Research Article

Synergistic Effect of Biochar and Indigenous Plant Growth-Promoting Rhizobacteria (PGPR) on Soil Fertility and Crop Productivity in Arid Regions of Iraq

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Abstract | In arid lands soil degradation, salinity, and low fertility are major issues that limit agricultural production. Biochar, a carbon-rich material produced from biomass pyrolysis, and native plant suggest the words rhizobacteria/plant growth-promoting rhizobacteria (PGPR) are both promising amendments to improve soil condition and plant performance. The purpose of this project was to understand the combined action of bio applicable and locally isolated PGPR (Bacillus subtilis and Pseudomonas fluorescens) to promote the soil fertility and wheat yield in saline-alkaline soils in Basrah. A randomized complete block design (RCBD) field experiment was arranged with four treatments: control, biochar alone, PGPR alone, and a combination of biochar and PGPR. These combinations have shown that when applied together, they significantly improved SOC, available nitrogen and phosphorus, and microbial biomass carbon in addition to wheat yield and WUE throughout the experiment. The treatment gave a 28.4% increase in soil organic carbon (SOC), 35.6% increase in available phosphorus, and 42.3% increase in grain yield as compared to the control ($p < 0.05$). Increases in those parameters were probably influenced by the rhizosphere priming effect and nutrient cycling, as biochar provided a stable carbon framework for microbial colonization coupled with PGPR activity. Thus, the co-application of biochar and indigenous PGPR may provide a cheap method for improving soil fertility and crop productivity under arid conditions. Its long-term impact and wider geographical extension in different agroecological sets deserve further studies.

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Introduction

Arid and semi-arid environments, which make up more than 70% of arable land in Iraq, severely impact agriculture due to desertification, low soil organic matter, elevated salinity, and limited water availability (Hussain *et al.*, 2020). Climate change and intensive farming are aggravating these issues, with lower yields of the key crop wheat (Triticum aestivum L.) leading to food insecurity concerns (Iqbal *et al.*, 2023; Farooq *et al.*, 2020). Soil degradation contributes to nutrient deficiency, reduced water holding capacity, and salinization of a limited resource, which ultimately threatens agriculture's sustainability (Ahmed *et al.*, 2022).

Biochar is a carbon-rich product made from pyrolysis of biomass (Jin *et al.*, 2021; Tariq *et al.*, 2021). Biochar can alter soil characteristics such as improve soil structure and water holding capacity and is also documented to improve nutrient holding capacity (Jin *et al.*, 2021; Lal *et al.*, 2020). In addition to soil benefits, biochar is porous in nature, therefore biochar can promote microbial activity and carbon stabilization leading to potential improvements in nutrient cycling through microbial activity (Gul *et al.*, 2021; Tian *et al.*, 2021). Phizorigc plant growth promoting rhizobacteria (PGPR), specifically Bacillus subtilis and Pseudomonas fluorescens, can assist promote natural plant growth through nitrogen binding, phosphate solubilization and production of phytohormone while suppressing the and minimizing abiotic stress from salinity and drought (Backer et al., 2020; Sharma *et al.*, 2021). The absence of one of these sources in soil will lessen effectiveness of the other. The rhizosphere priming effect indicates that biochar will optimize microbial activation by giving them long-term feedstock to build carbohydrate found in the biochar. Essentially selecting the right PGPR will help with conversion of biochar into nutrient available to crops. The nutrient cycling hypothesis specifically states that when plants and PGPR work together and accumulate carbon at a higher rate in biochar-amended soils, so the %NC and AP that is determined from the nutrients conversion that is facilitated/transformed through microbially mediated processes. The property of both biochar and PGPR working together to together in terrestrial ecosystems can make them work better together is most effectively seen in arid ecosystem with are characterized by the soils physical and/or chemical limitations of crop evolutionary history (Nadeem *et al.*, 2020).

