RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2025, **15**, 29509

Biochar-microbe synergy enhances auxin-mediated soil–plant interactions for canola productivity in alkaline calcareous soil

Adnan Mustafa,[ab] Qudsia Saeed,[a] Muhammad Naveed,[b] Iqra Abid,[b] Abdul Ghafoor,*[c] Faiza Bano,[b] Martin Brtnicky,[d] Muhammad Munir,[e] Zulfiqar Ahmad,[b] Mohsin Mahmood,[f] Muhammad Mehran,[g] Nimra Maqsood[a] and Xiankai Lu*[a]

Biochar and microbial bio-fertilizers, such as endophytic fungi and plant growth-promoting endophytes (PGPEs), offer sustainable alternatives to chemical fertilizers by enhancing soil fertility and plant performance. However, their synergistic effects particularly those involving auxin (IAA) biosynthesis and nutrient uptake, remain underexplored in calcareous soils. This study investigates how the integration of biochar with auxin-producing microbial inoculants influences soil–plant interactions and canola productivity. A pot experiment was conducted using two *Brassica napus* (canola) cultivars (DGL and Faisal canola) grown under alkaline calcareous soil. Treatments included individual and combined applications of *A. baumannii* MN24 and *P. indica*, with and without biochar. Physiological traits, auxin concentrations (IAA), root and shoot biomass, nutrient uptake (N, P, K), seed quality, and soil microbial and enzymatic activities were measured. Results revealed that the combined application of biochar, MN24, and *P. indica* significantly enhanced plant biomass, stem diameter, and grain yield by up to 203%, 127%, and 212%, respectively, in Faisal canola compared to the control. Biochar also amplified the microbial colonization and enzymatic activity in soil, leading to higher microbial biomass, enhanced nutrient uptake, and reduced electrolyte leakage and osmotic potential, indicating improved plant performance. Notably, these improvements translated to higher canola seed quality, with elevated fat, ash, and fiber contents. Enhanced auxin synthesis, particularly in the presence of L-tryptophan, was linked to improved root architecture, microbial colonization, and reduced plant stress indicators. These findings suggest that biochar–microbe–L-tryptophan synergy offers a powerful tool for sustainable crop intensification in challenging soils by optimizing hormone-mediated soil–plant processes.

Received 8th May 2025
Accepted 1st August 2025

DOI: 10.1039/d5ra03240g

rsc.li/rsc-advances

1 Introduction

Modern agricultural systems face mounting pressures from soil degradation, nutrient depletion, and climate-induced abiotic stresses, which collectively undermine crop productivity and nutritional quality. Oilseed crops like canola (*Brassica napus* L.) are particularly vulnerable due to their high demand for nutrient-rich soils and sensitivity to osmotic stress.[1] Canola production has spread across various climatic regions, including temperate and semi-arid zones, where soil and environmental constraints challenge optimal growth and yield.[2] Despite its agronomic potential, canola cultivation in many regions is hindered by soil degradation, nutrient deficiencies, and abiotic stress factors. Alkaline soils with low organic matter and poor nutrient availability restrict plant growth and oilseed yield.[3] High soil pH impacts nutrient solubility, diminishing the bioavailability of essential macro and micronutrients.[4–6] This ultimately results in decreased photosynthetic efficiency, compromised root development, and lower seed quality. Sustainable strategies to enhance soil–plant resilience while reducing reliance on synthetic inputs are urgently needed. In this context, integrating bio-based soil amendments and microbial inoculants into crop management systems offers

[a]*Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China. E-mail: luxiankai@scbg.ac.cn*

[b]*Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, 38040, Pakistan. E-mail: muhammad.naveed@uaf.edu.pk*

[c]*Centre for Water and Environmental Studies, King Faisal University, Al-Ahsa, 31982, Saudi Arabia. E-mail: aghafoor@kfu.edu.sa*

[d]*Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemedelska 1, Brno 61300, Czech Republic*

[e]*Date Palm Research Center of Excellence, King Faisal University, Al-Ahsa 31982, Saudi Arabia*

[f]*Key Laboratory of Grassland Ecology, School of Ecology and Environment, Inner Mongolia University, Hohhot 010020, China*

[g]*Key Laboratory of Arable Land Conservation (Middle and Lower Reaches of Yangtze River), Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan 430070, China*

a promising pathway toward ecological intensification of agriculture.[7,8]

Biochar is a carbon-rich product derived from the pyrolysis of organic materials and has gained great consideration as a soil amendment due to its ability to improve soil physicochemical characteristics, improve microbial activity, and promote water retention potential.[9] The application of biochar has been shown to stabilize soil pH, enhance cation exchange capacity, and facilitate nutrient cycling, leading to enhanced plant growth and productivity.[10] When combined with plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF), biochar can act as a carrier to enhance microbial survival and functionality, creating a synergistic effect on nutrient cycling.[11] Notably, such combinations may amplify phytohormone production, particularly auxin (indole-3-acetic acid, IAA) which plays a pivotal role in root development, nutrient acquisition, and stress tolerance.[12] However, the mechanisms linking biochar–microbe interactions to auxin-mediated improvements in soil–plant systems remain poorly understood, especially in oilseed crops.

Alongside biochar, PGPE and PGPF have emerged as powerful tools in biofertilization strategies.[13,14] Endophytic fungi are an important class of beneficial microorganisms that inhabit the internal tissues of plants without causing apparent harm. They play pivotal roles in promoting plant growth, enhancing stress tolerance, and stimulating the biosynthesis of phytohormones such as abscisic acid (ABA), indole-3-acetic acid (IAA).[12,15,16] These symbiotic relationships often result in improved nutrient acquisition, modulation of plant defense pathways, and enhanced resilience against biotic and abiotic stressors.[14,17] Among various endophytes, *Piriformospora indica* has emerged as a well-characterized root-colonizing fungus with remarkable potential to improve plant health and productivity across diverse agroecosystems. Its ability to establish a mutualistic relationship with a wide range of host plants, promote root architecture, and enhance hormonal crosstalk makes it an ideal candidate for biofertilizer formulations.[12,18] The synergistic interaction of *P. indica* with PGPE such as *Acinetobacter baumannii*, when applied with biochar, may further amplify beneficial soil–plant processes through coordinated hormone signaling and nutrient mobilization. However, the synergistic potential of these microbial inoculants with biochar remains poorly understood, especially in relation to soil–plant hormone dynamics and crop productivity.

Canola plants require a balanced supply of essential nutrients to maximize yield and oil content.[19] However, conventional fertilization practices often result in low nutrient use efficiency due to leaching, volatilization, and fixation losses. Integrating biochar with microbial inoculants can enhance nutrient-use efficiency by reducing nutrient losses and improving soil nutrient-holding capacity.[20] Recent studies suggest that biochar may not only serve as a carrier matrix and microbial niche, but also indirectly modulate plant–microbe interactions and hormone (auxin) production by improving soil biochemical conditions.[21] However, the downstream implications of this auxin boost remain underexplored. For instance, its effects on soil biological activity, nutrient uptake efficiency, physiological resilience, and crop quality are poorly documented. While biochar and microbial inoculants (PGPE/PGPF) have been studied in isolation, their synergistic effects on auxin-mediated soil–plant dynamics and the subsequent impact on canola seed quality are rarely quantified. This gap hinders the optimization of integrated soil amendments for sustainable oilseed production. Therefore, this study aims to assess the effect of biochar and microbial inoculants on the agronomic performance, physiological responses, and nutrient uptake efficiency of two canola cultivars. We hypothesize that biochar will enhance nutrient solubilization, improve the functional capacity of PGPE and PGPF to stimulate auxin production, thereby improving root growth, nutrient uptake, soil microbial activity, and promote canola growth and yield by alleviating soil-related constraints. The outcomes of this study will contribute valuable insights into the development of sustainable soil management strategies to optimize oilseed production in challenging agroecosystems.

2 Materials and methods

2.1. Preparation of bacterial and fungal inoculum

A pre-characterized strain of *Acinetobacter baumannii* sp. MN24, originally isolated as an endophyte from wheat roots, was obtained from the University of Agriculture Faisalabad (UAF), Pakistan. To prepare bacterial inoculum, the strain was first cultured in a 10% lysogenic broth (LB) medium as described by ref. 4. The resultant medium was autoclaved for 20 minutes at 121 °C before inoculation. The bacterial culture was incubated in a 500 mL Erlenmeyer flask at 28 ± 2 °C with shaking at 200 rpm in an orbital incubator for 48 hours. Cell density was adjusted to an optical density (OD_{600}) of 0.5 ($\sim 10^8$ CFU mL^{-1}) before application.

For fungal inoculum preparation, *Piriformospora indica* was grown on potato dextrose agar (PDA) plates at 28 ± 2 °C. Fresh fungal discs, 5 mm in diameter, were excised from actively growing edges of fungal colonies and transferred to sterilized potato dextrose broth (PDB). The PDB medium was prepared and autoclaved under the same conditions as the LB medium. The fungal culture was incubated at 30 °C for 7 days under a static environment. To prepare the fungal inoculum, the entire broth culture including mycelium and spores was homogenized in sterile distilled water using a sterile glass homogenizer. This method ensured a uniform suspension containing both fungal structures, with a final spore concentration of approximately 10^7 spores per mL. This approach was chosen based on established protocols demonstrating that both hyphae and spores contribute to early root colonization and successful symbiosis formation with host plants. Similar homogenization methods have been reported as effective in *P. indica* research.[12,22] In addition, both microbial strains *A. baumannii* sp. MN24 and *P. indica*, were examined for auxin production potential in the presence of L-tryptophan (L-TRP) following a process reported by ref. 23.

2.2. Production and characterization of biochar

Biochar was prepared using wheat straw. The wheat straw was carefully washed with distilled water for the removal of

impurities and oven-dried at 80 °C for 24 hours. The dried straw was then crushed into smaller pieces for uniform pyrolysis. Biochar production followed the technique described by ref. 24. The processed wheat straw was placed in a muffle furnace and heated under an oxygen-limited condition at a temperature of 400 °C. The temperature increased at a rate of 10 °C min^{-1}, with a retention period of 20 minutes. The produced biochar was further characterized by its surface functional groups and crystalline structure. Fourier Transform Infrared (FTIR) spectroscopy (Tensor 27 FTIR, Bruker Optics GmbH, Germany) was employed to discover surface functional groups, providing insights into the chemical properties of the biochar. X-ray Diffraction (XRD) analysis (Jeol JDX, Japan) was conducted to identify the crystalline structures present in the biochar. These characterizations confirmed the structural and functional properties of the biochar for its intended use in the experiment.

2.3. Experimental design, microbial inoculation, and plant growth trial

A pot experiment was established to evaluate the effect of *Acinetobacter baumannii* sp. MN24, root endophytic fungi *Piriformospora indica*, (*P. indica*), and their combined application with and without biochar on the growth, physiology, and nutrient acquisition of two canola varieties: dark green leaves (DGL) and Faisal canola. Each pot was filled with 8 kg of well-sieved (2 mm) soil. Biochar, produced from wheat straw at 400 °C (described in the biochar production section), was applied at a rate of 1% (w/w) to designated treatments. The physical and chemical characteristics of tested soil and applied biochar are mentioned in (Table 1). Before seed sowing the recommended doses of N, P, and K (120, 55, and 60 mg kg^{-1}) were added using urea, diammonium phosphate, and sulfate of potash to meet the nutrient requirements of plants. The experiment followed a two-factor factorial design as follows. Factor A comprised four microbial treatments: control (uninoculated), *A. baumannii* sp. MN24, *P. indica*, and a combination of *A. baumannii* sp. MN24 + *P. indica* with and without biochar. Factor B included two canola cultivars: Dark Green Leaves (DGL) and Faisal Canola. Each treatment combination was replicated three times (4 microbial treatments × 2 biochar levels × 2 cultivars × 3 replicates). Canola seeds were surface sterilized by immersion in 70% ethanol for 3–5 minutes, followed by 0.2% $HgCl_2$ for 2–3 minutes, and then rinsed three times with sterilized distilled water. For inoculation, sterilized seeds were dipped in 10 mL of bacterial or fungal inoculum (or their combination) for 2 hours. In the control treatment, seeds were soaked in 10 mL of sterilized water to maintain comparable moisture conditions for germination. The use of water ensured a natural environment for control plants without external nutrient inputs that could interfere with treatment effects.

Primarily, 6 seeds were sown in each pot, and after germination, seedlings were thinned to maintain 3 plants. Daily irrigation with tap water was provided to avoid moisture stress. Light, frequent irrigation was applied during the seedling stage to prevent surface crusting, while irrigation was adjusted to field capacity during vegetative and reproductive growth stages to prevent water stress at critical phases (*e.g.*, spiking and flowering). Manual weeding was performed to minimize competition. The crop was sown on 15 October 2021 and harvested at physiological maturity on 15 March 2022. Growth parameters, biomass production, root and shoot nutrient acquisition, and physiological traits were recorded to evaluate the combined effects of microbial treatments and biochar application on canola performance and rhizosphere interactions.

