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ORIGINAL PAPER

Biochar from Agricultural Waste Enhances Rhizosphere Microbial Activity Over Bulk Soil

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Abstract

Understanding how biochar influences microbial processes in the rhizosphere compared to bulk soil remains limited. This study assessed the effects of biochars produced from different agricultural wastes at two pyrolysis temperatures on microbial properties in loamy-clay soil. Biochars were derived from vineyard prunings (VB), tomatoes (TB), cloves (CB), and bananas (BB) at 300 °C and 500 °C. A Completely Randomized Design (CRD) with five replications and nine treatments, including a control, was used. Soil samples were analyzed at harvest (8 weeks) for pH, electrical conductivity (EC), and microbial activities. Biochar significantly enhanced microbial activity, with stronger effects in the rhizosphere than in bulk soil. In the first growing period, biochar increased pH (by up to 1.2 units), EC (by 35%), alkaline phosphatase (by 42%), β-glucosidase (by 51%), denitrification (by 28%), and bacterial counts (by 63%) in the rhizosphere compared to bulk soil. In the second growing period, pH, urease, β-glucosidase, and bacterial counts remained higher in the rhizosphere, although denitrification declined by 19%. Among treatments, CB300, CB500, and BB500 significantly increased dehydrogenase activity (by 47%), while TB300 and CB300 promoted bacterial growth (by 58%) compared to the control. Biochar had a stronger impact on microbial activity in the rhizosphere than in bulk soil, with effects influenced by pyrolysis temperature and feedstock type. These findings highlight biochar's potential to enhance soil microbial functions, providing insights for optimizing its application to improve soil health and crop productivity.

Highlights

- Biochar enhanced microbial activity more strongly in the rhizosphere than in bulk soil, particularly stimulating soil enzyme activities and bacterial abundance.
- Low-temperature biochars (300 °C) provided labile carbon that boosted urease and β-glucosidase activities, while high-temperature biochars (500 °C) mainly improved pH and EC, indirectly affecting microbial functions.
- Biochar-mediated improvements in microbial activity were positively correlated with plant growth parameters (root diameter, leaf number, biomass, chlorophyll).
- Feedstock type and pyrolysis temperature jointly shaped soil microbial processes and nitrogen cycling, highlighting the potential of biochar as a climate-smart soil amendment.

Keywords Biochar · Rhizosphere · Microbial activity · Pyrolysis temperature · Soil enzymes

1 Introduction

The rhizosphere is a highly dynamic soil zone directly influenced by plant roots through the release of exudates, stimulation of microbial activity, and acceleration of nutrient cycling, making it central to plant growth and soil health (Bulgarelli et al. 2013; Kuzyakov and Razavi 2019). Compared with bulk soil, the rhizosphere is enriched in microbial diversity and enzymatic activities, creating unique biochemical conditions (Bell et al. 2013). The spatial

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gradient of enzyme activity from the root surface to bulk soil is often used to define the rhizosphere, as microbial processes tend to stabilize with increasing distance from the roots (Yan et al. 2022). Soil enzymes are widely recognized as sensitive indicators of soil quality because they mediate organic matter decomposition and nutrient availability (Raiesi and Beheshti 2014; Mendes et al. 2019). For example, β-glucosidase contributes to carbon cycling by breaking down cellulose, phosphatases facilitate phosphorus release from organic compounds, dehydrogenase reflects microbial respiratory activity, and urease regulates nitrogen availability through urea hydrolysis (Wu et al. 2022; Sun et al. 2024). These activities are closely linked to microbial communities, which also drive nitrogen transformations via nitrification and denitrification processes (Borchard et al. 2019; Zhang et al. 2025). Functional genes such as *amoA* (ammonia oxidation), *nirK* and *nirS* (nitrite reduction), and *nosZ* (N_2O reduction) play critical roles in controlling nitrogen turnover rates and greenhouse gas emissions (Francis et al. 2007; Liu et al. 2022).