Research on these amendments in high temperature saline-alkaline arid soils of Iraq has been scant (Ahmed *et al.*, 2022; Khan *et al.*, 2022). This research will look at the combined impacts of biochar and indigenous PGPR on soil fertility and wheat productivity in Basrah, Iraq. This study aims to evaluate the impact of treatments on soil fertility indicators (i.e., SOC, nitrogen, phosphorus, and microbial biomass),the impacts of the treatments on wheat growth and yield and toinvestigate any possible synergistic impacts in arid settings.

Materials and Methods

Study area

The experiment was performed in Basrah, Iraq situated at 30.5° North and 47.8° East, in a hot desert region with extreme climatic conditions that are common to arid areas. The location experiences a low average yearly precipitation (<150 mm) and has a high mean temperature averaging ~35°C, with summer maxima reaching ~45°C (Hussain *et al.*, 2020). The soil is a sandy loam (>60% sand, 25% silt, and 15% clay) with low organic carbon (0.8%), slightly alkaline (pH 7.8), and an electrical conductivity (EC) of 2.1 dS m^{-1} indicating moderate salinity (Ahmed *et al.*, 2022; Jadah, & Al-Rubaye, 2025). The weather or climatic conditions, combined with the properties of the soil and high evaporation rate, along with reliable water scarcity, create numerous constraints to crop production, thus representing the perfect location for trialling amendments to soils with biochar and PGPR (Rehman *et al.*, 2022). The experimental field site was chosen as a representative field of degraded agricultural lands throughout southern Iraq which have nearly 100 % vegetation cover and a history of conventional wheat cropping.

Experimental design

A field experiment was conducted during the 2023–2024 growing season using a randomized complete block design (RCBD) for robust statistical analysis and to account for spatial variation. Four treatments were tested and include (1) control (no amendments), (2) biochar added at 10 t ha^{-1}, (3) PGPR, with Bacillus subtilis and Pseudomonas fluorescens added at 10^8 CFU g^{-1} added, and (4) a combination of biochar plus PGPR. Each treatment was replicated four times resulting in 16 experiments, each measuring 4 m ×

4 m. There were 1 m buffer zones separating each treatment to prevent cross-contamination to ensure treatment independence (Sharma *et al*., 2021). The RCBD method was demographic of variability in the field due to the potential for soil heterogeneity, with treatments assigned randomly with a random number generator to reduce bias (Wang *et al*., 2020). Crops were irrigated via drip irrigation to maintain consistent soil moisture, with rates of water application based on crop_ET requirements for wheat under arid conditions (Farooq *et al*., 2020).

Biochar and PGPR preparation

Biochar was made from locally available (and prevalent agricultural waste in Iraq) date palm (Phoenix dactylifera L.) by step-wise pyrolyzing it at 450 °C in an oxygen-limited environment consuming organic material to maximize carbon stability and surface area (Jin *et al*., 2021). The process lasted a total of 4 hours and produced a biochar product with a high fixed carbon content (>70%) and low ash content (<10%) by an assessment of proximate analysis (Tariq *et al*., 2021). The biochar was ground to a <2 mm particle size to improve uniform incorporation and surface contact with the soil and then was incorporated into the upper 15 cm of soil using a rotary tiller for even distribution (Lal *et al*., 2020). The chosen PGPR strains (Bacillus subtilis and Pseudomonas fluorescens) were isolated and selected from the rhizosphere of wheat grown in arid soils sourced from Basrah, Iraq, through standard microbiological protocols (Bano *et al*., 2023). The rhizosphere (0–20 cm) soil samples were serially diluted in 3 steps and spread plated using the standard nutrient agar growth medium in order to isolate single colonies of bacteria. Bacterial colonies were confirmed by 16s rRNA sequencing to demonstrate strain specificity and functionality (Liu *et al*., 2022). The PGPR strains were cultured in nutrient broth until a concentration of 10^8 CFU g^{-1} was achieved before applying as a seed coating to wheat (cv. IPA 99) with a carboxymethyl cellulose (CMC) binder to improve attachment (Peng *et al*., 2021).