Table 1 Physical and chemical properties of tested soil and biochar

Parameters	Soil	Parameters	Biochar
Texture	Sandy clay loam	pH	8.96
Clay (%)	24.10	ECe (dS m^{-1})	5.17
Silt (%)	15.92	Total P (g kg^{-1})	2.06
Sand (%)	56.5	Total K (g kg^{-1})	0.62
ECe (dS m^{-1})	6.70	Total N (%)	1.30
pHs	7.88	OM (%)	3.88
N (%)	0.47	C (%)	64.3
K^+ (%)	2.04	Ash (%)	9.13
Olsen P (mg kg^{-1})	4.8		
Ca^{2+} + Mg^{2+}	12.8		
HCO_3^-	2.93		
$CaCO_3$	2.05		
CEC	15.8		
OM (%)	0.59		

Here, N = nitrogen; P = phosphorus; K = potassium; C = carbon; OM = organic matter.

2.4. Measurement of growth, physiology, and biochemical attributes of plant

Physiological attributes of the crop, including relative water content (RWC), relative membrane permeability (RMP), osmotic potential, and electrolyte leakage (EL), were measured to evaluate plant stress tolerance. Chlorophyll concentration was measured using a portable SPAD-502 plus meter to reflect photosynthetic efficiency and stem diameter was assessed with a Vernier caliper to indicate plant structural development. At maturity (150 DAS), plant height, and root and shoot lengths were assessed by a measuring tape. Additionally, root and shoot biomass were collected after drying at 60 °C until a consistent weight was attained. The number of pods per plant was measured by counting manually. Following the sun-drying of shoot samples, seeds were extracted through hand threshing and seed biomass was quantified using an electronic weighing scale to determine total seed yield.

2.5. Auxin production and seed quality profiling

Auxin production potential was assessed from plant tissues (roots and shoots), both with and without the precursor L-tryptophan, following the methods outlined by Sarwar *et al.* (1992 (ref. 23)). Seed quality-related attributes such as crude fat level, crude fiber concentration, and ash contents were also determined. Crushed seed samples (5 g) were analyzed for crude fat content using a Soxhlet extraction apparatus, where petroleum ether was used as the solvent, and the extraction was carried out at a rate of 3–4 drops (second^{-1}) for 8 hours. The extraction was then heated for 30 minutes at 900 °C until a consistent mass

was achieved. Crude fiber content was evaluated by boiling 2 g of crushed seed in 1.25% H_2SO_4 and NaOH solutions for 30 minutes, followed by drying the residues in an oven at 130 °C until a consistent weight was noted. The residues were further heated at 600 °C to attain the ignitable pool content and reweighed. For ash content estimation, 2 g of seed sample was put in a crucible and heated in a muffle furnace at a temperature of 550 °C until the sample turned grayish-white and achieved a consistent mass.

2.6. Soil microbiological analyses

Soil microbial biomass carbon (MBC) was quantified by a fumigation-extraction process documented by ref. 25. Soil samples were exposed to chloroform fumigation for 24 hours, followed by extraction with 0.5 M K_2SO_4 solution. Phenanthroline monohydrate was applied as a color indicator, and the resultant extraction was titrated alongside ferrous ammonium sulfate (0.033 M) till the solution turned red. The MBC was calculated by multiplying the microbial flush by a conversion factor of 0.45. Fluorogenic substrates including MUF-phosphate monoester, MUF-β-D-glucopyranoside, and MUF-*N*-acetyl-β-D-glucosaminide were used to calculate the levels of alkaline phosphatase, β-glucosidase, and acid phosphatase, respectively. Soil pastes were made by mixing 0.5 g of fresh soil with 50 mL of sterile water and pipetting them into 96-well plates. A buffer solution (50 μL) was mixed with 100 μL of a 4-methylumbelliferone substrate, and the plates were kept in an incubator for 2 hours at 20 °C. Fluorescence was estimated using a microplate reader at 360/460 nm. Enzyme activity was calculated using the Michaelis–Menten equation.[26]

2.7. Assessment of bacterial colonization in rhizosphere soil, root and shoot

Bacterial colonization was evaluated after harvest from the same pots used in the experiment, which had received various treatment combinations: *A. baumannii* MN24, *P. indica*, and biochar. No additional amendments were applied for the colonization assay. Indigenous microbes were not intentionally suppressed, as the goal was to assess colonization performance of the introduced PGPE under realistic, competitive soil conditions. For this purpose, serial dilution and plate counting methods were used. According to this method, soil samples were obtained from the rhizosphere area, and the soil paste was prepared by mixing soil with saline water with a 1 : 3 ratios. The resultant mixture was kept in a shaking incubator (180 rpm) at 28 °C for 30 min. After reaching soil sedimentation, serial dilutions were plated in the TSB media. To find the final values of colonization, the bacterial colonies were calculated after incubating the plate for 48 h at a temperature of 28 °C. For assessing root and shoot colonization, 2 g surface-disinfected crushed samples of root and shoot were homogenized in 10 mL (0.9% w/v) saline solution. Then, the resulting culture was put in a shaking incubator for 30 min at a temperature of 28 °C. After the settlement of the solid segment, serial dilutions were plated on a TSA medium. The 20 colonies were considered for each sample, and the attributes of the bacterial strains were confirmed by restriction fragment length polymorphism exploration of the 16S–23S rRNA intergenic spacer (IGS) region.[27]

2.8. Statistical analyses

All data were analyzed using one-way ANOVA under a factorial design to test the effects of microbial treatments, biochar application, and canola cultivars (varieties). Interaction effects among factors were assessed using factorial ANOVA with interaction terms up to three-way (microbial treatment × biochar × cultivar), depending on the dataset. *Post hoc* comparisons of treatment means were conducted using the least significant difference (LSD) test at a significance level of $P < 0.05$ using Statistix 8.1 software.[28] Graphical illustration of data was performed in GraphPad Prism 8.0. To evaluate relationships among soil, microbial, and plant parameters, the Mantel test and Pearson's correlation were performed using R studio (version 4.0.2).

3 Results

3.1. Characterization of biochar and applied strains

FTIR analysis (Fig. 1A) showed strong absorption bands at 3338.13 and 2886.78 cm^{-1}, related to O–H and C–H stretching, respectively, suggesting the retention of hydroxyl and aliphatic functional groups. The band at 760.65 cm^{-1} is consistent with Si–O–Si vibrations and the absorption at 1576.06 cm^{-1} is attributed to aromatic C=C stretching, signifying partially graphitized or lignin-derived aromatic domains, whereas the peaks at 1382.89 and 1107.99 cm^{-1} indicate carboxyl (COO^-) and C–O functionalities, respectively. The XRD spectra of wheat straw biochar (Fig. 1B) indicated the presence of inorganic minerals. The peak represents the incidence of quartz (SiO_2) at $2\theta = 26.54°$. The sharp peaks at $2\theta = 28.26°$ and 40.46° suggest the presence of sylvite (KCl) and the peak at 50.14 approves the existence of calcite ($CaCO_3$). The silicates of Ca, Mg, Mn, and quartz are verified by the different peaks at the range of 50.14–66.42°. These diverse peaks revealed in the XRD plot confirmed the existence of several inorganic constituents in biochar. Moreover, the applied strains have shown significant increase in auxin (IAA) synthesis in the presence of L-TRP (Fig. 2), whereby, highest increase in IAA was observed by MN24 followed by *P. indica*.

3.2. Effects of applied amendments on plant agronomic performance

The application of *A. baumannii* sp. MN24 and *P. indica*, individually and in combination, significantly ($P < 0.05$) enhanced plant height in both canola cultivars, particularly with biochar addition (Table 2). The highest increase in plant height was recorded under the combined application of *A. baumannii* sp. MN24 + *P. indica* with biochar, showed an increase of 160% for DGL and 153% for Faisal canola compared to their respective controls. The incorporation of *A. baumannii* sp. MN24 + *P. indica* in combination with biochar considerably improved studied growth parameters for both canola cultivars compared to the control. For shoot length, the combined treatment increased by 111% and 116% for DGL and Faisal Canola, respectively

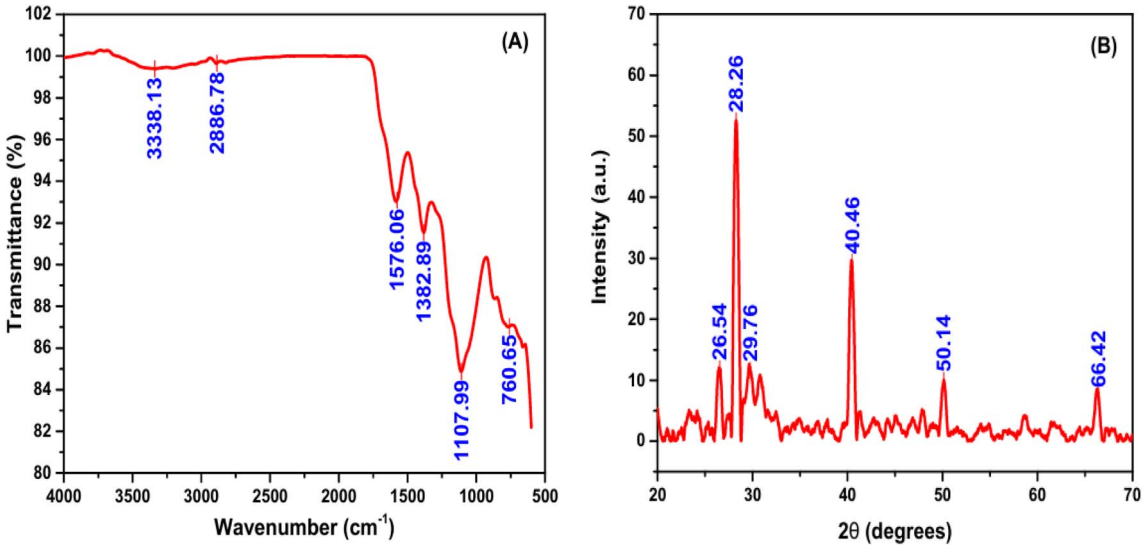

Fig. 1 FTIR spectrum (A), and XRD spectrum (B) of biochar.

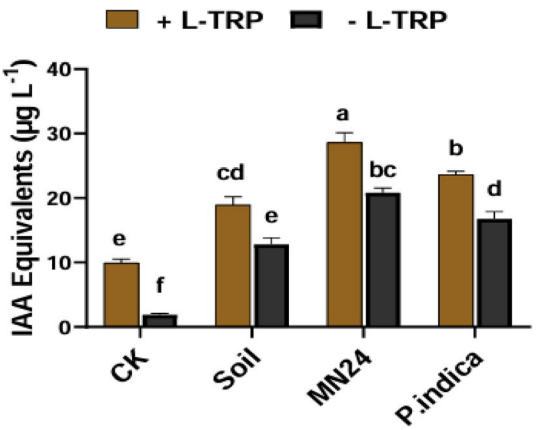

Fig. 2 Auxin production potential of selected strains. CK = control; bacteria used = *Acinetobacter baumannii* sp. MN24; fungus used = *Piriformospora indica*.

compared to control as well as other treatments giving smaller incremental gains. Root length exhibited a substantial improvement, with increases of 226% and 199% under combined treatment for DGL and Faisal canola, respectively compared to respective controls.

Regarding biomass production, the integration of MN24 + *P. indica* with biochar increased shoot and root fresh biomass by 179 and 175% and 218 and 158% for DGL and Faisal canola, respectively, compared to the untreated control (Table 2). Root fresh biomass showed enhancements for DGL and Faisal canola, respectively. Similarly, root and shoot dry biomass were increased considerably by 258 and, 203%, and 149 and 127% for DGL and Faisal canola cultivars respectively, under the same treatment, compared to the untreated control. Yield-related parameters also exhibited notable enhancements under the combined treatment. The number of pods per plant under the same treatment were increased by 106% and 108% for DGL and Faisal canola, respectively, compared to the untreated control. The 1000-seed weight increased by 142% for DGL and 126% for Faisal canola under the combined treatment, compared to the untreated control. Similarly, the seed yield exhibited substantial improvements, with increases of 212% and 209% for DGL and Faisal canola, respectively, compared to the untreated control (Table 2).

3.3. Effects of applied amendments on physiology and nutrient uptake of canola

The incorporation of *A. baumannii* sp. MN24 + *P. indica* with biochar significantly influenced physiological parameters in both canola cultivars (Fig. 3). This integration increased stem diameter by 167% and 118% for DGL and Faisal canola, respectively compared to the untreated control. Leaf area showed dramatic improvements, with increases of 769.1% and 769.3% for DGL and Faisal canola, respectively, while specific leaf area increased by 307% and 281% for the two cultivars when compared to their respective controls. Similarly, chlorophyll content was also boosted under the same treatment by 118% in DGL and 125% in Faisal canola and enhanced relative water content (RWC) by 133% and 127% for the respective cultivars compared to the untreated control. Conversely, reductions were observed in stress-related parameters. Electrolyte leakage (EL) decreased by 60% in DGL and 65% in Faisal canola, while relative membrane permeability (RMP) was reduced by 50% and 52%, respectively relative to their respective controls. Additionally, 69% and 71% of osmotic potential declined for DGL and Faisal canola, respectively, compared to the untreated control (Fig. 3).

