean rhizospheres during growth in the field. PLoS One 9:1–9

Su W, Ma Y, Hua M, Chen H, Liu Z, Wang S, Wang Y (2025) Enhancing microbial carbon use efficiency via exogenous carbon inputs: implications for soil carbon sequestration and phosphorus availability. Appl Soil Ecol 211:106160. https://doi.org/10.1016/j.apsoil.2025.106160

Tagore GS, Namdeo SL, Sharma SK, Kumar N (2013) Effect of *Rhizobium* and phosphate-solubilizing bacterial inoculants on symbiotic traits, nodule leghaemoglobin, and yield of chickpea genotypes. Int J Agron 2013:1–18. https://doi.org/10.1371/journal.pone.0100709

Valverde A, Burgos A, Fiscella T, Rivas R, Velázquez E, Rodríguez-Barrueco C, Igual JM (2006) Differential effects of coinoculations with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacterium) and *Mesorhizobium ciceri* C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. Plant Soil 287:43–50. https://doi.org/10.1007/s11104-006-9057-8

Wang M, Bian Z, Shi J, Wu Y, Yu X, Yang Y, Ni H, Chen H, Bian X, Li T, Zhang Y, Jiang L, Tu Q (2020) Effect of the nitrogen-fixing bacterium *Pseudomonas protegens* CHA0-ΔretS-nif on garlic growth under different field conditions. Ind Crops Prod 145:111982. https://doi.org/10.1016/j.indcrop.2019.111982

Wang Z, Li T, Wen X, Liu Y, Han J, Liao Y, DeBruyn JM (2017) Fungal communities in rhizosphere soil under conservation tillage shift in response to plant growth. Front Microbiol 8:1301. https://doi.org/10.3389/fmicb.2017.01301

Wani PA, Khan MS, Zaidi A (2007) Co-inoculation of nitrogen-fixing and phosphate-solubilizing bacteria to promote growth, yield and nutrient uptake in Chickpea. Acta Agron Hung 55:315–323. https://doi.org/10.1556/AAgr.55.2007.3.7

Wei Z, Gu Y, Friman VP, Kowalchuk GA, Xu Y, Shen Q, Jousset A (2019) Initial soil Microbiome composition and functioning predetermine future plant health. Sci Adv 5:1–11

Xu L, Naylor D, Dong Z, Simmons T, Pierroz G, Hixson KK, Kim Y-M, Zink EM, Engbrecht KM, Wang Y, Gao C, DeGraaf S, Madera MA, Sievert JA, Hollingsworth J, Birdseye D, Scheller HV, Hutmacher R, Dahlberg J, Jansson C, Taylor JW, Lemaux PG, Coleman-Derr D (2018) Drought delays development of the sorghum root Microbiome and enriches for monoderm bacteria. Proc Natl Acad Sci U S A 115:E4284–E4293. https://doi.org/10.1073/pnas.1717308115

Yao X, Guo H, Zhang K, Zhao M, Ruan J, Chen J (2023) *Trichoderma* and its role in biological control of plant fungal and nematode disease. Front Microbiol 14:1–15. https://doi.org/10.3389/fmicb.2023.1160551

Zaidi A, Ahemad M, Oves M, Ahmad E (2010) Microbes for legume improvement. Microbes Legume Improv :273–292

Zhang C, van der Heijden MGA, Dodds BK, Nguyen TB, Spooren J, Valzano-Held A, Cosme M, Berendsen RL (2024a) A tripartite bacterial-fungal-plant symbiosis in the mycorrhiza-shaped microbiome drives plant growth and mycorrhization. Microbiome 12:1–22. https://doi.org/10.1186/s40168-023-01726-4

Zhang J, Wang J, Zhu C, Singh RP, Chen W (2024b) Chickpea: its origin, distribution, nutrition, benefits, breeding, and symbiotic relationship with *Mesorhizobium* species. Plants 13:1–13. https://doi.org/10.3390/plants13030429

Zhang J, Wang N, Li S, Wang J, Feng Y, Wang E, Chen W (2023) The effect of different rhizobial symbionts on the composition and diversity of rhizosphere microorganisms of chickpea in different soils. Plants 12:3421. https://doi.org/10.3390/plants12193421

Zhang J, Xing P, Niu M, Wei G, Shi P (2021) Taxonomic compositions and co-occurrence relationships of protists in bulk soil and rhizosphere of soybean fields in different regions of China. Front Microbiol 12:738129. https://doi.org/10.3389/fmicb.2021.738129