



Synergistic Influence of Biochar, Vesicular Arbuscular Mycorrhizae (VAM), and Plant Growth-promoting Rhizobacteria (PGPR) on the Physicochemical and Nutrient Dynamics of Wheat Grown Soils

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Wheat is a major cereal crop, but intensive use of chemical fertilisers has led to soil degradation and reduced fertility. Sustainable approaches using biochar, VAM, and PGPR are gaining importance for improving soil health and nutrient dynamics. In this context, the present study examines their combined effect on the physicochemical properties of wheat-grown soils. A field experiment conducted during the 2023-24 Rabi season at Udai Pratap College, Varanasi, assessed wheat performance with various applications of Biochar, VAM, and PGPR in a randomized block design of 18 treatments across 3 replications. Results showed that Biochar decreased soil bulk density, enhanced porosity, and acted as a stable carbon reservoir. PGPR improved nutrient acquisition through nitrogen fixation and nutrient solubilization, while VAM improved

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water and phosphorus transport to plants. Together, they lowered soil pH from 7.88 to 6.86, enhanced long-term soil health, and significantly increased the bioavailability of essential nutrients. The optimal management strategy for wheat involved applying 6.0 tons ha⁻¹ of biochar, along with VAM and either *Bacillus megaterium* (T₁₈) or *Pseudomonas fluorescens* (T₁₇).

Keywords: Biochar; PGPR; VAM; wheat; sustainable agriculture.

1. Introduction

Wheat (*Triticum aestivum* L.) is the most important agricultural commodity in human history and a member of the Poaceae family. It typically contains 70% carbohydrates, 12% protein, and 12% water, with these proportions varying based on factors like soil nitrogen, moisture, and temperature. With a projected world population increase of over 2.3 billion by 2050, global food production, particularly cereals, must rise significantly—by about 70% (Food and Agriculture Organization of the United Nations, 2009). In the 2024-25 marketing year, wheat was grown on approximately 220.7 million hectares, producing 800.8 million metric tons, making it the second most-produced cereal after maize, with China, the European Union, and India as leading producers supplying nearly half of the global market (USDA, 2025). The overuse of nitrogenous fertilizers and underuse of organic matter have led to a harmful NPK ratio of 7.7:3.1:1, deviating from the ideal 4:2:1 (Yumkhaibam et al., 2025). This results in soil pH changes, reduced microbial diversity, and water pollution due to nutrient leaching (Rehali et al., 2026). Conventional practices neglect soil structure and health, causing compaction and loss of organic carbon. To enhance wheat yields, a combined approach utilizing organic amendments and biological stimulants is essential. A notable strategy is the integration of biochar, vesicular arbuscular mycorrhizae (VAM), and plant growth-promoting rhizobacteria (PGPR), which promotes a biologically driven agricultural model. Biochar improves soil structure and carbon retention while enhancing nutrient availability for crops. The combined use of biochar, VAM, and PGPR results in substantial physical and chemical enhancements in wheat-grown soils, particularly in post-harvest properties like bulk density, porosity, and water-holding capacity. This tripartite interaction improves nutrient availability and stability in the soil, reshaping nitrogen, phosphorus, and potassium cycles. Biochar functions as a nutrient reservoir, while PGPR solubilizes nutrients and VAM facilitates their transport to plant roots (Malik et al., 2024, Raghav and Rastogi, 2025). Moreover, this combination significantly boosts soil enzymatic activities, indicators of effective nutrient cycling (Rehali et al., 2026). The focus of modern agriculture on maximizing yield through heavy chemical inputs has harmed long-term soil health. With global soil facing pressures from climate stressors like droughts and temperature fluctuations, a shift to biologically driven soil restoration is critical. Biochar, VAM, and PGPR offer various advantages but also have limitations. Biochar can introduce heavy metals or toxic compounds based on feedstock. VAM strains may not form effective symbiotic relationships with all crop species, and PGPR often experiences reduced performance when moving from controlled laboratory conditions to complex field environments. Keeping above facts in mind, this study evaluated the effectiveness of combining biochar with biological inoculants, specifically Vesicular-Arbuscular Mycorrhiza and Plant Growth Promoting Rhizobacteria, to enhance the physico-chemical properties of soil in wheat cultivation.

2. Materials and Methods

The present study was undertaken at Research farm of Udai Pratap College, Varanasi, Uttar Pradesh, during the Rabi Season of 2023-24. The soils of Varanasi formed on alluvial deposited by river Ganga have pre dominance of illite, quartz and feldspars. Illite minerals are partly inherited from micas, which are predominant in the sand and silt fractions. It falls under the humid subtropical climate zone, characterized by hot summers, cold winters, and a strong southwest monsoon season. It is part of the Middle Gangetic Plain region, typically featuring intense, dry heat in April-June and high humidity in the monsoon months. The soil of the experiment site was sandy-loam in texture having a pH of 7.88, available nitrogen 241.15 kg ha⁻¹, available phosphorus 19.65 kg ha⁻¹ and available potassium 226.25 kg ha⁻¹. The experiment was conducted in a randomized block design (RBD) with three replications. The treatments comprised 3 levels of Biochar (0, 3.0, 6.0 tons ha⁻¹), 2 levels of VAM [(T-Treated) and (NT-Not Treated)] and 3 levels of PGPR [(NT-Not Treated), *Pseudomonas fluorescens* and *Bacillus megaterium*] (Table 1).