The combined application of *A. baumannii* sp. MN24 + *P. indica* with biochar notably enhanced nutrient concentrations as well as nutrient uptake in both canola cultivars (Table 3). For nitrogen (N), root concentrations increased by 116% in DGL and 130% in Faisal Canola, while shoot concentrations rose by 104% and 119%, respectively, compared to the untreated control. Phosphorus (P) concentrations saw significant increases, with roots showing 436% in DGL and 413% in Faisal canola, and shoots recording 693% and 547%, respectively, compared to the untreated control. Similarly,

Table 2 Impact of applied treatments on agronomic performance of canola crop[a]

	Plant Height (cm)		Shoot length (cm)		Root length (cm)		Root Fresh biomass (g)		Root Dry biomass (g)	
Treatments	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2
Without Biochar										
CK	47 ± 1.41^{i}	52 ± 1.65^{i}	86 ± 1.73^{k}	91 ± 2.31^{jk}	9 ± 0.57^{j}	10.7 ± 0.33^{ij}	48.7 ± 1.45^{k}	51 ± 1.15^{k}	3.53 ± 0.46^{h}	4.93 ± 0.58^{gh}
MN-24	79 ± 1.35^{fg}	86 ± 1.70^{ef}	136 ± 2.31^{ef}	141.7 ± 2.60^{de}	$18.7 \pm 1.20^{e-g}$	$20.3 \pm 0.88^{d-g}$	66 ± 1.15^{h}	70.3 ± 1.46^{g}	$7.7 \pm 0.61^{c-g}$	$8.27 \pm 0.58^{b-f}$
P. indica	75 ± 1.31^{g}	80 ± 1.53^{fg}	111.7 ± 2.60^{h}	115.3 ± 2.61^{h}	14.3 ± 0.88^{hi}	16.7 ± 0.88^{gh}	59 ± 1.46^{i}	63.3 ± 1.45^{hi}	$6 \pm 0.58^{e-h}$	$6.7 \pm 0.58^{d-h}$
MN-24 + *P. indica*	95 ± 1.41^{cd}	101 ± 1.45^{c}	171.7 ± 2.90^{c}	176.3 ± 3.18^{bc}	24.3 ± 0.88^{cd}	27 ± 1.15^{bc}	90 ± 1.16^{d}	95.3 ± 1.45^{c}	$9.49 \pm 0.64^{a-d}$	$9.99 \pm 0.61^{a-c}$
With Biochar										
CK	61 ± 1.70^{h}	61 ± 1.44^{h}	96.3 ± 2.61^{ij}	101 ± 1.73^{i}	11.7 ± 0.88^{ij}	12.3 ± 0.89^{ij}	52.3 ± 1.45^{jk}	55.3 ± 1.20^{j}	$5 \pm 0.98^{f-h}$	$5.43 \pm 0.58^{e-h}$
MN-24	86 ± 1.67^{ef}	90 ± 1.56^{de}	146 ± 2.89^{d}	150.3 ± 2.03^{b}	$21 \pm 0.58^{d-f}$	22.3 ± 0.88^{de}	79 ± 1.15^{e}	82 ± 1.15^{e}	$7.97 \pm 0.57^{b-g}$	$8.51 \pm 0.58^{b-e}$
P. indica	$83 \pm 1.56^{e-g}$	86 ± 1.45^{ef}	121 ± 2.31^{gh}	127.7 ± 2.60^{fg}	$17.7 \pm 0.88^{f-h}$	$18.3 \pm 0.89^{e-h}$	71 ± 1.15^{g}	75 ± 1.15^{f}	$6.67 \pm 0.57^{d-h}$	$6.83 \pm 0.58^{c-g}$
MN-24 + *P. indica*	122 ± 1.53^{b}	132 ± 1.70^{a}	181.3 ± 2.60^{b}	196.7 ± 2.61^{a}	29.3 ± 0.88^{ab}	32 ± 0.57^{a}	100.3 ± 1.46^{b}	106 ± 1.16^{a}	11.21 ± 0.56^{ab}	12.73 ± 0.59^{a}

	Shoot fresh biomass (g)		Soot dry biomass (g)		No. of pods per plant		1000-seeds weight (g)		Seed yield (g per plant)	
Without Biochar										
CK	44.7 ± 2.91^{n}	48.3 ± 4.33^{mn}	22.3 ± 0.88^{i}	24.2 ± 1.59^{hi}	1.67 ± 0.34^{h}	2.14 ± 0.20^{gh}	12.3 ± 0.33^{m}	13.7 ± 0.33^{lm}	2.08 ± 0.18^{m}	2.26 ± 0.16^{lm}
MN-24	78.3 ± 3.18^{gh}	82.3 ± 2.61^{fg}	37.5 ± 0.76^{ef}	41.2 ± 2.11^{de}	$3.33 \pm 0.28^{c-g}$	$3.62 \pm 0.21^{c-e}$	22.3 ± 0.33^{ef}	$23 \pm 0.57^{d-f}$	4.09 ± 0.16^{h}	4.34 ± 0.17^{g}
P. indica	63.7 ± 2.34^{k}	69.3 ± 4.92^{j}	31.3 ± 0.88^{g}	34.7 ± 1.20^{fg}	$2.43 \pm 0.40^{e-h}$	$2.94 \pm 0.28^{d-g}$	17.7 ± 0.34^{ij}	18.7 ± 0.33^{hi}	3.10 ± 0.15^{j}	3.33 ± 0.16^{j}
MN-24 + *P. indica*	100 ± 4.05^{d}	105.7 ± 3.76^{c}	50 ± 1.13^{bc}	52.8 ± 1.54^{b}	5.39 ± 0.22^{ab}	5.94 ± 0.19^{a}	25.7 ± 0.88^{bc}	27 ± 0.58^{b}	5.41 ± 0.23^{d}	5.89 ± 0.21^{c}
With Biochar										
CK	52 ± 2.31^{lm}	56 ± 4.94^{l}	25.2 ± 0.73^{hi}	29 ± 1.16^{gh}	$2.41 \pm 0.32^{f-h}$	$2.72 \pm 0.18^{d-h}$	15 ± 0.57^{kl}	16 ± 0.57^{jk}	2.49 ± 0.23^{kl}	2.71 ± 0.15^{k}
MN-24	85.7 ± 2.33^{ef}	89 ± 2.31^{e}	41.7 ± 1.20^{de}	44.5 ± 2.02^{cd}	3.8 ± 0.61^{cd}	4.26 ± 0.20^{bc}	$23.7 \pm 0.33^{c-e}$	$25 \pm 0.57^{b-d}$	4.68 ± 0.18^{f}	4.93 ± 0.19^{e}
P. indica	72.7 ± 4.34^{ij}	75 ± 4.04^{hi}	33.3 ± 1.45^{fg}	37.5 ± 1.04^{ef}	$3.3 \pm 0.38^{c-g}$	$3.57 \pm 0.21^{c-f}$	20 ± 0.58^{gh}	21 ± 0.58^{fg}	3.59 ± 0.20^{i}	3.86 ± 0.15^{h}
MN-24 + *P. indica*	124.7 ± 2.91^{b}	133 ± 4.05^{a}	62.8 ± 0.60^{a}	66 ± 1.16^{a}	5.97 ± 0.52^{a}	6.48 ± 0.25^{a}	29.7 ± 0.88^{a}	31 ± 0.57^{a}	6.49 ± 0.27^{b}	6.98 ± 0.23^{a}

[a] Means with different letters differ significantly according to LSD at a 5% probability level; crop = canola; V1 = DGL (dark green leaves) and V2 = Faisal canola; CK = control; bacteria used = *Acinetobacter baumannii* sp. MN-24; fungus used = *Piriformospora indica*

Table 3 Impact of applied treatments on nutrients uptake in plant tissues[a]

	N uptake shoot mg/plant		N uptake root mg/plant		P uptake shoot mg/plant		P uptake root mg/plant		K uptake shoot mg/plant		K uptake root mg/plant	
Treatments	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2
Without Biochar												
CK	247.7 ± 13.64^{j}	277.5 ± 14.10^{j}	1.84 ± 0.37^{h}	2.41 ± 0.07^{gh}	20.23 ± 5.28^{i}	28.80 ± 10.68^{i}	2.45 ± 1.07i	3.42 ± 0.78^{hi}	263.50 ± 29.57^{j}	302.53 ± 23.43^{j}	2.27 ± 0.45^{k}	3.17 ± 0.47^{jk}
MN-24	546.2 ± 32.59^{gh}	642.7 ± 26.92^{fg}	4.98 ± 0.48$^{e–g}$	5.60 ± 0.06$^{d–f}$	119.23 ± 18.34^{fg}	157.93 ± 20.83^{ef}	11.73 ± 2.18$^{e–h}$	14.06 ± 1.51$^{d–g}$	675.53 ± 64.48$^{f–h}$	869.95 ± 97.63^{ef}	6.93 ± 0.25$^{f–j}$	8.31 ± 0.46$^{f–h}$
P. indica	400.7 ± 30.33$^{h–j}$	486.1 ± 27.63$^{g–i}$	3.31 ± 0.58$^{f–h}$	4.09 ± 0.38$^{e–h}$	69.57 ± 10.87$^{g–i}$	92.53 ± 10.62^{gh}	5.99 ± 1.54$^{g–i}$	8.30 ± 1.65$^{f–i}$	469.97 ± 7.53$^{h–j}$	563.93 ± 38.78$^{g–i}$	4.31 ± 0.45$^{h–k}$	5.53 ± 0.18$^{g–k}$
MN-24 + *P. indica*	966.5 ± 14.93^{cd}	1114.7 ± 5.55^{c}	11.17 ± 0.73^{bc}	13.40 ± 0.62^{b}	292.55 ± 6.52^{c}	340 ± 19.07^{c}	33.70 ± 1.03^{c}	40.72 ± 1.17^{bc}	1540.33 ± 31.19^{c}	1710.81 ±105.85^{c}	17.24 ± 1.04^{cd}	19.89 ± 0.13^{bc}
With Biochar												
CK	308.7 ± 23.42^{j}	362.9 ± 18.25^{ij}	3.07 ± 0.57$^{f–h}$	3.50 ± 0.16$^{f–h}$	34.82 ± 6.29^{i}	48.73 ± 10.05^{hi}	4.17 ± 1.13^{hi}	5.14 ± 0.93^{hi}	337.30 ± 9.35^{ij}	411.20 ± 33.40^{ij}	3.94 ± 0.64$^{i–k}$	4.73 ± 0.11$^{h–k}$
MN-24	772.7 ± 20.74^{ef}	842.6 ± 30.75^{de}	6.75 ± 0.86^{de}	8.29 ± 0.43^{cd}	200.20 ± 9.96^{de}	237.68 ± 16.36^{d}	18.68 ± 3.54^{de}	22.09 ± 2.05^{d}	1088.10 ± 38.58^{de}	1281.92 ± 52.74^{d}	10.92 ± 1.50^{ef}	13.32 ± 0.83^{de}
P. indica	533.9 ± 12.26^{gh}	636.7 ± 36.64^{fg}	5.59 ± 0.57$^{d–f}$	6.1 1± 0.41$^{d–f}$	120.97 ± 4.37^{fg}	158.40 ± 7.77^{ef}	13.56 ± 2.62$^{e–g}$	15.68 ± 1.02$^{d–f}$	749.80 ± 27.20^{fg}	913.05 ± 28.16^{ef}	8.12 ± 0.57$^{f–i}$	9.64 ± 0.58$^{e–g}$
MN-24 + *P. indica*	1424 ± 57.93^{b}	1659.5 ± 59.22^{a}	14.25 ± 1.70^{ab}	17 ± 1.30^{a}	448.13 ± 11.98^{b}	512.27 ± 1.39^{a}	44.98 ± 5.12^{b}	53.51 ± 4.67^{a}	2193.93 ± 77.68^{b}	2539.47 ± 15.78^{a}	21.52 ± 1.20^{b}	26 ± 2.30^{a}

[a] Means with different letters differ significantly according to LSD at a 5% probability level; N = nitrogen; P = phosphorus; K = potassium; crop = canola; V1 = DGL (dark green leaves) and V2 = Faisal canola; CK = control; bacteria used = *Acinetobacter baumannii* sp. MN-24; fungus used = *Piriformospora indica*.

potassium (K) concentrations in roots increased by 163% in DGL and 174% in Faisal canola, and shoots by 198% and 208%, respectively, compared to the untreated control (Table 3). Other treatments, such as MN24 or *P. indica* alone, showed only moderate increases, highlighting the synergistic effect of co-application.

3.4. Effects of applied amendments on auxin synthesis and seed quality attributes

The combined application of *A. baumannii* sp. MN24 + *P. indica* with biochar significantly improved seed quality parameters in both canola cultivars (Fig. 4). Ash content increased by 13% in DGL and 12.8% in Faisal canola, while fiber content rose by 170% and 148% for the respective cultivars compared to the untreated control. Additionally, fat content showed an increase of 133% in DGL and 129% in Faisal canola compared to the control (Fig. 4). Similarly, significant increases in auxin production were observed across all treatments in the presence of L-TRP for both cultivars (Fig. 5), with and without biochar application. The highest auxin synthesis in shoots was recorded under the combined application of *A. baumannii* sp. *MN24 + P. indica* with biochar, achieving increases of 149% and 159% for DGL and Faisal canola, respectively. Similarly, root auxin synthesis showed maximum enhancement under the same treatment, with increases of 144% for DGL and 139% for Faisal canola, compared to the untreated control.