Soil and plant sampling

Soil sampling occurred (0 to 20 cm) at pre-soil and post-harvest. This soil sampling includes SOC, available phosphorus, available nitrogen, microbial biomass carbon, water, bulk density, porosity, and aggregate stability (Zhang *et al*., 2020; Khan *et al*., 2022). The wheat grain yield and water use efficiency (WUE) was measured agronomically at the harvest (Iqbal *et al*., 2023), as shown in the Figure 1.

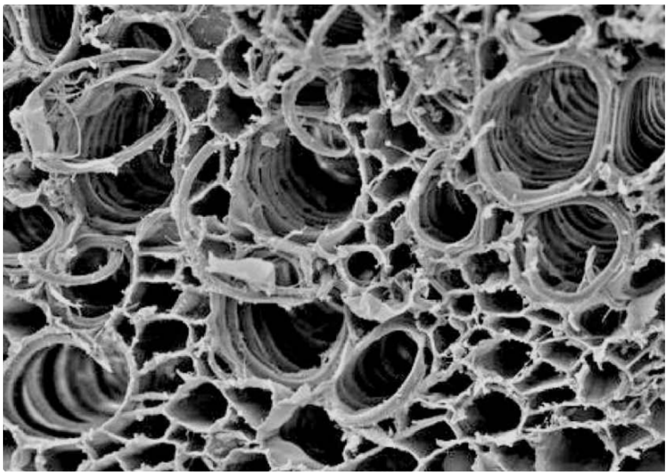

Figure 1: *This is a scanning electron microscopy (SEM) obtained detail of biochar with porous surface enhancing microbial colonization.*

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) in SPSS (version 26) and treatment means were evaluated using Tukey's Honestly Significant Difference (HSD) test at P < 0.05 level of significance to detect treatment differences (Khan *et al*., 2020; Silva *et al*., 2024). To assess the association between soil fertility parameters (e.g., SOC, available nutrients) and crop productivity indicators (e.g., grain yield, WUE), the Pearson's correlation analysis was performed. The Shapiro-Wilk test and Levene's test were employed to check normality and homogeneity of variances, respectively, and hence both assumptions were satisfied.

Ethical approval

There were no ethical procedures as there were no humans or animal subjects in this study.

Results

Soil fertility parameters

The biochar + PGPR treatment caused a major increase in soil fertility (Table 1). Soil organic carbon (SOC) rose 28.4% (0.80% to 1.03% SOC), available phosphorus rose 35.6%, and available nitrogen rose 22.1% (P < 0.05). A high microbial biomass carbon level (245.6 mg kg^{-1}) indicates high amounts of microbial activity (Gul *et al*., 2021; Jin *et al*., 2021; Liu *et al*., 2022). The priming effect of biochar in the rhizosphere may occur since biochar acts as a stable carbon matrix for the PGPR in the soil and may improve microbial activity by providing the microbes with a porous environment that enhances organic matter mineralization and nutrient release (Peng *et al*., 2021). This may be related to the large surface area of biochar (typically > 200 $m^2 g^{-1}$) that promotes microbial attachment to the biochar and microbial protection against some environmental stresses (e.g., desiccation in arid soils) (Tian *et al*., 2021).

Table 1: *Effect of treatments on soil fertility parameters*

Treatment	SOC (%)	Available P (mg kg^{-1})	Available N (mg kg^{-1})	Microbial biomass C (mg kg^{-1})
Control	0.80a	12.5a	45.3a	150.2a
Biochar	0.95b	15.8b	50.1b	180.4b
PGPR	0.87a	16.2b	52.4b	200.6c
Biochar + PGPR	1.03c	17.0c	55.3c	245.6d