Biochar, a carbon-rich product of biomass pyrolysis, has emerged as a multifunctional soil amendment that can improve soil aeration, pH, nutrient retention, and microbial habitat quality (Lehmann et al. 2021; Zhong et al. 2022). Its influence on microbial functions depends strongly on pyrolysis temperature and feedstock type, which together determine biochar's porosity, pH, and labile carbon content (Amoah-Antwi et al. 2020; Zhang et al. 2022). Low-temperature biochars (< 400 °C) generally retain more labile carbon, serving as an energy source for microbes, while high-temperature biochars (> 500 °C) are more stable, influencing soil chemistry mainly through alkalinity and cation exchange properties. Feedstocks with high mineral content, such as manures or crop residues, typically produce more alkaline biochars than woody materials (Lehmann 2007). Biochar has been shown to affect microbial communities and functional genes associated with nitrification and denitrification, sometimes enhancing ammonia-oxidizing bacteria abundance and altering nitrogen cycling pathways (Guo et al. 2022; Sun et al. 2024). Earlier studies have explored spatial heterogeneity in biochar effects by comparing rhizosphere and bulk soils (e.g., Chan et al. 2007; Sohi et al. 2010), showing that biochar can differentially influence microbial and biochemical processes across these zones. However, these works largely focused on single-season or short-term responses, leaving a gap in understanding how such zone-specific effects may vary across different environmental conditions or growth periods. Moreover, they did not systematically examine how pyrolysis temperature and feedstock type interact with seasonal variation to influence microbial activity and nutrient cycling in both rhizosphere and bulk soils.

To address these gaps, our study uses a dual-period greenhouse experiment spanning contrasting climatic conditions (spring–summer vs. autumn–winter) to evaluate how biochar type and pyrolysis temperature affect microbial enzyme activities, nitrogen transformation processes, and soil chemical properties in rhizosphere and bulk soils. By incorporating two growth periods with differing temperature and humidity regimes, we provide insight into the temporal stability or variability of biochar's effects, a dimension absents from most prior rhizosphere–bulk soil comparisons. Specifically, this work addresses two research questions: How do different biochar types and pyrolysis temperatures influence microbial enzyme activities and bacterial abundance in the rhizosphere compared with bulk soil? How do these biochar treatments affect soil chemical properties such as pH and electrical conductivity under contrasting growth periods? We hypothesize that: H_1: Biochar application will enhance microbial enzyme activities and bacterial abundance more in the temperature rhizosphere than in bulk soil due to higher microbial biomass and root-derived carbon inputs. H_2: Low-biochars (300 °C) will stimulate microbial activity more strongly by providing labile carbon, whereas high-temperature biochars (500 °C) will primarily influence soil pH, electrical conductivity, and nitrogen transformation pathways. H_3: Soil enzyme activities (urease, alkaline phosphatase, and β-glucosidase) in the rhizosphere will be positively correlated with plant growth parameters (root diameter, leaf number, biomass), reflecting the role of microbial nutrient cycling in supporting plant performance. By evaluating enzyme activities (urease, β-glucosidase, dehydrogenase), nitrogen transformation rates (nitrification, denitrification), and soil chemical parameters (pH, electrical conductivity) over two distinct growth periods, this study advances understanding of the mechanisms by which biochar influences rhizosphere and bulk soil processes. The findings will inform targeted biochar use for improving soil fertility and enhancing sustainable nutrient management strategies in agroecosystems.

2 Materials and Methods

2.1 Greenhouse Conditions

The study was conducted as a pot experiment in a controlled greenhouse at Akdeniz University, Faculty of Agriculture, Antalya, Türkiye. The first growing period lasted from April to June 2019, while the second took place from October to December 2019. The greenhouse was maintained under natural light with an average photoperiod of 14 h during the first period and 10 h during the second period. Average daily light intensity at canopy height ranged from 350 to 500 µmol

m^{-2} s^{-1} photosynthetically active radiation (PAR). Relative humidity was maintained at 55–65% in the first period and 60–70% in the second period, monitored with automated hygrometers. During the first experiment, drought-like conditions prevailed, with average greenhouse temperatures of 25.68 °C in April, 29.73 °C in May, and 35.22 °C in June. In contrast, the second experiment experienced milder conditions, with average temperatures of 29.29 °C in October, 22.33 °C in November, and 17.17 °C in December. Temperature and humidity were automatically regulated using a combination of ventilation fans, evaporative cooling pads, and heating units to minimize daily fluctuations.