3.5. Effects of applied amendments on soil microbiological attributes

The incorporation of *A. baumannii* sp. MN24 + *P. indica* noticeably influenced enzymatic activities in both canola cultivars (Fig. 6). The significantly ($p < 0.05$) highest glucosidase activity was recorded in the treatment combining *A. baumannii* sp. MN24 + *P. indica*, showing an increase of 79% and 151% with biochar addition in DGL and Faisal canola relative to biochar control respectively. For alkaline phosphatase activity, the treatment combining *A. baumannii* sp. MN24 + *P. indica* with biochar resulted in an increase of 14.8% and 16.4% in DGL and Faisal canola, respectively, compared to the biochar control. Similarly, the same treatment enhanced acid phosphatase activity, showing a 10% increase in DGL and a 26% increase in Faisal canola relative to the control. On the other hand, the incorporation of *A. baumannii* sp. MN24 and *P. indica*, individually or in combination, significantly enhanced MBC in both canola cultivars, with and without biochar addition (Fig. 6D). However, the highest increase in MBC was observed under the combined incorporation of *A. baumannii* sp. MN24 + *P. indica* with biochar resulted in an increase of approximately 36% for DGL and 33% for Faisal canola relative to the control.

3.6. Effects of applied amendments on bacterial colonization in rhizosphere, roots and shoots

After incorporating the selected amendments, the highest bacterial colonization was determined in the rhizosphere and plant tissues including root, and shoot (Fig. 7) under the integration of biochar. However, the MN24 + *P. indica* treatment in the presence of biochar displayed the highest microbial persistence in the rhizosphere soil and plant tissues. Under this combination, the bacterial colonization of the rhizosphere, root, and shoot was significantly improved up to 104.8 and 139.2%, 271.4 and 221%, and 450 and 205.8% respectively as compared to respective control treatments in the presence of biochar (Fig. 7).

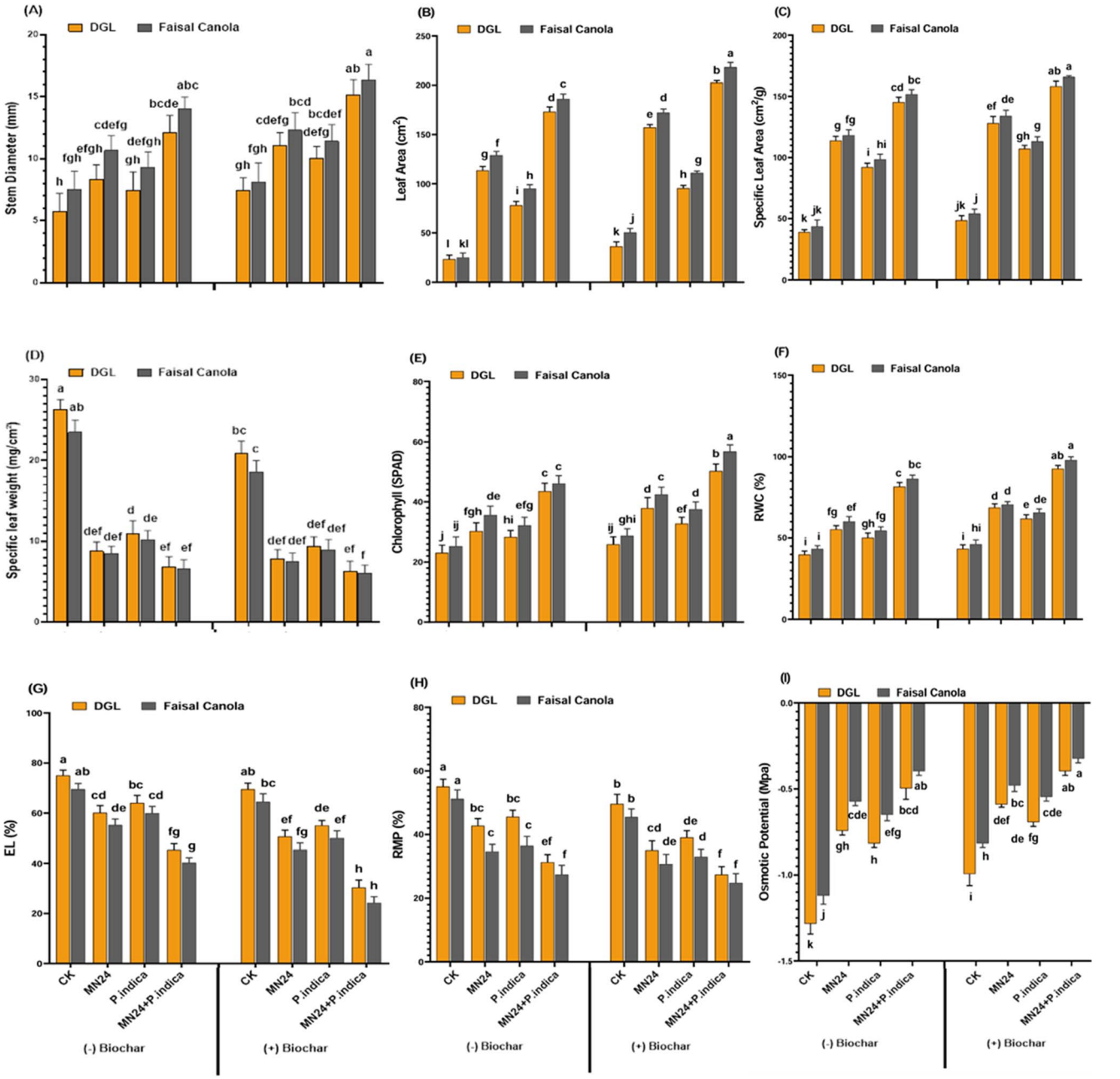

Fig. 3 The impact of applied treatments with and without biochar application on crop morphological and physiological attributes. CK = control; bacteria used = *Acinetobacter baumannii* sp. MN24; fungus used = *Piriformospora indica*; RWC = relative water content; EL = electrolyte leakage; RMP = relative membrane permeability. Different letters above the bars indicate significant differences at $p < 0.05$ according to LSD test. Treatments sharing at least one letter are not significantly different.

3.7. Biotic and abiotic factors regulating plant–microbe–soil interactions

Fig. 8 presents a hierarchical clustering analysis of soil enzymatic activities under different treatments, comparing conditions with and without biochar. The color gradient in both panels represents the relative enzyme activity levels, with red indicating high activity, blue representing low activity, and intermediate shades showing moderate activity. In panel (without Biochar), the treatments show a dominance of red hues, particularly for acid phosphatase, alkaline phosphatase, and microbial biomass carbon (MBC), suggesting higher enzymatic activity in these parameters across different treatments (Fig. 8a). The combined treatment of *A. baumannii* sp. MN24 and *P. indica* exhibited the highest enzymatic activities, whereas the CK showed comparatively lower activity, with more neutral and yellowish shades. The clustering pattern indicates a strong association between microbial inoculation and enhanced phosphatase-related enzyme activities, which play a crucial role in phosphorus cycling and organic matter decomposition. In panel (with Biochar), the color distribution shifts, with notable blue hues in glucosidase V1 and V2,

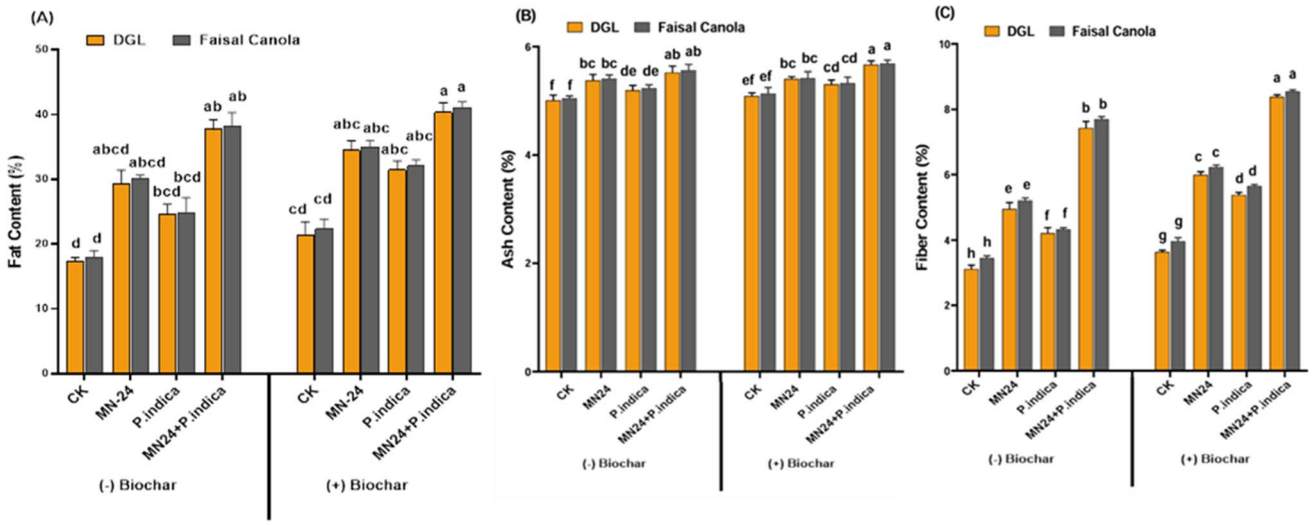

Fig. 4 The impact of applied treatments with and without biochar on seed quality parameters. CK = control; bacteria used = *Acinetobacter baumannii* sp. MN24; fungus used = *Piriformospora indica*. Different letters above the bars indicate significant differences at $p < 0.05$ according to LSD test. Treatments sharing at least one letter are not significantly different.

indicating a significant reduction in their activity compared to phosphatases (Fig. 8b). Acid phosphatase remained highly active, as denoted by red-colored clusters, suggesting its critical role in phosphorus mobilization in these treatments. The presence of biochar appears to modify enzymatic responses, potentially by altering microbial interactions and nutrient availability in the soil.

Furthermore, the Mantel test correlation matrices were also estimated by comparing the relationships between different soil and plant parameters under applied treatments with and without the application of biochar (Fig. 9). The results further show the relation between auxin synthesis, nutrient concentrations, and seed quality attributes with and without biochar. Darker blue indicates strong positive correlations, while lighter shades represent weaker ones. Auxin synthesis (with and without L-tryptophan) showed substantial positive correlations with N, P, and K levels in shoots and roots, with stronger correlations in biochar-treated conditions. The Mantel test (orange lines) highlights enhanced auxin–nutrient interactions with biochar. Seed quality traits (fiber, fat, ash) also correlated positively with nutrient uptake, emphasizing biochar's role in improving metabolic efficiency and resource allocation.

4 Discussion

4.1. Effects of applied amendments on plant agronomic performance

The combined application of *Acinetobacter baumannii* sp. MN24 and *Piriformospora indica* with biochar significantly enhanced the agronomic performance of both canola cultivars, with a more pronounced impact observed in DGL compared to Faisal canola (Table 1). The effectiveness of biochar was confirmed through FTIR and XRD analytics (Fig. 1). The FTIR and XRD results revealed that the synthesized biochar contains both organic functional groups and inorganic mineral phases,[29,30] which can influence its physicochemical attributes and possible synergism with microbial inoculants for sustainable agriculture. Furthermore, the employed statistical analysis (Fig. 6 and 7), also suggests that biochar application enhances or stabilizes the relationships between nutrient dynamics and plant hormonal responses. Practically, the incorporation of biochar in combination with inoculants may endorse more synergistic relations among nutrients, root/shoot growth regulators, and overall plant development, leading to a more cohesive physiological response compared to plants grown without biochar. This synergistic improvement can be attributed to multiple factors, including increased nutrient bioavailability, enhanced microbial colonization in the rhizosphere, and improved soil moisture retention. These findings are consistent with previous studies by ref. 31–33, which demonstrated that the integration of biochar with plant growth-promoting microorganisms (PGPMs) enhances the soil microenvironment and contributes to increased plant biomass and productivity.

The enhanced agronomic traits observed under the combined treatment, including significant increases in plant height, root length, stem diameter, and biomass production, compared to control and sole application of endophytes highlight the beneficial interaction between biochar and microbial inoculants.[34,35] The porous structure of biochar likely contributed to improved root architecture and soil–root interactions, facilitating better nutrient and water uptake. Former studies reported by ref. 36 and 37, also verified that biochar-amended soils enhance root elongation and microbial activity, thereby promoting efficient nutrient cycling and plant development. Moreover, studies by ref. 38 and 39 emphasize that biochar-mediated improvements in soil structure and aeration enhance rhizosphere microbial diversity, further supporting root health and function.