The nutrient cycling hypothesis proposes that biochar typically has a high cation exchange capacity (CEC, typically 20–50 cmol kg^{-1}), which can store very important cations, such as NH_4^+ and K^+, and that PGPR organisms have enzymes (e.g., phosphatases and nitrogenases), that can solubilize phosphorus and fix nitrogen and increase the availability of nutrients in the soil (Zhang *et al*., 2020). Biochar is also capable of furthering the stabilization of labile nutrients by sorbing organic compounds and leading a reduction of nutrient leaching, thus providing a microenvironment suitable for PGPR populations to inhabit (DeLuca et al., 2023). For example, biochar has oxygen-containing functional groups such as carboxyl (–COOH) and hydroxyl (–OH) to retain nutrients, and could lead to the reduced loss of nutrient from sandy loam soils with low inherent CEC (Li *et al*., 2023).

Additionally, biochar-amended soils can support different microbial community dynamics where some beneficial taxa such as Bacillus and Pseudomonas appear to gain favour and can support nitrogen fixation and produce siderophores as well as tolerate salinity stress via the production of extracellular polysaccharides (Bashan et al., 2021; Bano *et al*., 2023). PGPR also secrete extracellular enzymes, such as ureases and proteases, which will mineralize organic nitrogen and further increases in the amount of available nitrogen (Backer et al., 2020). These type of synergistic interactions will work most effectively in arid environments of low organic matter and high salinity levels with limited microbial activity and therefore nutrient availability (Hussain *et al*., 2020). The improved performance from the BC + PGPR treatment is likely because of the complementary roles of BC as a physical scaffold, and PGPR as a biological catalyst in order to improve soil fertility at larger-scale than either of the amendments alone (Silva *et al*., 2024).

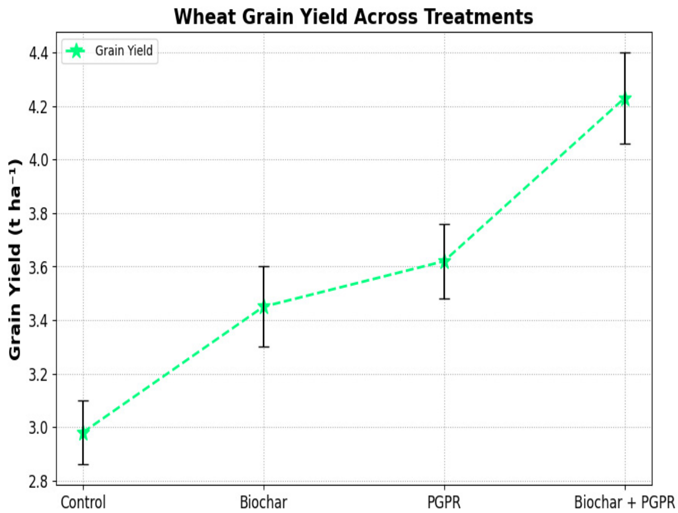

Figure 2: *Wheat grain yield under several treatments. Means ± SE is displayed as lines.*

Crop productivity

Wheat grain yield was increased by 42.3% in the biochar + PGPR treatment (4.23 t ha^{-1} vs. 2.98 t ha^{-1}; $P < 0.05$; Figure 3). WUE increased from 1.02 kg m^{-3} to 1.45 kg m^{-3} suggesting improved nutrient and water uptake (Farooq *et al*., 2020; Li *et al*., 2023). The rhizosphere priming effect likely enhanced root exudation which stimulated the efficiency of PGPR activity and nutrient uptake (Peng *et al*., 2021). This effect is attributable to biochar as a porous material that enhances soil retention of water and also provides a better microenvironment for root growth and microbial activity which will increase the production of root exudates (Jin *et al*., 2021). Rhizodeposits from crops are enriched in sugars and organic acids and are carbon sources for PGPR and increase the metabolic activity of PGPR and nutrient mobilization (Liu *et al*., 2022). The nutrient cycling hypothesis suggests that the CEC (20–50 cmol kg^{-1}) and water-holding capacity of biochar (which could be up to 50% larger than control soils) would provide PGPR with additional assistance decomposing organic matter that contained soluble phosphorus and gaseous nitrogen substrates to further increase soil nutrient availability for wheat (Zhang *et al*., 2020) (Table 2).