Sowing of Wheat variety 'HD- 2967' was performed at a spacing of 22.5 cm x 10 cm. The recommended dose of N: P₂O₅: K₂O for wheat @ 120:60:60 kg ha⁻¹ was applied uniformly in all the plots. Half dose of nitrogen, full dose of Phosphorous, Potash was applied through Urea, SSP, MOP as per treatment at the time of sowing. The rest of the half dose of nitrogen was applied in two splits as top dressing, first at 30 DAS and second at ear head

initiation stage. Biochar was applied in the field after ploughing @ 3.0- and 6.0-tons ha⁻¹ in respective treatments. VAM was broadcasted @5 kg ha⁻¹ by mixing with organic manure and in the field before sowing. PGPRs, i.e., *Pseudomonas fluorescens* and *Bacillus megaterium* were applied in the soil before sowing @1.5 kg ha⁻¹ and 3.0 kg ha⁻¹, respectively. Bulk density and Particle density of soil was determined at various growth stages of wheat, i.e., 30 DAS, 60 DAS and harvest stage, by the Core sampler method and the Pycnometer method (Blake and Hartge, 1986 & 1986a), respectively. Porosity at all the growth stages is measured by calculating the ratio of pore volume (void space) to the total bulk volume of a material, expressed as a percentage. Sand, silt and clay content was estimated by using the Hydrometer Method (Bouyoucos, 1930). Water holding capacity was determined by Keen's Raczkowski box method by saturating a dry sample (soil or food), allowing excess water to drain or spinning it out, and calculating the difference in weight. Soil Temperature was measured at 6AM and 2 PM for all the growth stages at various depths, i.e., 5, 10 and 15 cm using a soil thermometer (Taylor and Jackson, 1986). The pH, EC and Soil Organic Carbon content of soil was measured by pH meter, EC meter and the Walkley and Black rapid titration method at all the growth stages. Available Nitrogen, Phosphorus and Potassium were estimated by using the Alkaline permanganate method (Subbajah and Asija, 1956), Olsen's extract method (Olsen et al., 1954) and Neutral Normal ammonium acetate Method (Stanford and English, 1949), respectively. The data obtained from the present investigation were statistically analyzed for critical difference (CD) and standard mean error (SEm) by using the Randomized Block Design (RBD) to test the significance of treatment effect on various soil and plant parameters. The analysis of variance (ANOVA) procedure was performed as described by Fisher and Yates (1953).

Table 1. Treatment details

Treatments	Biochar (tons ha ⁻¹)	VAM (Vesicular-arbuscular Mycorrhiza)	Plant Growth Promoting Rhizobacteria (PGPR)
		NT- Not Treated T- Treated (5 kg ha ⁻¹)	NT- Not Treated <i>Pseudomonas fluorescens</i> @ 1.5 kg ha ⁻¹ <i>Bacillus megaterium</i> @ 3 kg ha ⁻¹
T ₁	0	NT	NT
T ₂	0	NT	<i>Pseudomonas fluorescens</i>
T ₃	0	NT	<i>Bacillus megaterium</i>
T ₄	3.0	NT	NT
T ₅	3.0	NT	<i>Pseudomonas fluorescens</i>
T ₆	3.0	NT	<i>Bacillus megaterium</i>
T ₇	6.0	NT	NT
T ₈	6.0	NT	<i>Pseudomonas fluorescens</i>
T ₉	6.0	NT	<i>Bacillus megaterium</i>
T ₁₀	0	T	NT
T ₁₁	0	T	<i>Pseudomonas fluorescens</i>
T ₁₂	0	T	<i>Bacillus megaterium</i>
T ₁₃	3.0	T	NT
T ₁₄	3.0	T	<i>Pseudomonas fluorescens</i>
T ₁₅	3.0	T	<i>Bacillus megaterium</i>
T ₁₆	6.0	T	NT
T ₁₇	6.0	T	<i>Pseudomonas fluorescens</i>
T ₁₈	6.0	T	<i>Bacillus megaterium</i>

3. Results and Discussion

3.1 Bulk Density

The experimental results (Table 2) showed that applying biochar, both alone and with VAM and PGPR, significantly reduced soil density over three-time intervals (30, 60 days after sowing, and harvest). The control treatment had the highest density, starting at 1.41 Mg m⁻³ and decreasing to 1.37 Mg m⁻³ by harvest. In contrast, 6.0 tons ha⁻¹ of biochar with VAM and *Bacillus megaterium* (T₁₈) led to the lowest density of 1.15 Mg m⁻³, marking a significant 16.06% reduction from the control. This reduction is attributed to the "dilution effect" of biochar's low bulk density and enhanced biological interactions, including EPS secretion by PGPR (Li et al.,

2022) and the structural role of VAM fungi. These processes contribute to stable soil aggregates, thereby lowering bulk density during the wheat growth cycle (Liao et al., 2019; Jing et al., 2025).