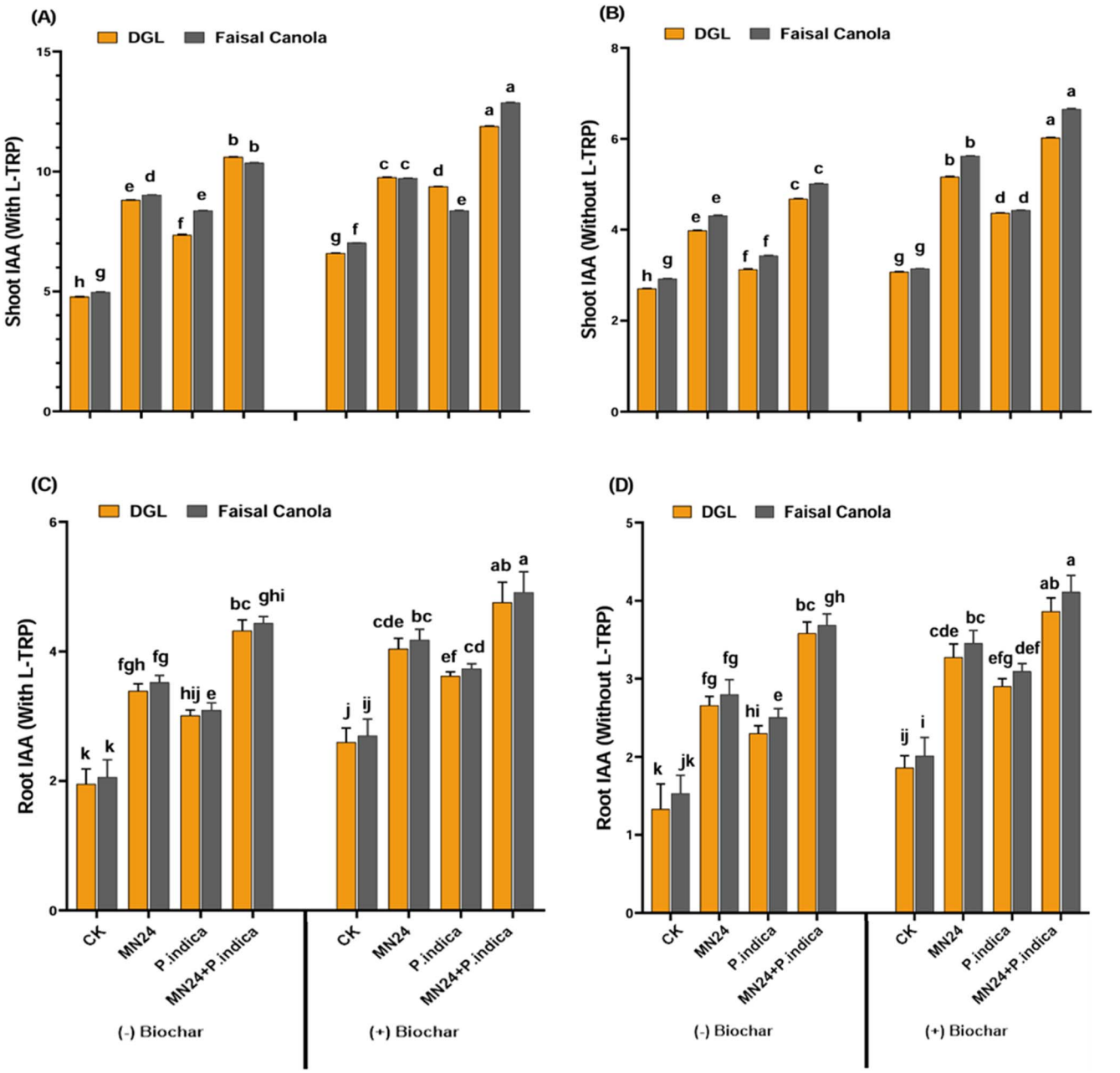

Fig. 5 The impact of applied treatments with and without biochar on auxin accumulation in shoot and root of Canola varieties. CK = control; bacteria used = *Acinetobacter baumannii* sp. MN-24; fungus used = *Piriformospora indica.* Different letters above the bars indicate significant differences at $p < 0.05$ according to LSD test. Treatments sharing at least one letter are not significantly different.

The combined amendments also led to notable enhancements in leaf area and specific leaf area, indicating improved photosynthetic efficiency and water-use optimization (Fig. 3). Biochar's role in modulating soil moisture dynamics and nutrient bioavailability likely contributed to these improvements, as suggested by ref. 40–42, who reported that biochar-enriched soils enhance light interception and gas exchange capacity. Additionally, microbial inoculants have been found to stimulate chlorophyll biosynthesis and antioxidant enzyme activity, reducing oxidative stress and improving overall plant vigor.[43,44]

Reproductive success and yield-related parameters were also significantly impacted by the combined incorporation of biochar and microbial inoculants. Increased numbers of pods per plant, higher 1000-seed weight, and improved seed yield suggest more efficient nutrient partitioning and translocation to reproductive structures (Table 2). Similar outcomes were reported,[35,45] where biochar–microbial interactions facilitated enhanced rhizosphere nutrient dynamics, boosting pod formation and seed filling. The role of microbial inoculants in enhancing hormonal signaling pathways associated with

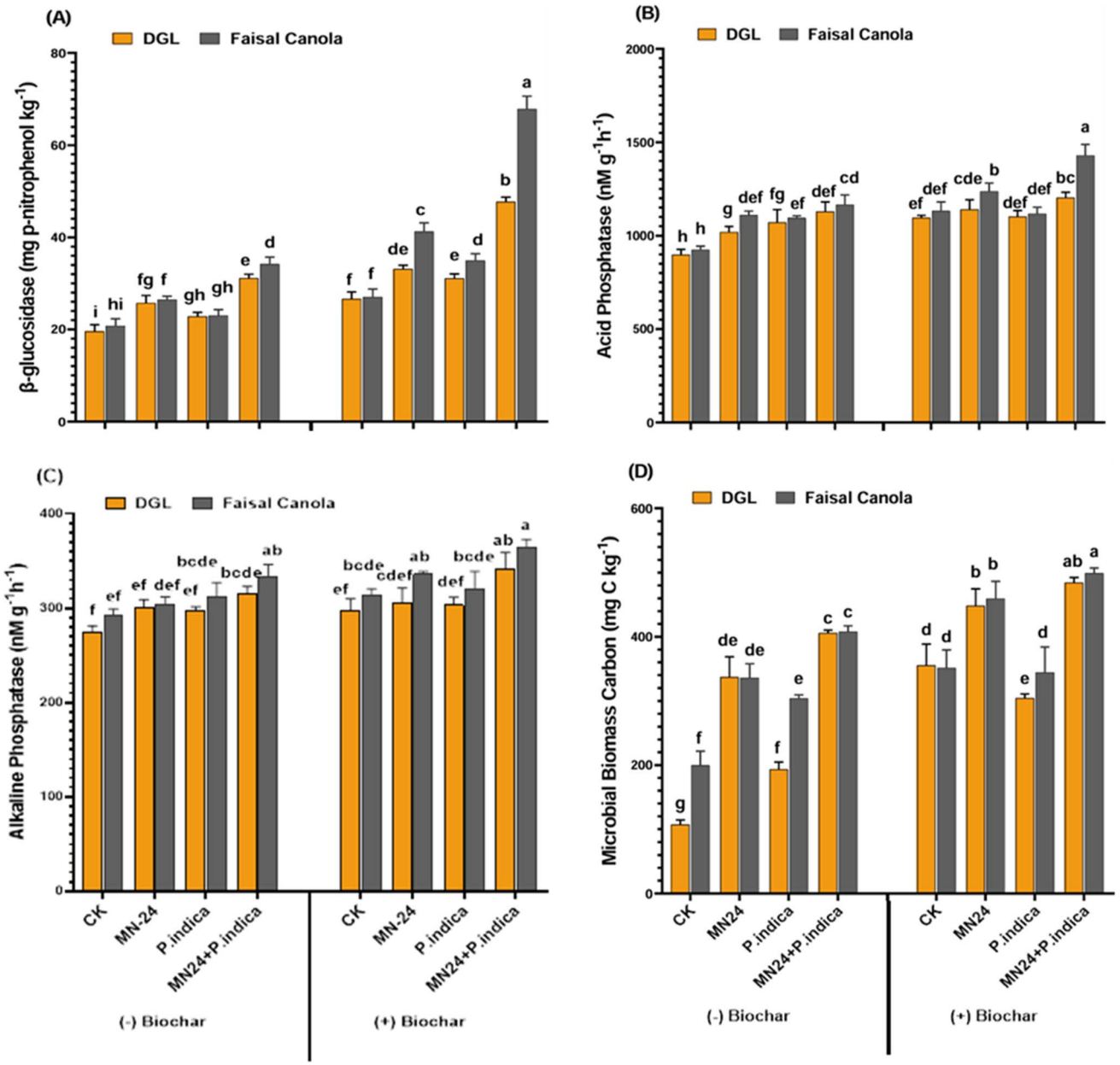

Fig. 6 The impact of applied treatments with and without biochar on soil microbial and biochemical properties. CK = control; bacteria used = *Acinetobacter baumannii* sp. MN24; fungus used = *Piriformospora indica.* Different letters above the bars indicate significant differences at $p < 0.05$ according to LSD test. Treatments sharing at least one letter are not significantly different.

flowering and seed development has also been documented by ref. 46 and 47 further supporting the observed yield improvements. These results reinforce the importance of biochar as a microbial carrier that protects and sustains beneficial microbial populations, thereby enhancing their efficacy in promoting plant growth.[48,49] Without biochar, microbial effects were less pronounced, likely due to constraints in soil moisture and nutrient availability. Overall, the integration of *A. baumannii* sp. MN24, *P. indica*, and biochar present a sustainable approach to improving agronomic performance across vegetative and reproductive growth stages in canola cultivars. The improvements observed in plant performance highlight the potential of biochar-microbial amendments in advancing sustainable agricultural practices.

4.2. Effects of applied amendments on physiological and nutrient uptake performance of canola

The combined application of *Acinetobacter baumannii* sp. MN24, *Piriformospora indica*, and biochar significantly enhanced physiological performance and nutrient uptake in both canola cultivars compared to individual treatments or control. The increase in chlorophyll content and relative water content (RWC) under the combined treatment indicates improved photosynthetic efficiency and water retention capacity (Fig. 3).

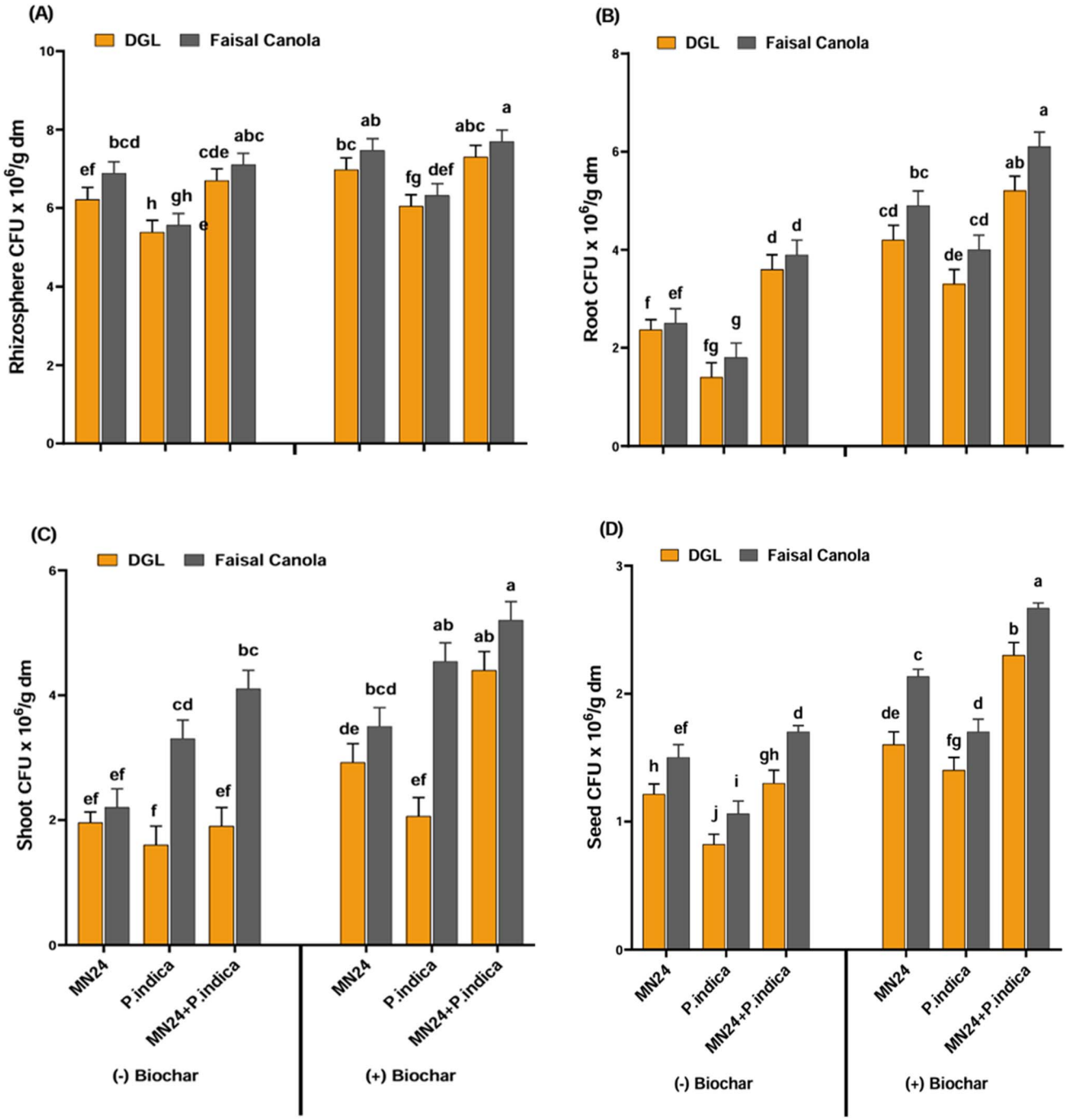

Fig. 7 The impact of applied treatments with and without biochar on microbial colonization in rhizosphere soil (A); root (B); shoot (C) and seed (D) of canola varieties. CK = control; bacteria used = *Acinetobacter baumannii* sp. MN24; fungus used = *Piriformospora indica*. Different letters above the bars indicate significant differences at $p < 0.05$ according to LSD test. Treatments sharing at least one letter are not significantly different.