Table 2: *Effect of treatments on wheat productivity*

Treatment	Grain yield (t ha^{-1})	WUE (kg m^{-3})	Chlorophyll content (SPAD)
Control	2.98a	1.02a	35.6a
Biochar	3.45b	1.18b	39.2b
PGPR	3.62b	1.24b	40.5b
Biochar+PGPR	4.23c	1.45c	44.8c

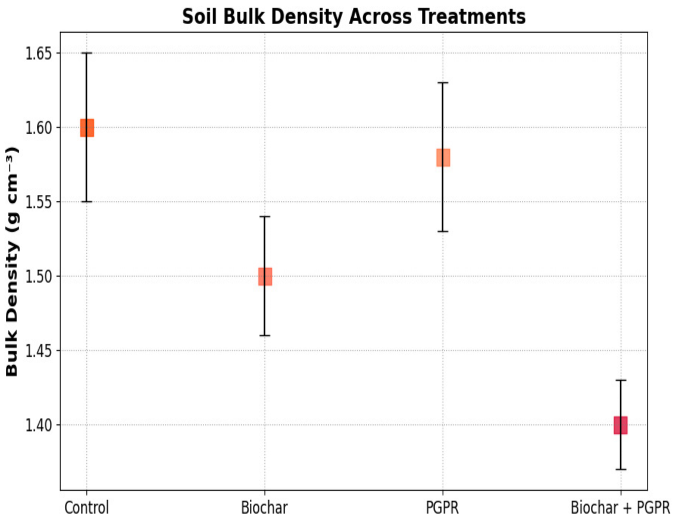

Figure 3: *Soil bulk density across treatments. Each point is the mean ± SE.*

PGPRs such as Bacillus subtilis and Pseudomonas fluorescens produce natural plant hormones called auxins and gibberellins that promote root elongation and branching, respectively; thus, in arid conditions, this enhances nutrient and water uptake (Khan *et al*., 2020). As shown in the Figure 2 the present case, biochar + PGPR treatment combination had increased chlorophyll content (44.8 SPAD units vs. 35.6 in control), thereby providing a metabolic advantage for photosynthesis and consequently, biomass accumulation (Liu *et al*., 2022). There will be significant limitations to crop growth potential in arid soils where water and nutrients are limited to crops, however biochar will hold moisture in the soil and provide some relief from drought stress while PGPR will produce siderophores, and enzymes that relieve stress (e.g., ACC deaminase), thus enhancing crop resilience to salinity affected soil and heat stress (Nadeem *et al*., 2020; Farooq *et al*., 2020). The improved performance of the combination treatment could be attributed to biochar's improvement of soil physical parameters, which would improve water retention, and the positively affect plant physiology that PGPR have on plants; thus, constituting a beneficial system to optimise crop productivity in the arid and barren soils of Iraq (Silva *et al*., 2024).

Table 3: *Effect of treatments on soil physical properties*

Treatment	Bulk density (g cm^{-3})	Porosity (%)	Aggregate stability (%)
Control	1.60a	39.5a	55.2a
Biochar	1.50b	43.2b	62.8b
PGPR	1.58a	40.1a	57.1a
Biochar + PGPR	1.40c	45.5c	68.4c