2.2 Soil Collection and Characterization

The soil utilized in this pot experiment was collected from a depth of 0–30 cm from an Entisol in the Tehnelli series, located at the Faculty of Agriculture Research and Application Center in Aksu District, Antalya, Türkiye. Approximately 500 kg of this soil was transported to the Venlo-type modern greenhouse at Akdeniz University, Faculty of Agriculture. The soil was air-dried, sieved through a 4 mm mesh, and thoroughly mixed before being distributed into pots, each containing 10 kg of soil. Before the first cultivation period, the soil's properties were determined, and the initial soil analysis data were published by Abdul Aziz and Uz (2025). The soil has a loam-clay texture and is slightly alkaline in reaction (Soil Survey Staff, 1951). It is very calcareous (Kellog, 1952) and classified as salt-free (Evliya 1960). The analysis revealed that the soil is high in total nitrogen (Loue, 1968), low in organic matter (Thun et al., 1955), sufficient in exchangeable potassium and magnesium, and high in calcium (Pizer, 1967; Loue, 1968). Furthermore, the trial soil was classified as high in available manganese and copper, sufficient in iron (Lindsay and Norvell 1978), low in available phosphorus (Olsen and Sommers 1982), and very low in zinc (Lindsay and Norvell 1978) (see Table 1). These characteristics align with typical soils of the Antalya region, which are slightly alkaline, calcareous, and low in organic matter. Such soil properties are common in the Mediterranean climate of southern Türkiye, influencing nutrient availability and microbial activity.

Table 1 Physicochemical properties of the soil used in the study

Parameter	Results
pH	7.7
Lime (%)	29.8
Salt (%)	0.018
Sand (%)	10.88
Clay (%)	46.72
Silt (%)	42.4
Organic matter (%)	1.94
Total nitrogen %	0.12
Plant available P (kg P_2O_5 ha^{-1})	26.2
Plant available K (kg K_2O ha^{-1})	350
Exchangeable Ca (Kg CaO ha^{-1})	14.07
Exchangeable Mg (Kg MgO ha^{-1})	744
DTPA extractable Fe (mg kg^{-1})	7.95
DTPA extractable Mn (mg kg^{-1})	5.43
DTPA extractable Zn (mg kg^{-1})	0.12
DTPA extractable Cu (mg kg^{-1})	1.70
Urease activity (µg NH_4^+-N h^{-1})	24.31
Alkaline phosphatase activity (µg PNP h^{-1})	51.13
β-glucosidase activity (µg PNG h^{-1})	1.50
Dehydrogenase activity (µg TPF h^{-1})	0.53
Nitrification activity (µg NO_2^- -N h^{-1})	0.15
Denitrification activity (µg NO_2^- -N h^{-1})	0.10
Bacteria count (cfu g^{-1})	1.1×10^4

Abdul Aziz and *Uz* (2025)

2.3 Biochar Characteristics

In this study, we utilized four different feedstocks to produce biochar: vineyard prunings (*Vitis vinifera*, VB), tomatoes (*Solanum lycopersicum*, TB), cloves (*Dianthus caryophyllus*, CB), and bananas (*Musa* spp., BB). These feedstocks were chosen because they represent widely available agricultural and horticultural residues in the Mediterranean region, are often underutilized or disposed of as waste, and differ substantially in lignocellulosic composition, enabling a comparative assessment of how feedstock type influences biochar properties. The biochar was produced through slow pyrolysis at two temperatures—300 °C and 500 °C—for 12 h. The choice of these temperatures was based on their frequent use in biochar research to represent low- and high-temperature pyrolysis regimes: 300 °C to retain more volatile compounds and functional groups, and 500 °C to enhance aromatic carbon structures and stability (Lehmann and Joseph 2015). After production, the biochar was ground to a particle size of less than 1 mm using a sieve and stored in a sealed plastic container until use. No sterilization process was applied to the biochar, as the study aimed to mimic practical application conditions where biochar is incorporated directly into soil without prior microbial removal. Before planting lettuce seedlings, the biochar was thoroughly incorporated into the topsoil (0–20 cm) to ensure even distribution within the growth medium. A sample of each biochar was taken for characterization. Proximate analysis followed ASTM standards, measuring moisture content (ASTM D3173-03), volatile matter (ASTM D3175-07), and ash content (ASTM D3174-02). Fixed carbon content was calculated by difference (ASTM D3172-07a). The elemental composition was determined using a CHNS-932 LECO Elemental Analyzer. The pH and electrical conductivity (EC) of the biochar were measured using a 1:10 biochar-to-water ratio, following the method outlined by Jindo

et al. (2014). The key physical and chemical properties of the pyrolyzed biochar are presented in Table 2.