3.2 Particle Density

Particle density, which measures the density of the solid phase of soil, is largely influenced by the specific gravity of minerals like quartz, feldspar, and micas (Rivenshield & Bassuk, 2007). It is generally stable and less affected by management practices compared to bulk density (Afroz et al., 2023). Experimental data (Table 2) from wheat cultivation shows particle density values ranged narrowly from 2.66 to 2.69 Mg m⁻³, without significant differences between control and bio-amended plots. This suggests that biochar application rates (up to 6.0 tons ha⁻¹) do not fundamentally alter soil solid phase specific gravity (Afroz et al., 2023). Instead, increases in porosity and reductions in bulk density are attributed to structural changes in the soil, creating new pore volumes (Głab et al., 2016) rather than altering the mineral composition. Biochar, in conjunction with microbial agents, reconfigures soil architecture to enhance pore networks while retaining the mineral foundation.

3.3 Porosity

Soil porosity, defined as the ratio of pore volume to bulk volume (Afroz et al., 2023), is crucial for root respiration, soil solution movement, and microorganism habitat. Integrating biochar, VAM, and PGPR markedly increased soil porosity (Table 2) during wheat growth stages. The control treatment showed the lowest porosity (47.32% - 48.81%), whereas combined treatments T₁₇ and T₁₈ reached a maximum of 57.21%, indicating an 8.4% increase, critical for water retention in late growth stages. This rise in porosity results from both "intra-particle" porosity from biochar and enhanced "inter-particle" voids due to the irregular shape of biochar disrupting soil mineral packing (Liu et al., 2017). Additionally, microbial activity from VAM and PGPR stabilizes soil micro-aggregates, improving pore distribution for better aeration and drainage (Liao & Thomas, 2019; Brikmans et al., 2025; Głab et al., 2016). Overall, the findings highlighted that bio-inoculants promote increased porosity and soil health.

3.4 Water Holding Capacity

The Water Holding Capacity (WHC) of soil is essential for agricultural productivity, especially in rainfed wheat systems (Liu et al., 2017). Biochar enhances WHC due to its sponge-like properties, significantly increasing moisture retention when combined with biological treatments. Data from this study (Table 2) revealed that, the maximum WHC of 62.08% was achieved with 6.0 t ha⁻¹ biochar, VAM fungi, and *Pseudomonas fluorescens*, reflecting a 21.18% improvement over the control. Biochar's effectiveness stems from its surface area and microporosity, which create capillary forces for water retention (Zhang et al., 2013; Głab et al., 2016). VAM fungi expand root absorption by accessing water in small soil pores (Diagne et al., 2020), while PGPR improve soil wetting and promote systemic plant resistance (Li et al., 2022), thereby enhancing moisture availability and crop resilience in drought conditions (Sagar et al., 2021; Jing et al., 2025).

3.5 Soil Temperature

Soil temperature is a primary driver of plant physiological processes and microbial metabolic rates (Xiong et al., 2020). It exhibits strong diurnal and depth-dependent fluctuations, which are influenced by the soil's albedo (reflectance), thermal conductivity, and heat capacity. Biochar's unique physical properties—specifically its dark color and low thermal conductivity—make it a powerful tool for regulating the soil thermal regime. At 30 DAS, the bio-amended soils exhibited significantly higher temperatures than the control across all depths and times (Table 3). For instance, at the 5 cm depth at 6 AM, treatment T₁₈ was 1.29 °C warmer than the control. During the early growth stages of wheat, which often occurring in cooler months, maintaining adequate soil warmth is essential for vigorous root development and nutrient uptake. This "heat preservation" effect is a result of biochar's low thermal conductivity; because biochar is highly porous and air-filled, it acts as an insulator, slowing the loss of soil heat to the cold atmosphere during the night and early morning (Zhang et al., 2013). By 2 PM, the difference becomes even more pronounced. Treatment T₁₈ was 2.35 °C warmer at 5 cm than the control. This is largely attributed to biochar's low albedo; the black color of the biochar particles increases the absorption of incoming solar radiation, effectively "heating up" the surface layer (Zhang et al., 2013). This warming is particularly beneficial in early spring, as it can trigger microbial P-solubilization and N-mineralization earlier in the season, giving the wheat crop a head start in biomass accumulation (Xiong et al.,