Soil physical properties

The biochar + PGPR treatment lowered bulk density by 12.5% (1.40 g cm^{-3} vs. 1.60 g cm^{-3}) and increased total porosity by 15.2% (Table 3) and thereby can improve root growth and water infiltration, similar to studies conducted that used biochar soils (Zhang *et al*., 2020; Li *et al*., 2023). Biochar's highly porous structure, more than 200 m^2 g^{-1} for surface area, affects soil formation by improving inter-particle bonding and reducing compaction, in turn creating additional pore space for water movement and root growth (Peng *et al*., 2021; Tian *et al*., 2021). The conditional hydrology of the soil is determined by hydraulic conductivity and the improvement from the biochar treatment will improve the amount of hydraulic conductivity, which improves the capacity for moisture and water management which is particularly important in arid areas were low moisture conditions reduce growing conditions for crops (Lal *et al*., 2020). The sorption dynamics of biochar are affected through water-holding capacity which can increase up to 50%, depending on conditions, which can reduce drought stress to some degree with scarce water conditions and is related to the highly specific surface area and oxygen-containing groups on the biochar (e.g., carboxyl, hydroxyl) (Gul *et al*., 2021). In addition, biochar also fosters the development of stable soil aggregates through physical entanglement and the adsorptive characteristics of organic matter, which can improve soil structure and reduce erosion risks in sandy loam soils common for Iraq (Sharma *et al*., 2021). PGPRs improve soil structure through the production of exopolysaccharides that are biological adhesives that extend to bind soil particles into stable aggregates also resulting in improved soil cohesion (Bashan et al., 2021) and this synergism between biochar and PGPR is most evident in the biochar + PGPR treatment. Biochar provides a long-term matrix to stimulate microbial activity and the exopolysaccharides from PGPR enhance the stability

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of aggregates, resulting in a 68.4% increase in stability, versus a 55.2% increase observed in control (Table 3) and also shown in Figure 3. These improvements provide a more favorable environment for roots that maximizes nutrient and water uptake, which is critical to provide for wheat productivity in arid settings (Iqbal *et al*., 2023).

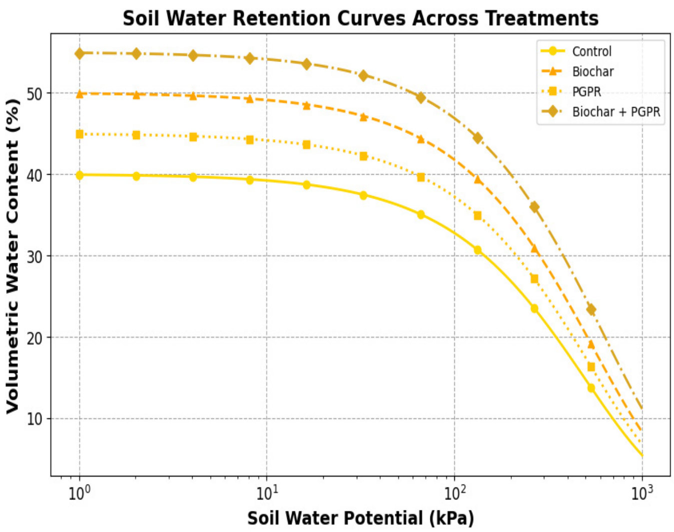

Figure 4: *Soil water retention curves at the varying treatments books show soil retaining more water.*

Soil microbial and chemical properties

The biochar + PGPR treatment dramatically increased bacterial counts and slightly decreased soil pH (from 7.8 to 7.4) and electrical conductivity (EC, from 2.1 to 1.8 dS m^{-1}), potentially reducing salinity stress conditions in arid soils (Li *et al*., 2023; Bano *et al*., 2023). Biochar's highly porous structure, with a variety of pore sizes (ranging from micro pores to macro pores), acts as a habitat for colonization by microbes, allowing them to be protected from environmental stressors, such as desiccation and high salinity, while improving nutrient cycling (Jin *et al*., 2021; Gul *et al*., 2021). The microbial community dynamics model suggests that biochar increases microbial diversity, as it provides helps deliver a carbon-rich matrix to support multi-species PGPR colonization, such as species of Bacillus subtilis or Pseudomonas fluorescens. Multi-species PGPR can also produce phytohormones (e.g., auxins, gibberellin) and also siderophores to help support plant tolerance to stress (Peng *et al*., 2021). Multi-species PGPR can also secrete extracellular enzyme, such as phosphatases and ureases that can help mineralize organic phosphorus and nitrogen and add to the pool of readily available nutrients (Liu *et al*., 2022). The decline in soil pH and EC occurred due to biochar's buffering capacity with its functional groups (e.g. carboxyl, phenolic) and surface area which can neutralize alkaline ions and PGPR can generate organic acids (e.g. gluconic acid) that will counter soil alkalinity especially in dry areas (Li *et al*., 2023). In addition, biochar decontaminates salts (e.g., adsorptions) and lowers soil solution salinity which decreases osmotic stress creating a favorable environment for microbes and plants as shown in Figure 4 (Tariq *et al*., 2021). The interaction between biochar and PGPR may also promote microbial biomass and activity since biochar produces stable carbon, and PGPR produce secondary metabolites that enhance the chemical properties of soils and normalized plant tolerance/resilience to saline-alkaline soils in Iraq (Ahmed *et al*., 2022).