2.4 Experimental Design and Treatments

The experiment was conducted using a completely randomized design (CRD), comprising five replications across nine treatments: Control, VB300, VB500, TB300, TB500, CB300, CB500, BB300, and BB500. Due to the limited availability of biochar, the study was carried out in pots under greenhouse conditions instead of in an open field. A total of 45 pots were utilized, representing four types of agricultural waste, two pyrolysis temperatures, five replications, and five control treatments. Biochar was manually applied at a rate of 20 tons per hectare, which is equivalent to 80 g per pot, and it was thoroughly mixed with 10 kg of soil. Lettuce (*Lactuca sativa* var. *crispa*) was selected because it is a key leafy vegetable in the Mediterranean region, valued for its short growth cycle and high market demand. Its shallow roots and rapid nutrient uptake make it highly responsive to soil amendments. Sensitivity to soil property changes makes it an effective indicator for assessing biochar effects. Chemical fertilizers were applied according to standard agricultural practices concerning timing and dosage. One week before planting the seedlings, biochar was applied to the designated pots, mixed thoroughly with the soil, and watered. A week later, a single lettuce seedling was transplanted in each pot and irrigated. A balanced fertilizer with equal amounts of 20 g of NPK 18-18−18 was applied at the rate according to the soil's nutrient content to enhance lettuce leaf production. Every 10 days, 0.5 g of NPK were added to each pot through manual irrigation. Additionally, 1 g of calcium nitrate [$Ca(NO_3)_2$] was applied to each pot midway through the lettuce growing stage to ensure adequate nitrogen and calcium supply during peak vegetative growth. The experiment was repeated in the subsequent growing season using the same treated soils, and new lettuce seedlings were planted while keeping the same biochar and fertilizer application rates.

2.5 Soil sampling, Chemical and Biological Analysis

Soil samples were collected from each pot at the time of lettuce harvesting, specifically from a depth of 0–10 cm. These samples were placed in small, transparent polyethene bags. Air-dried soil samples were utilized for pH and electrical conductivity (EC) analysis, while fresh, moist soil samples were used to measure enzyme activity. Before experimenting, we analyzed the physical and chemical properties of the soil using several established methods. Soil pH and electrical conductivity (EC) were measured in samples taken at the end of the experiment using a 1:2.5 soil-to-water ratio, as recommended by Jackson (1967). Soil texture was assessed using the Bouyoucos hydrometer method (Bouyoucos 1951). The organic matter content was determined through the Modified Walkley-Black method (Black 1965). pH and electrical conductivity (EC) were measured with a 1:2.5 soil-to-water mixture, following the protocol established by Jackson (1967). Total nitrogen was quantified using the modified Kjeldahl method (Kacar 1995), while available phosphorus was

Table 2 Physical and chemical properties of Biochar used in the experiment

Parameter	Biochar							
	VB300	TB300	CB300	BB300	VB500	TB500	CB500	BB500
pH	8.13	8.92	9.82	8.72	9.19	9.67	9.56	10.01
EC (dS m^{-1})	0.288	4.405	4.915	3.41	0.564	4.45	6.23	3.64
Moisture (%)	3.34	4.89	4.8	3.79	4.03	3.01	4.15	2.72
Ash (%)	5.39	23.82	23.92	17.65	8.93	30.62	32.48	25.55
Volatile matter (%)	33.26	30.87	38.66	37.13	14.01	19.86	22.55	17.38
Fixed C (%)	58.01	40.42	33.05	41.42	73.03	46.51	40.82	54.35
% C	63.57	60.00	54.00	51.75	54.75	51.62	49.93	47.69
% H	4.526	3.765	4.547	3.962	2.617	2.280	1.879	1.922
% N	1.373	1.777	3.217	0.672	0.860	1.393	2.491	1.038
% S	-	0.310	0.127	0.141	-	0.438	0.279	0.174
P (%)	0.26	0.62	0.41	0.14	0.27	0.83	0.48	0.33
K (%)	1.17	5.23	6.23	3.56	1.19	6.52	8.80	3.88
Ca (%)	1.31	2.48	3.25	2.95	2.00	2.62	0.44	4.63
Mg (%)	0.17	0.48	0.33	0.91	0.25	0.52	0.60	1.26
Fe (g kg^{-1})	817.7	2378	608.9	288.6	1041	3067	1332	1755
Mn (g kg^{-1})	61.7	109.2	342.8	213.9	63.4	145.7	412	434.6
Zn (g kg^{-1})	32.8	123.8	182.3	47.3	78.9	163	158	48.1
Cu (g kg^{-1})	26.2	36.1	44.2	17.2	24.1	39.7	26.2	21.0