2020). As the season progresses and the sun's intensity increases, the soil's thermal profile becomes increasingly critical for preventing heat stress in the root zone. By harvest, afternoon temperatures at 5cm in the biochar-rich plots (T_{17} , T_{18}) exceeded 40 °C, nearly 7 °C higher than the control (Table 3). While these peak temperatures seem high, it is important to observe the depth gradient. At 15 cm, the harvest temperature in T_{18} at 2 PM was 37.37 °C, whereas at 30 DAS it was only 11.54 °C. The insulating properties of biochar create a significant thermal lag between the surface and the deeper root zones (Zhang et al., 2013). Furthermore, the higher Water Holding Capacity of the bio-amended soils (as seen in Table 2) plays a vital role in thermal stabilization. Water has a high specific heat capacity ($4.18 \text{ J g}^{-1}\text{K}^{-1}$); therefore, soils with 60% moisture content (T_{17} , T_{18}) require much more energy to change temperature than soils with 51% moisture (Control). This moisture-mediated thermal buffering "dampens" the diurnal temperature amplitude, protecting the core root mass from rapid, shocking temperature swings (Zhang et al., 2013). The synergistic combination thus ensures that while the surface might be warmer (benefitting early growth), the deeper rhizosphere remains a more stable environment for root function and microbial persistence (Ding et al., 2019).

3.6 Soil pH

The production of wheat in arid and semi-arid regions faces challenges due to alkaline soils (pH 7.5 to 9.0), which limit nutrient availability, especially phosphorus (Çiğ et al., 2021). Data from the present study (Table 4) reported that, Integrated applications of biochar and biological inoculants can reduce soil pH, enhancing nutrient acquisition. The control treatment shows a static pH of 7.88. However, using PGPR (*P. fluorescens* or *B. megaterium*) reduces pH to approximately 7.50-7.51, primarily due to organic acids secreted by bacteria (Pan et al., 2023). Biochar application further lowers pH to 7.21 at 3.0 t ha^{-1} and 7.09 at 6.0 t ha^{-1} , functioning to neutralize alkalinity (Yao et al., 2025; Liu XiangHong et al., 2012). The optimal results occur with a combination treatment (6.0 t ha^{-1} biochar + VAM + *B. megaterium*), reducing pH to 6.86, which enhances nutrient availability through a multi-layered acidification mechanism involving bacterial and fungal interactions (Meng et al., 2025).

3.7 Soil Electrical Conductivity

Soil Electrical Conductivity (EC) serves as an indicator of dissolved salts in soil. While some ions are needed for plant growth, high EC levels can cause osmotic stress, impact water uptake and potentially leading to ion toxicity (Ultanbekova et al., 2025). In the present study, the control (T_1) EC remained stable at 0.76 dSm^{-1} , favourable for wheat growth, while treatments with biochar and VAM consistently resulted in lower EC levels, reaching 0.65 dSm^{-1} at harvest (Table 4). Biochar functions as an ion sink, helping to reduce EC by adsorbing excess salts, particularly sodium, and improving the K^+/Na^+ ratio (Guo et al., 2020; Malik et al., 2024). Additionally, microbial interactions, such as those with PGPR and VAM fungi, promote ion consumption, further lowering EC.

These treatments enhance nutrient-use efficiency and contribute to a healthier soil environment, minimizing risks associated with high ion concentrations and supporting better wheat development ((Meng et al., 2025; Zou et al., 2024).

3.8 Soil Organic Carbon

Soil Organic Carbon (SOC) is vital for soil health, influencing water-holding capacity and nutrient exchange. Intensified farming has led to significant SOC loss, with many soils losing up to 60% of their original content (Kumar & Bisht, 2025). Findings from the current study (Table 4) revealed that biochar and microbial interactions can effectively reverse this trend. The control (T_1) shows a concerning decline in SOC from 0.31% to 0.28% by harvest. In stark contrast, all biochar-containing treatments (T_4 - T_9 and T_{13} - T_{18}) show a steady and significant accumulation of SOC. Treatments containing biochar, VAM and *Bacillus megaterium* (T_{18}) showed substantial SOC accumulation 0.41%, with a 46% increase in SOC content compared to control that exhibited a decline. This enhancement is attributed to three mechanisms: the stable carbon input from biochar (Çiğ et al., 2021), the "negative priming effect" that protects indigenous organic matter (Habibullah & Sahib Alam, 2018), and the biological contribution from VAM fungi, which increase carbon storage through stable micro-aggregates (Mason et al., 2025).