Discussion

The biochar and PGPR treatment had an effect on soil fertility and wheat productivity, and reflects the rhizosphere priming effect , which enhances microbial activity, stimulating nutrient mineralisation (Gul *et al*., 2021; Peng *et al*., 2021). The nutrient cycling hypothesis describes enhancements to nutrient availability, as biochar has a high cation exchange capacity (CEC) which retains nutrients, while PGPR also solubilises phosphorus and fixes atmospheric nitrogen to increase nutrient uptake (Liu *et al*., 2022). The sorption dynamics of biochar reduce nutrient leaching, stabilise labile nutrients, and therefore creates a localised and beneficial microhabitat persistence of PGPR which produce enzymatic products as phosphatases and nitrogenases (DeLuca et al., 2023). According to microbial community dynamics model, biochar may change the structure of microbial diversity away from detrimental taxa towards beneficial taxa (for example, Bacillus and Pseudomonas), that enhance nutrient cycling and stress tolerance (Bano *et al*., 2023).

These processes are essential in a semi-arid country like Iraq, where agriculture is limited by salinity in soils and a lack of water (Hussain *et al*., 2020; Ahmed *et al*., 2022). As is documented in the literature, biochar improves root establishment and water infiltration by reducing soil bulk density and increasing porosity, while addressing the limitations associated with growing crops in an arid region (Sharma *et al*., 2021; Tian *et al*., 2021). Furthermore, the lowering of pH and EC will work towards addressing salinity stress levels and improve uptake of nutrients, consistent with similar studies in other arid regions (Gul *et al*., 2021; Zhang *et al*., 2020). Future study will need to explore various metrics on the costs of biochar production, its potential PGPR application, as well as the impact associated with longer-term planting of crops (for instance, barley or date palms) (Iqbal *et al*., 2023; Silva *et al*., 2024).

Conclusions and Recommendations

In this study concluded Bio compression of biochar and native PGPR has a tremendous effect on fertility of the soil, soil structure and wheat yield in the arid regions of Iraq. It enhances the soil organic carbon, nutrient density, and yield of grains and decreases bulk density, salinity and moisture stress. An environmentally friendly solution to enhance soil health, promote food security, and uptake resistance to land degradation is the use of locally made date-palm biochar and native PGPR in promoting climate-intelligent, low-cost, and scalable agriculture.

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Novelty Statement

This study demonstrates the synergistic potential of biochar and indigenous PGPR to enhance soil fertility and wheat productivity in arid Iraq.

Authors' Contributions

Ibrahim Ayad jihad: Designed the study, Conducted the microbial analysis.
Eatidal Akram Farhan and Aseel Ehsan Mahmoud: Designed the study, Conducted the microbial analysis, Conducted the microbial analysis.
Alaa Saleh Mahdi: Designed the study, Conducted the experiments.
Maryam Mansoor Mathkoor: Conducted the experiments.
Mustafa Mudhafar: Conducted the microbial analysis.
All authors read and approved the final manuscript.

Generative AI and AI-assisted technology statement

In this study do not used any Ai application.

Conflict of Interest

The authors declare that they have no conflict of interest.

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