Abdul Aziz and Uz (2025)

analyzed using the Olsen method (Olsen and Sommers 1982). Exchangeable potassium (K), calcium (Ca), and magnesium (Mg) were measured using the 1 N ammonium acetate method at pH 7 (Kacar 1995). Additionally, the concentrations of iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) were evaluated using the DTPA extraction method (Lindsay and Norwell, 1978). For enzyme activity analysis, 1 gram of soil was weighed to determine urease activity following the procedure described by Hoffman and Teicher (1961). β-glucosidase and alkaline phosphatase activities were analysed following the modified procedure outlined by Eivazi and Tabatabai (1988) and Tabatabai and Bremner (1969), respectively. Dehydrogenase activity was analysed following the procedure by Ross (1971). Nitrification activity was determined by incubating soil with ammonium sulfate as the substrate under aerobic conditions. Sodium chlorate ($NaClO_3$) was added to inhibit nitrite oxidation to nitrate, allowing nitrite produced during ammonium oxidation to accumulate. The accumulated nitrite was extracted with KCl and quantified colorimetrically at 520 nm, following a modified method of Berg and Rosswall (1985). Denitrification activity was measured by incubating soil under waterlogged (anaerobic) conditions with potassium nitrate (KNO_3) as the electron acceptor. 2,4-dinitrophenol (DNP) was added to inhibit nitrite reductase, thereby preventing further reduction of nitrite to gaseous products (NO, N_2O, and N_2). The accumulated nitrite was extracted with KCl and determined colorimetrically at 520 nm, following a modified procedure of Abdelmagid and Tabatabai (1987). For microbial enumeration, soil samples were serially diluted (10^{-3}–10^{-6}), and aliquots were plated on molten nutrient agar. Following incubation, mesophilic aerobic bacterial colonies were counted, and the results were expressed as colony-forming units per gram of soil, accounting for dilution factors (Parkinson et al. 1971).

2.6 Statistical Analysis

All data collected were subjected to statistical analysis using the SPSS 27.0 software package (IBM Corp., Armonk, NY, USA). Before analysis of variance (ANOVA), the data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. When these assumptions were not met, appropriate data transformations (log or square-root) were applied to stabilize variances and normalize distributions. ANOVA was then performed to determine differences among treatments, and significant means were separated using the Duncan Multiple Range (DMR) test at a 5% level of significance.

3 Results

3.1 Biochar Enhances Soil pH and Electrical Conductivity with Stronger Rhizosphere Effects

Biochar application significantly influenced soil chemical properties, with notable differences between rhizosphere and bulk soils (Fig. 1; Table 3). Across both experimental periods, rhizosphere soils consistently exhibited higher pH values than bulk soils. During the first period, biochar-treated rhizosphere soils (VB300, TB300, CB300, BB300, VB500, CB500, and BB500) showed pH increases relative to bulk soil, with the largest change observed under VB300 (2.1%). In the second period, BB300 produced the highest pH increase in the rhizosphere (2.4%), while CB500 exhibited the greatest overall change (6.0%) (Table 3). Bulk soil pH remained largely unchanged during the first period, indicating that biochar effects were more pronounced in root-associated zones. Similarly, biochar significantly enhanced soil electrical conductivity (EC), reflecting increased soluble ion availability. Rhizosphere EC consistently exceeded bulk soil values across treatments and periods. In the first period, VB300, CB300, TB500, CB500, and BB500 increased rhizosphere EC by 42.6–137.8%, with TB500 exhibiting the highest increase. In the second period, VB300, TB300, BB300, and VB500 enhanced rhizosphere EC by 13.6–50.2%, while bulk soil EC showed significant increases under BB300 (63.8%), TB500 (75.3%), and BB500 (80.9%) (Table 3). Overall, biochar-driven changes in pH and EC were more pronounced in rhizosphere soils, highlighting the synergistic influence of root exudates and biochar on soil chemical properties.