These enhancements are likely due to biochar's ability to enhance soil moisture retention and nutrient availability, fostering favorable conditions for plant metabolism. Previous studies have demonstrated that biochar improves photosynthetic efficiency by increasing soil carbon content and microbial diversity, enhancing root exudation and nutrient uptake.[50–52] This influence of biochar was also detected by Mona *et al.* 2024 (ref. 31) through biochar characterization by FTIR and XRD respectively. Moreover, biochar has been reported to alter microbial communities in rhizosphere soils, creating a favorable microenvironment for plant growth.[53,54] Microbial inoculants have also been shown to facilitate stomatal regulation, enhance nitrogen metabolism, and promote antioxidant enzyme activity, optimizing photosynthetic performance under various environmental conditions.[55–57]

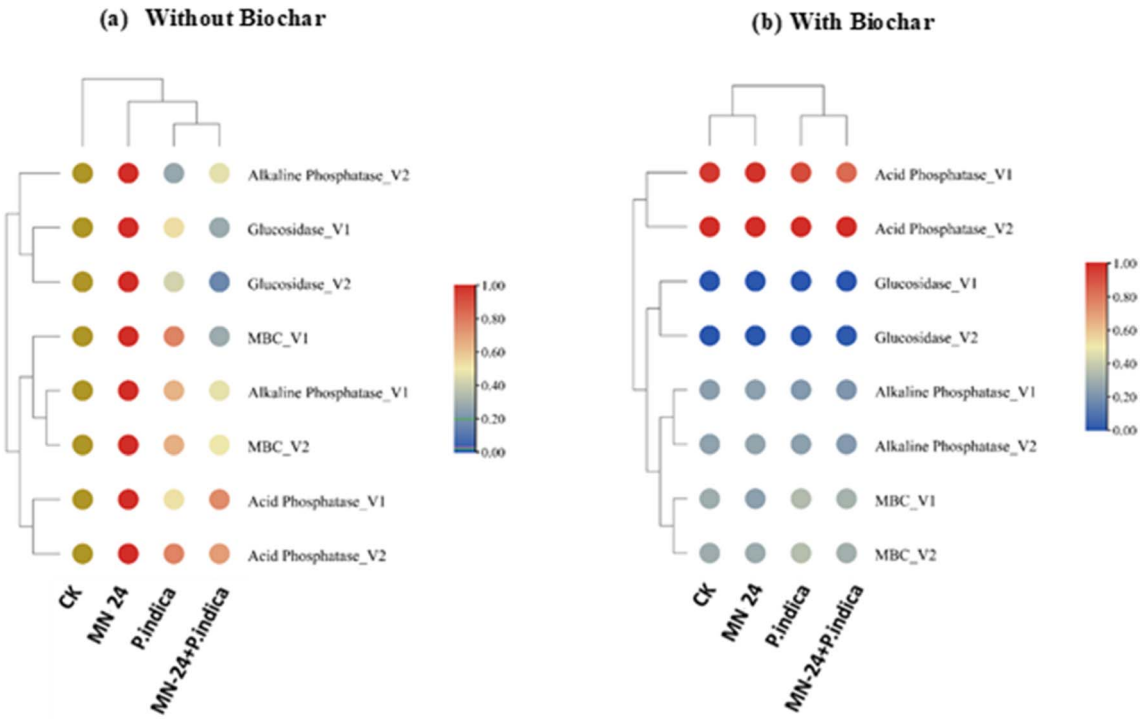

Fig. 8 The impact of applied treatments with and without biochar application on correlations among measured parameters of soil microbial and biochemical properties. CK = control; bacteria used = *Acinetobacter baumannii* sp. MN24; fungus used = *Piriformospora indica*.

Stress-related parameters, including electrolyte leakage (EL), relative membrane permeability (RMP), and osmotic potential, were significantly reduced in plants treated with the combined amendment (Fig. 3). These reductions suggest that biochar and microbial inoculants improved membrane stability and plant resilience to oxidative and osmotic stress. The stabilization of membrane integrity may be linked to biochar's ability to buffer soil pH, regulate ion homeostasis, and enhance the production of osmolytes such as proline and glycine betaine.[58–60] Additionally, biochar and microbial amendments have been found to modulate phytohormonal signaling pathways, triggering enhanced antioxidant responses and reducing lipid peroxidation under abiotic stress conditions.[61–63] The role of plant growth-promoting rhizobacteria (PGPR) in reducing oxidative damage by increasing peroxidase, superoxide dismutase, and catalase activities has also been well-documented.[64,65]

The synergistic effects of biochar and microbial inoculants also led to substantial improvements in nutrient uptake (Table 2). These improvements indicate the synergistic impact of biochar and microbial inoculants in improving nutrient availability and uptake efficiency.[66] The improved nutrient assimilation may be attributed to biochar's cation exchange capacity, which enhances nutrient retention, and microbial inoculants' ability to facilitate nutrient solubilization and mobilization.[37,67,68] This outcome could also have been supported by our FTIR outcomes, which confirmed the existence of functional groups such as hydroxyl/phenol (2886.78 cm^{-1}, 3338.13 cm^{-1}) and carboxyl (1107.99 cm^{-1} and 1382.89 cm^{-1}) in biochar strongly stimulate the establishment of ionic charges in the soil.[30] Furthermore, biochar has been found to modify the soil microbiome by promoting beneficial microbial populations that enhance nutrient mineralization and cycling, leading to increased nutrient bioavailability for plant uptake.[69–71] The increased uptake of P could be attributed to the enhanced activity of phosphate-solubilizing bacteria and mycorrhizal fungi colonization in biochar-amended soils.[72–74] Moreover, microbial inoculants have been reported to improve nitrogen fixation through the enhancement of root-associated nitrogenase activity, further supporting nutrient uptake efficiency.[37,75] Our results are consistent with these findings, as the combined treatment led to significantly higher N uptake than individual treatments, indicating enhanced nutrient-use efficiency under microbe–biochar synergy.

Collectively, our findings underscore the effectiveness of integrating biochar and microbial inoculants to enhance plant physiological health and increase their uptake capacity of nutrients and stress resilience in canola cultivars. Given the increasing focus on sustainable agricultural practices, these findings have significant implications for developing eco-friendly soil amendments that enhance crop yield while reducing environmental impact.

4.3. Effects of applied amendments on auxin synthesis and seed quality attributes

The application of *Acinetobacter baumannii* sp. MN24 and *Piriformospora indica*, both individually and in combination, significantly enhanced auxin synthesis in both shoots and roots of canola cultivars (Fig. 5). These findings suggest that the interaction between biochar and microbial inoculants not only

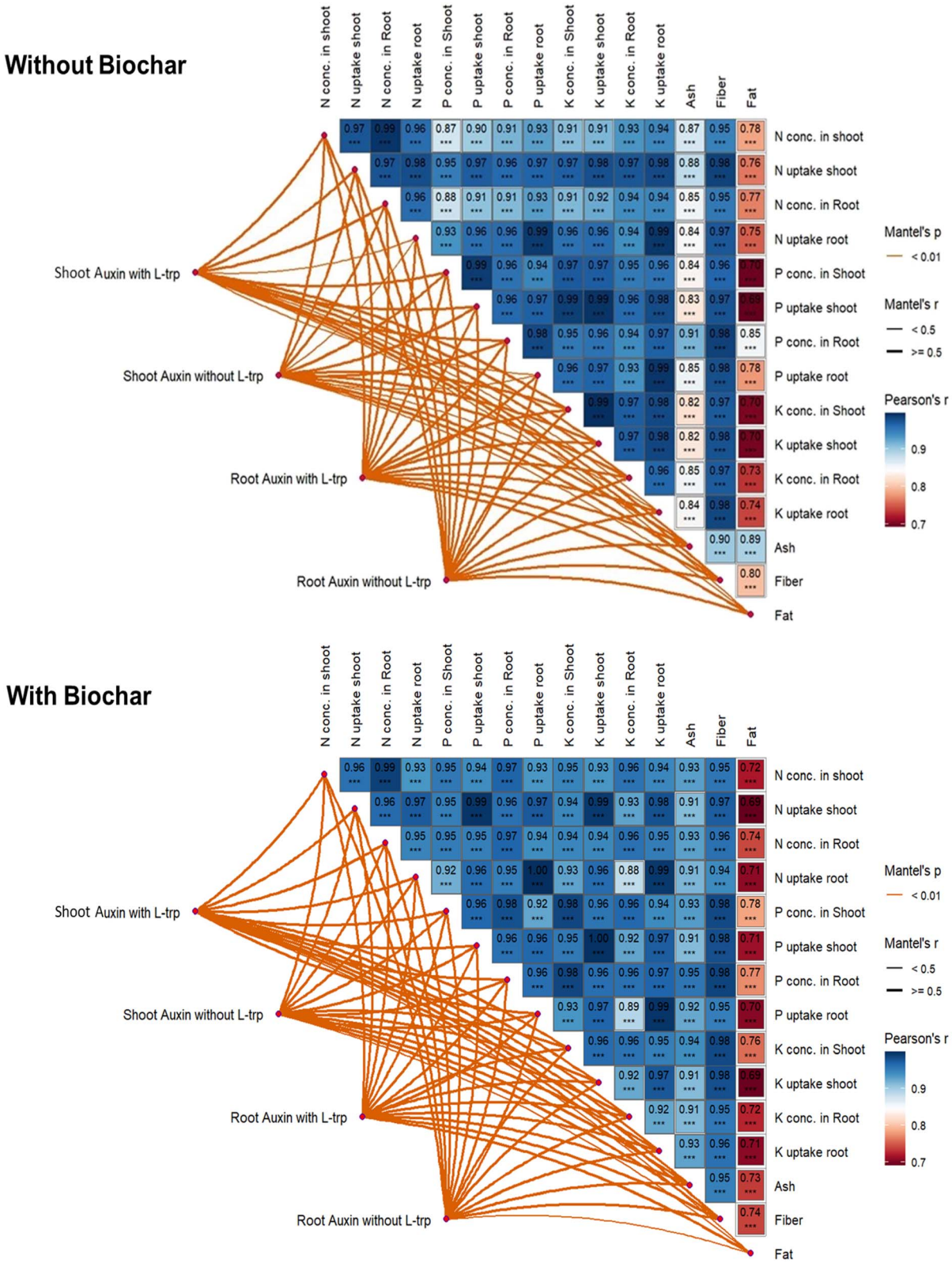

Fig. 9 Mantel test correlation matrices between different plant parameters under the influence of applied treatments with and without biochar application. Here, N = nitrogen; P = phosphorus; K = potassium.

boosts auxin synthesis but also enhances microbial colonization, root exudate composition, and nutrient bioavailability, factors essential for optimal plant growth and development.[76,77] The presence of biochar provided a favorable microhabitat for microbial persistence and enhanced production of auxin precursors in the rhizosphere, amplifying auxin biosynthesis through enhanced microbial metabolic activity.[78–80]

Without biochar, the individual treatments of *A. baumannii* sp. MN24 and *P. indica* still exhibited moderate increases in auxin synthesis, underscoring their role in promoting plant growth *via* phytohormone modulation. However, the combined microbial treatment showed a stronger synergistic effect, which was significantly amplified by the presence of biochar. The ability of biochar to act as a microbial carrier, protect beneficial microbes from environmental stress, and enhance their longevity and metabolic activity likely played a crucial role in increasing auxin synthesis. These results are aligned with previous studies demonstrating that biochar-amended soils create more favorable conditions for microbial activity and phytohormone production.[81,82] The enhancement of auxin synthesis under biochar and microbial inoculant treatment could also be attributed to the increased availability of tryptophan, an auxin precursor, facilitated by biochar's impact on microbial-driven nitrogen transformations.[83–85]

In terms of seed quality attributes, the combined application of *A. baumannii* sp. MN24 + *P. indica* with biochar significantly improved crude fiber, crude fat, and ash content in canola seeds (Fig. 4). These improvements are likely due to enhanced nutrient assimilation and metabolic efficiency facilitated by biochar's ability to modify soil physicochemical properties, improve cation exchange capacity, and stimulate beneficial microbial interactions.[86,87] The individual microbial treatments also contributed to enhanced seed quality traits, but the combined treatment with biochar consistently yielded superior results, demonstrating the importance of microbial synergy and soil amendments in improving seed biochemical composition.[88,89] Furthermore, the improved seed composition in our findings may be linked to biochar's role in modulating carbon and nitrogen dynamics, which are essential for lipid metabolism, fiber development, and overall seed nutritional quality.[90–92] These findings are consistent with the work of ref. 93, who demonstrated the potential of biochar to enhance the functional traits of microbial inoculants, leading to improved crop quality and productivity. The results emphasize the importance of integrated soil amendments for sustainable agriculture, where biochar and microbial inoculants can be utilized to maximize plant growth, stress resilience, and crop quality under varying environmental conditions.[94,95]