Table 2 Effect of Biochar, VAM and PGPR on Bulk Density (Mg m⁻³), Particle Density (Mg m⁻³), Porosity (%) and Water holding capacity of soil under Wheat

Treatments				Bulk Density (Mg m ⁻³)			Particle Density (Mg m ⁻³)			Porosity (%)			Water Holding Capacity (%)		
Biochar (tons ha ⁻¹)	VAM	PGPR		30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest
T ₁	0	NT	NT	1.41	1.39	1.37	2.67	2.67	2.68	47.32	48.10	48.81	51.21	51.22	51.23
T ₂	0	NT	<i>Pseudomonas fluorescens</i>	1.37	1.35	1.34	2.68	2.68	2.67	48.93	49.62	50.06	52.61	53.88	53.90
T ₃	0	NT	<i>Bacillus megaterium</i>	1.37	1.35	1.34	2.67	2.66	2.68	48.82	49.31	50.07	52.75	54.04	54.05
T ₄	3	NT	NT	1.33	1.31	1.30	2.68	2.69	2.67	50.35	51.26	51.44	53.75	55.06	55.07
T ₅	3	NT	<i>Pseudomonas fluorescens</i>	1.30	1.28	1.27	2.68	2.68	2.68	51.61	52.38	52.74	55.53	56.88	56.90
T ₆	3	NT	<i>Bacillus megaterium</i>	1.29	1.28	1.26	2.68	2.68	2.66	51.78	52.37	52.55	54.80	56.13	56.14
T ₇	6	NT	NT	1.25	1.23	1.22	2.67	2.68	2.68	53.28	54.08	54.43	56.47	57.84	57.85
T ₈	6	NT	<i>Pseudomonas fluorescens</i>	1.19	1.17	1.16	2.68	2.67	2.68	55.71	56.25	56.84	60.19	61.65	61.67
T ₉	6	NT	<i>Bacillus megaterium</i>	1.18	1.17	1.16	2.66	2.68	2.66	55.59	56.41	56.57	59.22	60.66	60.67
T ₁₀	0	T	NT	1.39	1.37	1.36	2.68	2.68	2.68	48.05	48.69	49.32	51.78	53.04	53.05
T ₁₁	0	T	<i>Pseudomonas fluorescens</i>	1.34	1.32	1.31	2.68	2.68	2.67	50.05	50.72	51.03	53.08	54.37	54.38
T ₁₂	0	T	<i>Bacillus megaterium</i>	1.34	1.32	1.31	2.67	2.66	2.68	49.81	50.24	51.04	52.82	54.10	54.11
T ₁₃	3	T	NT	1.32	1.31	1.29	2.66	2.68	2.67	50.25	51.34	51.58	53.78	55.09	55.10
T ₁₄	3	T	<i>Pseudomonas fluorescens</i>	1.29	1.27	1.26	2.68	2.67	2.68	52.04	52.51	53.05	55.09	56.43	56.44
T ₁₅	3	T	<i>Bacillus megaterium</i>	1.28	1.27	1.25	2.67	2.68	2.67	51.97	52.67	53.09	56.19	57.55	57.57
T ₁₆	6	T	NT	1.20	1.18	1.17	2.69	2.67	2.69	55.56	55.82	56.60	59.43	60.87	60.89
T ₁₇	6	T	<i>Pseudomonas fluorescens</i>	1.18	1.16	1.15	2.68	2.68	2.68	56.19	56.67	57.21	60.59	62.06	62.08
T ₁₈	6	T	<i>Bacillus megaterium</i>	1.17	1.16	1.15	2.68	2.67	2.68	56.12	56.66	57.21	59.93	61.39	61.40
CD				0.005	0.005	0.006	N/A	N/A	N/A	0.818	0.817	0.822	0.133	0.972	0.976
SE(m)				0.002	0.002	0.002	0.016	0.016	0.016	0.283	0.283	0.285	0.046	0.337	0.338

*T-Treated, NT-Not Treated, DAS-Days after sowing

Table 3. Effect of Biochar, VAM and PGPR on Soil Temperature (°C) at various depths of under Wheat at 30 DAS, 60 DAS and harvest stage

Treatments				30 DAS						60 DAS						Harvest					
Biochar (tons ha ⁻¹)	VAM	PGPR		Morning (6AM)			Afternoon (2 PM)			Morning (6AM)			Afternoon (2 PM)			Morning (6AM)			Afternoon (2 PM)		
				5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm
T ₁	0	NT	NT	7.04	8.55	10.35	11.39	10.19	9.57	19.63	20.21	21.5	22.34	21.49	20.19	30.75	31.93	33.00	33.40	32.42	30.99
T ₂	0	NT	<i>Pseudomonas fluorescens</i>	7.24	9.03	10.64	12.03	10.75	10.10	20.73	21.33	22.69	23.58	22.69	21.31	32.46	33.7	34.83	35.25	34.22	32.71
T ₃	0	NT	<i>Bacillus megaterium</i>	7.26	8.81	10.67	11.74	10.5	9.86	20.23	20.82	22.15	23.02	22.15	20.8	31.68	32.9	34.00	34.41	33.4	31.93
T ₄	3	NT	NT	7.39	9.22	10.87	12.28	10.98	10.31	21.16	21.77	23.16	24.07	23.16	21.75	33.13	34.4	35.55	35.99	34.93	33.39
T ₅	3	NT	<i>Pseudomonas fluorescens</i>	7.64	9.51	11.23	12.66	11.32	10.64	21.82	22.46	23.9	24.83	23.89	22.44	34.18	35.49	36.68	37.12	36.04	34.45
T ₆	3	NT	<i>Bacillus</i>	7.54	9.52	11.08	12.68	11.34	10.65	21.85	22.48	23.92	24.86	23.92	22.46	34.21	35.53	36.71	37.16	36.07	34.48