4.4. Effects of applied amendments on soil microbiological attributes

The application of *Acinetobacter baumannii* sp. MN24 and *Piriformospora indica*, particularly in combination with biochar, significantly influenced soil microbiological attributes by enhancing MBC and enzymatic activities associated with nutrient cycling (Fig. 6). In the absence of biochar, higher activities of acid phosphatase, alkaline phosphatase, and MBC were recorded, particularly in microbial-inoculated treatments, indicating improved P mobilization and microbial carbon turnover. These findings were consistent with the clustering analysis highlighting a strong association between microbial inoculation and enzymatic activity enhancement which could be supported by previous findings reported by ref. 96–98, where microbial consortia contributed significantly to soil biochemical processes through enhanced microbial persistence and functionality. Additionally, studies by ref. 99–101 also emphasized that microbial inoculation enhances soil enzyme activity by stimulating microbial respiration and exoenzyme production, further improving soil nutrient cycling and organic matter decomposition. The presence of biochar notably enhanced β-glucosidase activity, which plays a critical role in carbon cycling and organic matter decomposition (Fig. 5). These findings align with previous studies[102,103] that reported biochar's ability to create a more hospitable microenvironment for beneficial microbial communities, leading to improved enzyme-mediated nutrient transformations. Moreover, research by ref. 104 and 105 suggested that biochar enhances microbial exoenzyme synthesis by modulating soil pH, increasing cation exchange capacity, and improving soil aeration. In addition to enzyme activity, MBC levels significantly increased in soils receiving the combined application of *A. baumannii* sp. MN24 and *P. indica* with biochar, suggesting a synergistic effect on microbial biomass accumulation. This result is consistent with findings by ref. 106–108, who demonstrated that biochar amendments enhance microbial biomass by improving soil organic matter stabilization and microbial nutrient availability. In this context, these enhancements also reflect bacterial colonization (Fig. 7). Diverse environments had a considerable effect on bacterial colonization. In this present study, the combined incorporation of MN24 and *P. indica* along with biochar showed great potential for colonizing the plant ecosystem by effectively competing with the native microbes (Fig. 7). The favorable microbial habitat offered by biochar may be the reason for the improved colonization of soil treated with selected amendments. This notion could be supported by former studies conducted by ref. 109 and 110, which highlighted that biochar serves as a microbial habitat, providing a carbon-rich substrate that enhances microbial proliferation and enzymatic efficiency. This mechanism likely explains the more remarkable microbial persistence observed under biochar treatments, reinforcing its role in improving soil microbial ecology. Furthermore, these observations were also consistent in the Mantle correlation matrices (Fig. 9). Strong positive associations between nutrient concentrations, and microbial biomass are closely linked with soil microbiological transformation, enzyme activity, and better nutrient availability in soil. Concisely, these overall findings of the present study indicate that microbial inoculation, particularly in combination with biochar, significantly enhances soil microbiological attributes by improving enzymatic activity, microbial biomass, and nutrient cycling. The observed improvements highlight the potential of integrating microbial inoculants with biochar to promote sustainable soil fertility management, reinforcing the role of biochar in

stabilizing microbial populations and enhancing nutrient transformations.

6 Conclusion

This study underscores the transformative potential of biochar–microbe synergy in modulating auxin-mediated soil–plant interactions, offering a biologically driven path to enhanced canola productivity. The co-application of *Acinetobacter baumannii* MN24 and *Piriformospora indica*, especially in the presence of biochar, significantly boosted plant growth, nutrient uptake, seed quality, and biomass production in both canola cultivars. Notably, auxin synthesis was markedly elevated in the presence of L-tryptophan, highlighting the critical role of precursor availability in hormone-driven plant responses. The integration of biochar significantly magnified microbial colonization and auxin nutrient coordination, resulting in greater shoot and root biomass, higher yield attributes, and improved seed nutritional traits. These outcomes reflect a deeper mechanistic interaction between soil amendments and phytohormone signaling offering a biologically enriched, low-input strategy for enhancing crop productivity. This approach offers a cost-effective, eco-conscious alternative to input-heavy agronomic systems and a promising leap towards regenerative agriculture, where soil health and crop performance are enhanced hand in hand.

Author contributions

Conceptualization; Adnan Mustafa, Muhammad Naveed, Zulfiqar Ahmad, Abdul Ghafoor, data curation; Iqra Abid, Adnan Mustafa, Faiza Bano, formal analysis; Nimra Maqsood, Iqra Abid, Mohsin Mahmood, Adnan Mustafa, Martin Brtnicky, Muhammad Mehran, funding acquisition; Martin Brtnicky, Xiankai Lu, investigation; Muhammad Naveed, Iqra Abid, methodology; Muhammad Naveed, Iqra Abid, Adnan Mustafa, project administration; Muhammad Naveed, validation; roles/writing – original draft; Adnan Mustafa, Qudsia Saeed, and writing – review & editing; Xiankai Lu, Iqra Abid, Muhammad Munir, Muhammad Naveed.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Data availability

The data supporting this article have been included in the submission.

Funding

This work has been financially supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Project No. KFU252798).

Acknowledgements

The authors would like to acknowledge the Key Research and Development Program of Guangdong Province (No. 2022B1111230001) for facilitating this research.

References

1 I. A. Mohamed, N. Shalby, A. M. El-Badri, E. F. Awad-Allah, M. Batool, M. H. Saleem, Z. Wang, J. Wen, X. Ge, Z. Xu and J. Wang, *J. Agric. Food Res.*, 2025, 101794.
2 C. E. Borges, R. Von dos Santos Veloso, C. A. Da Conceição, D. S. Mendes, N. Y. Ramirez-Cabral, F. Shabani, M. Shafapourtehrany, M. C. Nery and R. S. Da Silva, *Sci. Rep.*, 2023, **13**, 12656.
3 M. A. Secchi, J. A. Fernandez, M. J. Stamm, T. Durrett, P. V. V. Prasad, C. D. Messina and I. A. Ciampitti, *Field Crops Res.*, 2023, **293**, 108848.
4 M. Naveed, I. Abid, F. Mustafa, H. R. Ahmad, S. Alamri, M. H. Siddiqui, A. T. Alfagham and A. Mustafa, *Chem. Biol. Technol. Agric.*, 2024, **11**, 168.
5 A. H. Hudhaifa, M. M. Aljumaily and M. A. Al-Obaidi, *Rev. Agric. Neotrop.*, 2024, **11**, e8543.
6 N. Younas, M. Naveed, M. Yaseen, M. Younas, M. Z. Mumtaz, M. H. Babar, M. Nadeem, S. Soysal, I. Al-Ashkar and A. Mustafa, *Agronomy*, 2024, **14**, 1289.
7 M. U. Muzaffar, M. Naveed, Z. Naseem, I. Abid, K. Z. Amir, S. Alamri, M. Siddique, M. Brtnicky and A. Mustafa, *Chemosphere*, 2024, **368**, 143797.
8 Y. Xing, X. Wang and A. Mustafa, *Ecotoxicol. Environ. Saf.*, 2025, **289**, 117703.
9 F. Zhang, B. Hu, L. Meng, M. Pang, Y. Chen, W. Song, Y. Feng and Y. Ma, *Soil Use Manag.*, 2025, **41**, e70044.
10 J. Holatko, J. Kucerik, A. Mustafa, K. Lonova, M. H. Siddiqui, M. Naveed, T. Hammerschmiedt, A. Kintl, O. Malicek, T. Chorazy and T. Baltazar, *BMC Plant Biol.*, 2025, **25**, 323.
11 Q. Zou, L. Zhao, L. Guan, P. Chen, J. Zhao, Y. Zhao, Y. Du and Y. Xie, *Front. Plant Sci.*, 2024, **15**, 1501400.
12 M. Iqbal, M. Naveed, M. Sanaullah, M. Brtnicky, M. I. Hussain, J. Kucerik, J. Holatko and A. Mustafa, *J. Soils Sediments*, 2023, **23**, 1233–1249.
13 Z. A. Virk, D. A. Al Farraj, M. Iqbal, K. Lewińska and S. Hussain, *Sustainability*, 2022, **14**, 6975.
14 Q. Saeed, W. Xiukang, F. U. Haider, J. Kučerik, M. Z. Mumtaz, J. Holatko, M. Naseem, A. Kintl, M. Ejaz, M. Naveed and M. Brtnicky, *Int. J. Mol. Sci.*, 2021, **22**, 10529.
15 R. Shahzad, S. Bilal, M. Imran, A. L. Khan, A. A. Alosaimi, H. A. Al-Shwyeh, H. Almahasheer, S. Rehman and I. J. Lee, *Biochem. J.*, 2019, **476**, 3385–3400.

16 F. Nazli, A. Mustafa, M. Ahmad, A. Hussain, M. Jamil, X. Wang, Q. Shakeel, M. Imtiaz and M. A. El-Esawi, *Sustainability*, 2020, **12**, 9056.
17 A. Kour, A. S. Shawl, S. Rehman, P. Sultan, P. H. Qazi, P. Suden, R. K. Khajuria and V. Verma, *World J. Microbiol. Biotechnol.*, 2008, **24**, 1115–1121.
18 S. Mourouzidou, G. K. Ntinas, A. Tsaballa and N. Monokrousos, *Sustainability*, 2023, **15**, 5959.
19 J. Akhtar, P. Upadhyay and H. Kumar, in *Hybrid Seed Production for Boosting Crop Yields: Applications, Challenges and Opportunities*, Springer Nature Singapore, Singapore, 2025, pp. 177–224.
20 T. Javed, I. I, R. K. Singhal, R. Shabbir, A. N. Shah, P. Kumar, D. Jinger, P. M. Dharmappa, M. A. Shad, D. Saha and H. Anuragi, *Front. Plant Sci.*, 2022, **13**, 877544.
21 T. Mushtaq, A. Bano and A. Ullah, *J. Plant Growth Regul.*, 2024, 1–22.
22 A. Varma, A. Singh, N. S. Sahay, J. Sharma, A. Roy, M. Kumari, D. Rana, S. Thakran, D. Deka, K. Bharti, Piriformospora indica: an axenically culturable mycorrhiza-like endosymbiotic fungus, in *Fungal Associations*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2001, pp. 125–150.
23 M. Sarwar, M. Arshad, D. A. Martens and W. Frankenberger, *Plant Soil*, 1992, **147**, 207–215.
24 M. E. Sánchez, E. Lindao, D. Margaleff, O. Martínez and A. Morán, *J. Anal. Appl. Pyrolysis*, 2009, **85**, 142–144.
25 E. D. Vance, P. C. Brookes and D. S. Jenkinson, *Soil Biol. Biochem.*, 1987, **19**, 703–707.
26 M. Sanaullah, E. Blagodatskaya, A. Chabbi, C. Rumpel and Y. Kuzyakov, *Appl. Soil Ecol.*, 2011, **48**, 38–44.
27 B. Reiter, U. Pfeifer, H. Schwab and A. Sessitsch, *Appl. Environ. Microbiol.*, 2002, **68**, 2261–2268.
28 R. G. Steel, J. H. Torrie, D. A. Dicky, *Principles and Procedures of Statistics*, 2nd edn, 1997.
29 C. Li, Q. Huang, H. Zhang, Q. Wang, R. Xue, G. Guo, J. Hu, T. Li, J. Wang and S. Hu, *Int. J. Environ. Res. Public Health*, 2021, **18**, 11413.
30 S. Mona, N. Saini, S. Kumar, A. Sharma, A. Yadav, N. Yadav and B. Deepak, *Bioresour. Technol. Rep.*, 2024, **27**, 101893.
31 J. Hou, A. Pugazhendhi, R. Sindhu, V. Vinayak, N. C. Thanh, K. Brindhadevi, N. T. Chi and D. Yuan, *Environ. Res.*, 2022, **14**, 113909.
32 N. Nurhidayati, A. S. Ansari, A. Sholihah and P. N. Chiangmai, *J. Hortic. Res.*, 2022, **30**, 55–66.
33 E. Yeboah, G. Asamoah, P. Ofori, B. Amoah and K. O. Agyeman, *Open Agric.*, 2020, **5**, 352–360.
34 A. A. Ajeng, R. Abdullah, T. C. Ling, S. Ismail, B. F. Lau, H. C. Ong, K. W. Chew, P. L. Show and J. S. Chang, *Environ. Technol. Innov.*, 2020, **20**, 101168.
35 M. Głodowska, T. Schwinghamer, B. Husk and D. Smith, *Agric. Sci.*, 2017, **8**, 1048–1064.
36 S. Bolan, D. Hou, L. Wang, L. Hale, D. Egamberdieva, P. Tammeorg, R. Li, B. Wang, J. Xu, T. Wang and H. Sun, *Sci. Total Environ.*, 2023, **886**, 163968.
37 H. Ma, V. Shurigin, D. Jabborova, J. A. dela Cruz, T. E. dela Cruz, S. Wirth, S. D. Bellingrath-Kimura and D. Egamberdieva, *Plants*, 2022, **11**, 423.
38 J. Lu, W. Zhang, X. Liu, A. Khan, W. Wang, J. Ge, S. Yan and Y. C. Xiong, *Environ. Technol. Innov.*, 2024, **35**, 103683.
39 M. Rafique, I. Ortas, I. A. Ahmed, M. Rizwan, M. S. Afridi, T. Sultan and H. J. Chaudhary, *J. Environ. Manage.*, 2019, **247**, 672–680.
40 J. Liu, Z. Chen, S. Wu, H. Sun, J. Xing and Z. Zhang, *Plants*, 2024, **13**, 614.
41 M. Sharifi and M. Hajiaghaei-Kamrani, *Can. J. Soil Sci.*, 2022, **103**, 200–212.
42 S. Winarso, B. Hermiyanto, S. Romadhona, M. H. Pandutama, T. C. Setiawati and I. Indasah, *J. Degraded Min. Lands Manage.*, 2020, **7**, 2263.
43 S. Sarapat, A. Longtonglang, K. Umnajkitikorn, T. Girdthai, N. Boonkerd, P. Tittabutr and N. Teaumroong, *J. Plant Interact.*, 2020, **15**, 322–334.
44 M. Singh, K. A. Qureshi, M. Jaremko, M. Rajput, S. K. Singh, K. D. Pandey, L. F. Ferreira and A. Kumar, *Plants*, 2022, **11**, 1787.
45 H. Yang, Q. G, B. H, H. Chen, X. P, R. F and J. Du, *J. Agric. Resour. Environ.*, 2023, **40**, 15.
46 N. S. Aggangan and E. A. Aguilar, *Philipp. J. Crop Sci.*, 2019, **44**, 89–97.
47 M. Azeem, T. U. Hassan, M. I. Tahir, A. Ali, P. G. Jeyasundar, Q. Hussain, S. Bashir, S. Mehmood and Z. Zhang, *Appl. Soil Ecol.*, 2021, **157**, 103732.
48 T. Kocsis, Z. Kotroczó, L. Kardos and B. Biró, *Environ. Technol. Innov.*, 2020, **20**, 101191.
49 Z. Ullah, S. Ali, N. Muhammad, N. Khan, M. Rizwan, M. D. Khan, N. Khan, B. Khattak, H. A. Alhaithloul, M. H. Soliman and H. Ullah, *Arab. J. Geosci.*, 2020, **13**, 757.
50 H. P. Schmidt, C. Kammann, C. Niggli, M. W. H. Evangelou, K. A. Mackie and S. Abiven, *Agric. Ecosyst. Environ.*, 2014, **191**, 117–123.
51 H. Schulz and B. Glaser, *J. Plant Nutr. Soil Sci.*, 2012, **175**, 410–422.
52 H. Yu, W. Zou, J. Chen, H. Chen, Z. Yu, J. Huang, H. Tang, X. Wei and B. Gao, *J. Environ. Manage.*, 2019, **232**, 8–21.
53 H. Chen, J. Ma, J. Wei, X. Gong, X. Yu, H. Guo and Y. Zhao, *Sci. Total Environ.*, 2018, **635**, 333–342.
54 D. Liu, Z. Ding, E. F. Ali, A. M. Kheir, M. A. Eissa and O. H. Ibrahim, *Sci. Rep.*, 2021, **11**, 8739.
55 E. T. Alori, M. O. Dare and O. O. Babalola, *Sustain. Agric. Rev.*, 2017, 281–307.
56 E. T. Alori and O. O. Babalola, *Front. Microbiol.*, 2018, **9**, 2213.
57 M. J. Salomon, R. Demarmels, S. J. Watts-Williams, M. J. McLaughlin, A. Kafle, C. Ketelsen, A. Soupir, H. Bücking, T. R. Cavagnaro and M. G. van der Heijden, *Appl. Soil Ecol.*, 2022, **169**, 104225.
58 M. Abid, S. Ali, L. K. Qi, R. Zahoor, Z. Tian, D. Jiang, J. L. Snider and T. Dai, *Sci. Rep.*, 2018, **8**, 4615.
59 N. A. Igbokwe, *Sokoto J. Vet. Sci.*, 2018, **16**, 1–23.