Treatments				30 DAS						60 DAS						Harvest					
Biochar (tons ha ⁻¹)	VAM	PGPR)		Morning (6AM)			Afternoon (2 PM)			Morning (6AM)			Afternoon (2 PM)			Morning (6AM)			Afternoon (2 PM)		
				5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm	5 cm	10 cm	15 cm
			<i>megaterium</i>																		
T ₇	6	NT	NT	7.77	9.69	11.42	12.91	11.55	10.85	22.25	22.9	24.36	25.31	24.36	22.88	34.84	36.18	37.39	37.84	36.74	35.12
T ₈	6	NT	<i>Pseudomonas fluorescens</i>	8.28	10.09	12.17	13.44	12.02	11.29	23.16	23.84	25.36	26.36	25.36	23.82	36.27	37.67	38.93	39.40	38.25	36.56
T ₉	6	NT	<i>Bacillus megaterium</i>	8.15	10.17	11.97	13.55	12.11	11.38	23.35	24.03	25.56	26.56	25.56	24.00	36.56	37.96	39.23	39.71	38.55	36.85
T ₁₀	0	T	NT	7.12	8.82	10.47	11.76	10.51	9.88	20.26	20.85	22.18	23.05	22.18	20.83	31.72	32.94	34.04	34.46	33.45	31.97
T ₁₁	0	T	<i>Pseudomonas fluorescens</i>	7.3	9.04	10.73	12.04	10.77	10.11	20.75	21.35	22.72	23.61	22.71	21.33	32.49	33.74	34.87	35.29	34.26	32.75
T ₁₂	0	T	<i>Bacillus megaterium</i>	7.27	9.06	10.68	12.07	10.8	10.14	20.8	21.41	22.78	23.67	22.78	21.39	32.58	33.83	34.96	35.39	34.35	32.84
T ₁₃	3	T	NT	7.40	9.15	10.87	12.19	10.9	10.24	21.01	21.62	23.01	23.91	23.00	21.6	32.90	34.17	35.31	35.74	34.70	33.16
T ₁₄	3	T	<i>Pseudomonas fluorescens</i>	7.58	9.56	11.14	12.74	11.39	10.70	21.95	22.59	24.04	24.98	24.03	22.57	34.38	35.70	36.89	37.34	36.25	34.65
T ₁₅	3	T	<i>Bacillus megaterium</i>	7.73	9.45	11.36	12.59	11.26	10.58	21.70	22.33	23.76	24.69	23.76	22.31	33.98	35.29	36.47	36.91	35.83	34.25
T ₁₆	6	T	NT	8.17	10.17	12.02	13.55	12.12	11.38	23.35	24.03	25.57	26.57	25.57	24.01	36.57	37.98	39.25	39.73	38.56	36.86
T ₁₇	6	T	<i>Pseudomonas fluorescens</i>	8.24	10.28	12.12	13.69	12.24	11.50	23.59	24.28	25.83	26.84	25.82	24.25	36.94	38.36	39.64	40.12	38.95	37.23
T ₁₈	6	T	<i>Bacillus megaterium</i>	8.33	10.31	12.25	13.74	12.29	11.54	23.68	24.37	25.93	26.94	25.92	24.34	37.08	38.5	39.79	40.27	39.1	37.37
		CD		0.013	0.004	0.011	0.007	0.017	0.012	0.036	0.017	0.038	0.024	0.020	0.027	0.049	0.025	0.039	0.065	0.036	0.028
		SE(m)		0.004	0.001	0.004	0.002	0.006	0.004	0.013	0.006	0.013	0.008	0.007	0.009	0.017	0.009	0.014	0.023	0.013	0.010

*T-Treated, NT-Not Treated, DAS-Days after sowing

Table 4. Effect of Biochar, VAM and PGPR on Soil pH, EC (dSm⁻¹) and Organic Carbon content (%) under Wheat

Treatments				Soil pH			Soil EC (dSm ⁻¹)			Soil Organic Carbon Content (%)		
Biochar (tons ha ⁻¹)	VAM	PGPR)		30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest
T ₁	0	NT	NT	7.88	7.88	7.88	0.76	0.76	0.76	0.31	0.30	0.28
T ₂	0	NT	<i>Pseudomonas fluorescens</i>	7.70	7.52	7.51	0.75	0.72	0.71	0.32	0.34	0.36
T ₃	0	NT	<i>Bacillus megaterium</i>	7.69	7.51	7.50	0.75	0.72	0.71	0.32	0.34	0.36
T ₄	3	NT	NT	7.39	7.22	7.21	0.72	0.69	0.68	0.33	0.35	0.37
T ₅	3	NT	<i>Pseudomonas fluorescens</i>	7.21	7.04	7.02	0.70	0.68	0.66	0.34	0.36	0.38
T ₆	3	NT	<i>Bacillus megaterium</i>	7.20	7.03	7.02	0.70	0.67	0.66	0.34	0.36	0.39
T ₇	6	NT	NT	7.27	7.10	7.09	0.71	0.68	0.67	0.35	0.37	0.39
T ₈	6	NT	<i>Pseudomonas fluorescens</i>	7.14	6.97	6.96	0.70	0.67	0.66	0.36	0.38	0.41
T ₉	6	NT	<i>Bacillus megaterium</i>	7.15	6.98	6.97	0.70	0.67	0.66	0.36	0.39	0.41

Treatments				Soil pH			Soil EC (dSm ⁻¹)			Soil Organic Carbon Content (%)		
Biochar (tons ha ⁻¹)	VAM	PGPR)		30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest
T ₁₀	0	T	NT	7.66	7.48	7.46	0.75	0.72	0.70	0.31	0.33	0.35
T ₁₁	0	T	<i>Pseudomonas fluorescens</i>	7.59	7.41	7.40	0.74	0.71	0.70	0.33	0.35	0.37
T ₁₂	0	T	<i>Bacillus megaterium</i>	7.60	7.42	7.41	0.74	0.71	0.70	0.33	0.35	0.37
T ₁₃	3	T	NT	7.12	6.95	6.94	0.69	0.67	0.65	0.33	0.35	0.37
T ₁₄	3	T	<i>Pseudomonas fluorescens</i>	7.08	6.91	6.90	0.69	0.66	0.65	0.34	0.36	0.38
T ₁₅	3	T	<i>Bacillus megaterium</i>	7.07	6.91	6.90	0.69	0.66	0.65	0.34	0.36	0.38
T ₁₆	6	T	NT	7.10	6.93	6.92	0.69	0.67	0.65	0.37	0.39	0.41
T ₁₇	6	T	<i>Pseudomonas fluorescens</i>	7.05	6.88	6.87	0.69	0.66	0.65	0.37	0.39	0.41
T ₁₈	6	T	<i>Bacillus megaterium</i>	7.04	6.87	6.86	0.69	0.66	0.65	0.37	0.39	0.41
CD				0.024	0.088	0.078	0.005	0.016	0.015	0.005	0.015	0.009
SE(m)				0.008	0.030	0.027	0.002	0.006	0.005	0.002	0.005	0.003

*T-Treated, NT-Not Treated, DAS-Days after sowing

Table 5. Effect of Biochar, VAM and PGPR on Soil Available Nitrogen, Phosphorus and Potassium (kg ha⁻¹) under Wheat

Treatments				Soil available Nitrogen (kg ha ⁻¹)			Soil available Phosphorus (kg ha ⁻¹)			Soil available Potassium (kg ha ⁻¹)		
Biochar (tons ha ⁻¹)	VAM	PGPR)		30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest	30 DAS	60 DAS	Harvest
T ₁	0	NT	NT	239.95	234.73	224.47	19.24	18.42	17.42	224.22	214.53	198.57
T ₂	0	NT	<i>Pseudomonas fluorescens</i>	286.05	272.87	255.22	22.93	21.87	20.46	228.44	218.97	202.34
T ₃	0	NT	<i>Bacillus megaterium</i>	285.75	272.58	254.95	22.91	21.85	20.44	228.20	218.73	202.13
T ₄	3	NT	NT	298.57	284.82	266.39	23.93	22.83	21.36	238.44	228.55	211.20
T ₅	3	NT	<i>Pseudomonas fluorescens</i>	301.61	287.71	269.10	24.18	23.06	21.57	240.86	230.87	213.34
T ₆	3	NT	<i>Bacillus megaterium</i>	305.05	291.00	272.17	24.45	23.33	21.82	243.61	233.51	215.78
T ₇	6	NT	NT	315.70	301.15	281.67	25.31	24.14	22.58	252.11	241.66	223.31
T ₈	6	NT	<i>Pseudomonas fluorescens</i>	327.32	312.24	292.04	26.24	25.03	23.41	261.39	250.55	231.53
T ₉	6	NT	<i>Bacillus megaterium</i>	328.87	313.72	293.43	26.36	25.15	23.52	262.64	251.74	232.63
T ₁₀	0	T	NT	282.83	269.80	252.34	22.67	21.63	20.23	225.86	216.50	200.06
T ₁₁	0	T	<i>Pseudomonas fluorescens</i>	298.28	284.54	266.13	23.91	22.81	21.33	238.21	228.33	210.99
T ₁₂	0	T	<i>Bacillus megaterium</i>	293.72	280.19	262.07	23.55	22.46	21.01	234.57	224.84	207.77
T ₁₃	3	T	NT	296.59	282.92	264.62	23.78	22.68	21.21	236.85	227.03	209.79
T ₁₄	3	T	<i>Pseudomonas fluorescens</i>	309.61	295.35	276.24	24.82	23.68	22.14	247.26	237.00	219.01
T ₁₅	3	T	<i>Bacillus megaterium</i>	304.05	290.04	271.28	24.37	23.25	21.75	242.81	232.74	215.07
T ₁₆	6	T	NT	325.20	310.21	290.15	26.07	24.87	23.26	259.70	248.93	230.03
T ₁₇	6	T	<i>Pseudomonas fluorescens</i>	328.48	313.35	293.08	26.33	25.12	23.49	262.33	251.45	232.36
T ₁₈	6	T	<i>Bacillus megaterium</i>	333.47	318.10	297.53	26.73	25.50	23.85	266.31	255.26	235.88
CD				0.620	1.750	1.315	0.056	0.345	0.254	0.253	1.422	1.676
SE(m)				0.215	0.606	0.456	0.020	0.120	0.088	0.088	0.493	0.581