60 P. S. Valentovic, M. S. Luxova, L. S. Kolarovic and O. S. Gasparikova, *Plant Soil Environ.*, 2006, **52**, 184.
61 F. Çığ, F. Sönmez, M. A. Nadeem and A. E. Sabagh, *Agronomy*, 2021, **11**, 2418.
62 M. Ijaz, M. Tahir, M. Shahid, S. Ul-Allah, A. Sattar, A. Sher, K. Mahmood and M. Hussain, *Braz. J. Microbiol.*, 2019, **50**, 449–458.
63 M. Saeed, N. Ilyas, K. Jayachandran, S. Shabir, N. Akhtar, A. Shahzad, R. Z. Sayyed and A. Bano, *Environ. Pollut.*, 2022, **305**, 119282.
64 G. Lalay, A. Ullah, N. Iqbal, A. Raza, M. A. Asghar and S. Ullah, *Environ. Dev. Sustain.*, 2024, **26**, 3457–3480.
65 M. Zhang, Y. Liu, Q. Wei, J. Gou, L. Liu, X. Gu and M. Wang, *Horticulturae*, 2023, **9**, 1104.
66 E. S. Desoky, M. Mahmood, D. Feng, A. F. Ahmed, L. M. Al Masoudi, A. S. Alahmari, R. S. Eid, M. M. Rady, A. Ayyoub and N. A. Taha, *BMC Plant Biol.*, 2025, **25**, 236.
67 H. M. Javeed, R. Naeem, M. Ali, R. Qamar, M. A. Sarwar, F. Nawaz, M. Shehzad, A. Farooq, S. Khalid, K. Mubeen and N. Masood, *Acta Physiol. Plant.*, 2022, **44**, 110.
68 D. C. Pallavi, A. K. Sharma, in *Plant-Microbe Interactions*, Springer, 2017, pp. 607–626.
69 Y. Xie, H. Wang, Y. Guo, C. Wang, H. Cui and J. Xue, *Waste Manage.*, 2023, **171**, 512–521.
70 H. Ren, Z. Wang, C. Lv, B. Huang, X. Sun and F. Qin, *Forests*, 2024, **15**, 1091.
71 K. Wang, S. Wang, X. Zhang, W. Wang, X. Wang, F. Kong and M. Xi, *Environ. Res.*, 2024, **240**, 117426.
72 D. Egamberdieva, M. Hua, M. Reckling, S. Wirth and S. D. Bellingrath-Kimura, *Environ. Sustain.*, 2018, **1**, 19–24.
73 M. Mahmood, Y. Wang, W. Ahmed, S. Mehmood, A. Ayyoub, A. S. Elnahal, W. Li and X. Zhan, *Front. Plant Sci.*, 2023, **14**, 1224583.
74 W. Y. Qi, H. Chen, Z. Wang, S. F. Xing, C. Song, Z. Yan and S. G. Wang, *J. Hazard. Mater.*, 2023, **458**, 131921.
75 J. Lehmann, M. C. Rillig, J. Thies, C. A. Masiello, W. C. Hockaday and D. Crowley, *Soil Biol. Biochem.*, 2011, **43**, 1812–1836.
76 P. Kannan, M. Paramasivan, S. Marimuthu and C. Swaminathan, *Agric. Ecosyst. Environ.*, 2021, **308**, 107258.
77 M. Schoebitz, M. D. López Belchí, in *Bioformulations*, Springer, 2016, 251–265.
78 L. Hale, M. Luth and D. Crowley, *Soil Biol. Biochem.*, 2015, **81**, 228–235.
79 A. K. Jaiswal, N. Alkan, Y. Elad, N. Sela, E. R. Graber and O. Frenkel, *Sci. Rep.*, 2020, **10**, 13934.
80 E. Yang, J. Meng, H. Hu, D. Cheng, C. Zhu and W. Chen, *Ecotoxicol. Environ. Saf.*, 2019, **170**, 338–345.
81 M. Z. Hossain, M. M. Bahar, B. Sarkar, S. W. Donne, Y. S. Ok, K. N. Palansooriya, M. B. Kirkham, S. Chowdhury and N. Bolan, *Biochar*, 2020, **2**, 379–420.
82 W. Yang, Y. Zhao, Y. Yang, M. Zhang, X. Mao, Y. Guo, X. Li, B. Tao, Y. Qi, L. Ma and W. Liu, *J. Soils Sediments*, 2023, **23**, 947–957.
83 M. C. Andrenelli, A. Maienza, L. Genesio, F. Miglietta, S. Pellegrini, F. P. Vaccari and N. Vignozzi, *Agric. Water Manage.*, 2016, **163**, 190–196.
84 M. Asif, F. Ahmad, T. Ansari and A. Khan, *J. Agric. Sci.*, 2017, 2017.
85 M. K. Hossain, V. Strezov and P. F. Nelson, *Pedosphere*, 2015, **25**, 680–685.
86 M. Cong, Y. Hu, X. Sun, H. Yan, G. Yu, G. Tang, S. Chen, W. Xu and H. Jia, *Front. Plant Sci.*, 2023, **14**, 1172425.
87 C. Wu, D. Zhi, B. Yao, Y. Zhou, Y. Yang and Y. Zhou, *Environ. Res.*, 2022, **212**, 113226.
88 W. J. Grichar, T. W. Janak, J. A. McGinty and M. J. Brewer, *Agronomy*, 2023, **13**, 1429.
89 N. E. Means, C. J. Starbuck, R. J. Kremer and L. W. Jett, *Compost Sci. Util.*, 2005, **13**, 116–121.
90 R. D. Alixandre, P. A. Lima, T. F. Almeida, J. S. Oliveira, M. B. Pereira, F. T. Alixandre, G. R. Jacomino, R. S. Dias, R. S. Alexandre, A. Ferreira and R. R. Passos, *Braz. J. Biol.*, 2023, **83**, e277437.
91 M. Caldara, M. Gullì, S. Graziano, N. Riboni, E. Maestri, M. Mattarozzi, F. Bianchi, M. Careri and N. Marmiroli, *Sci. Total Environ.*, 2024, **917**, 170168.
92 C. T. Primaz, A. Ribes-Greus and R. A. Jacques, *Energy*, 2021, **235**, 121363.
93 X. Hu, J. Song, H. Wang, W. Zhang, B. Wang, W. Lyu, Q. Wang, P. Liu, L. Chen and J. Xing, *Water Sci. Technol.*, 2019, **80**, 2260–2272.
94 Z. Hamzah and S. N. Shuhaimi, *IOP Conf. Ser. Earth Environ. Sci.*, 2018, **215**, 012011.
95 D. Olszyk, T. Shiroyama, J. Novak, K. Cantrell, G. Sigua, D. Watts and M. G. Johnson, *Agrosyst. Geosci. Environ.*, 2020, **3**, e20067.
96 J. Ali, A. S. Mohammed and A. B. Mekonnen, *Adv. Agric.*, 2023, **2023**, 1483976.
97 Z. Khan, X. J. Yang, Y. Fu, S. Joseph, M. N. Khan, M. A. Khan, I. Alam and H. Shen, *Biochar*, 2023, **5**, 52.
98 Y. Zhang, J. Ding, H. Wang, L. Su and C. Zhao, *BMC Plant Biol.*, 2020, **20**, 288.
99 M. D. Vázquez Vázquez, S. César and J. M. Barea Navarro, *Microb. Ecol.*, 2000, **39**, 200–208.
100 G. A. Querejeta, *Int. J. Methodol.*, 2023, **2**, 34–40.
101 P. Trivedi, K. Singh, U. Pankaj, S. K. Verma, R. K. Verma and D. D. Patra, *Ecol. Eng.*, 2017, **102**, 127–136.
102 S. Ayyar, S. Appavoo, M. Basker, P. Pandiyarajan and R. Kavimani, *Int. J. Curr. Microbiol. Appl. Sci.*, 2019, **8**, 1804–1814.
103 A. E. Cherni, D. Trabelsi, S. Chebil, F. Barhoumi, I. D. Rodríguez-Llorente and K. Zribi, *Water, Air, Soil Pollut.*, 2015, **226**, 145.
104 W. T. Frankenberger Jr and J. B. Johanson, *Soil Biol. Biochem.*, 1982, **14**, 433–437.
105 X. Liu and S. Zhang, *Plant Soil*, 2019, **440**, 11–24.
106 F. U. Haider, J. A. Coulter, S. A. Cheema, M. Farooq, J. Wu, R. Zhang, S. G. Shuaijie and C. Liqun, *Ecotoxicol. Environ. Saf.*, 2021, **214**, 112112.
107 M. Li and L. Cai, *Sustainability*, 2021, **13**, 3244.

108 M. Rafique, I. Ortas, M. Rizwan, H. J. Chaudhary, A. R. Gurmani and M. F. Munis, *Chemosphere*, 2020, **238**, 124710.
109 D. K. Abdulrahman, R. Othman and H. M. Saud, *Malays. J. Soil Sci.*, 2016, **20**, 177–194.
110 K. Saranya, K. Kumutha and P. S. Krishnan, *Madras Agric. J.*, 2011, **98**, 158–164.