*T-Treated, NT-Not Treated, DAS-Days after sowing

Additionally, PGPR inoculants promote root growth, leading to increased rhizodeposition, further contributing to carbon sequestration (Zou et al., 2024). The synergistic effects of biochar and microbial activity present a promising approach for sustainable agriculture and carbon credit generation in wheat rotations.

3.9 Soil Available Nitrogen

The data in Table 5 demonstrates that the combination of biochar and biological agents has been shown to significantly enhance nitrogen availability, with T₁₈ treatment resulting in a 32.5% increase in nitrogen by harvest compared to control. The control (T₁) shows a decline in available N from 239.95 to 224.47 kg ha⁻¹ by harvest. Across all treatments, the maximum N availability is observed in T₁₈ (6.0 tons ha⁻¹ biochar + VAM + *B. megaterium*), which peaks at 333.47 kg ha⁻¹ at 30 DAS and maintains 297.53 kg ha⁻¹ at harvest. Biochar's high cation exchange capacity allows it to retain ammonium ions, preventing nitrogen loss during rainfall. Additionally, bacteria like *Pseudomonas fluorescens* and *Bacillus megaterium* aid in nitrogen mineralization (Çiğ et al., 2021; Guo et al., 2025). Despite a general decline in nitrogen availability from 30 days after sowing (DAS) to harvest, treated plots maintain a higher nitrogen floor, reducing the necessity for chemical fertilizers and promoting grain yield and protein content (Habibullah & Sahib Alam, 2018).

3.10 Soil Available Phosphorus

Phosphorus (P) is referred to as the "unavailable nutrient" due to its predominance in insoluble complexes in soil, particularly calcium phosphates in alkaline conditions, which hinder plant absorption (Ultanbekova et al., 2025). The biochar-VAM-PGPR complex effectively extracts phosphorus, as shown by the treatment T₁₈, which increased available P from 19.24 to 23.85 kg ha⁻¹—a 37% improvement over the control (Table 5). This process involves a two-step mechanism: the phosphate-solubilizing bacteria (*Bacillus megaterium* and *Pseudomonas fluorescens*) solubilize P into forms like H₂PO₄⁻ (Pan et al., 2023) and VAM fungi transport it to plant roots (Meng et al., 2025). Biochar aids this process by supplying soluble phosphorus and enhancing VAM growth while preventing P from forming insoluble precipitates by adsorbing calcium ions (Hammer et al., 2015).

3.11 Soil Available Potassium

Potassium (K) is crucial for wheat's enzyme activation, photosynthesis, and turgor pressure (Chen et al., 2023). An integrated treatment significantly improves available K, with control plots showing 198.57 kg ha⁻¹ compared to 235.88 kg ha⁻¹ in the most effective treatment (T₁₈), an increase of 18.8% (Table 5). Biochar contributes substantially to available K by concentrating K during pyrolysis, making it a slow-release fertilizer (Hammer et al., 2015). Potassium-solubilizing bacteria like *Bacillus megaterium* and *Pseudomonas fluorescens* release K from soil minerals, while VAM fungi enhance K uptake by up to 2.2-fold (Meng et al., 2025).

4. Conclusion

The combined use of biochar and microbial inoculants enhances the wheat rhizosphere by offering a sustainable alternative to chemical fertilizers. This method fosters a robust ecosystem, advancing climate-resilient agriculture. Agronomists are encouraged to refine biochar-microbe formulations tailored to specific soils and wheat types. Nutrient-enriched biochars could significantly support soil fertility, carbon sequestration, and global food security amid climate challenges. Emphasizing integrated biological synergy will help transition agricultural practices towards sustainability.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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