3.2 β-glucosidase Was Significantly Enhanced in the Rhizosphere Soils

Biochar application significantly altered soil enzyme dynamics, with effects varying by enzyme, soil type, and period (Figs. 2 and 3; Tables 4 and 5). Urease activity, critical for nitrogen mineralization, was significantly higher in biochar-treated soils. In the first period, bulk soils showed increased activity under VB300, BB300, VB500, and CB500 (20.1–49.2%), while rhizosphere soils remained lower. By the second period, rhizosphere urease activity surpassed bulk soil across all treatments, with increases ranging from 45.5% (CB300) to 92.3% (BB300) (Table 4). Alkaline phosphatase activity was consistently elevated in rhizosphere soils during the first period, with increases from 39.3% (VB500) to 67.4% (control) (Table 5). Interestingly,

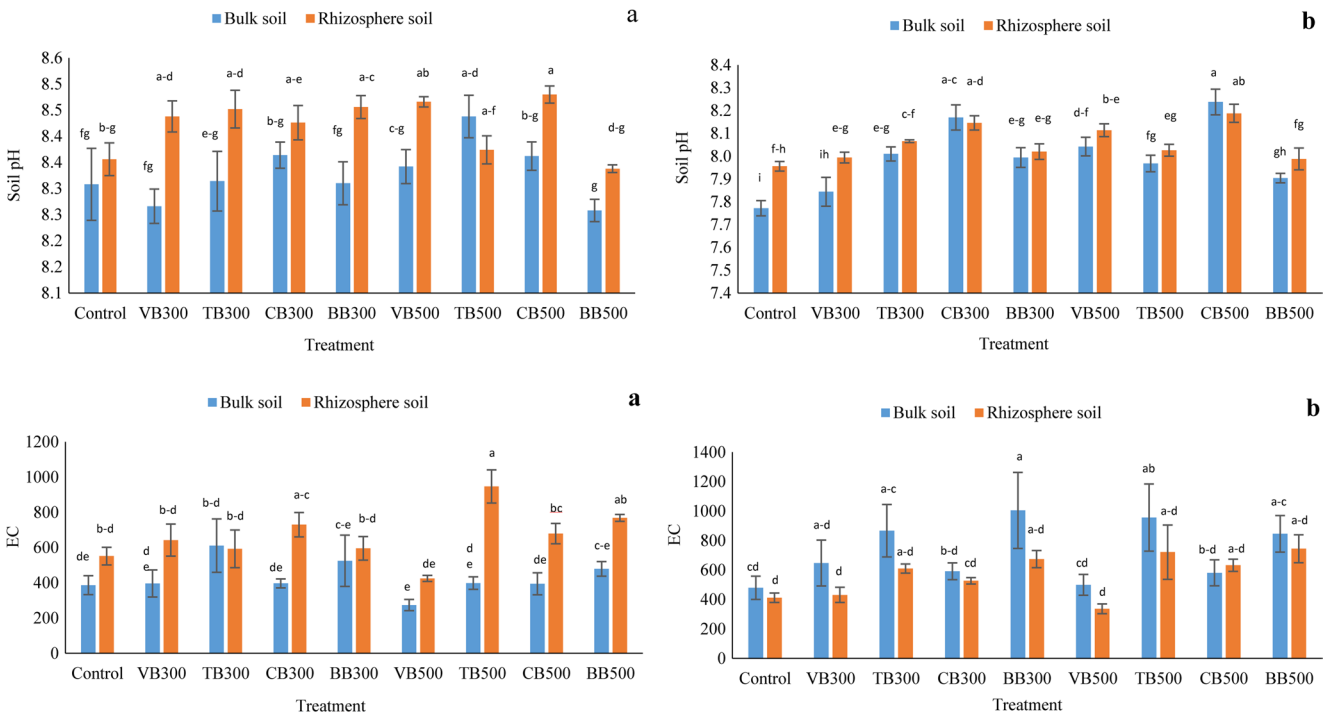


Fig. 1 Effects of biochar applications on bulk and rhizosphere soil pH and EC (dS m^{-1}) across two growing periods (A: first period, B: second period). Values represent means ± standard error ($n = 3$). Means with different letters are significantly different at the 5% level of probability. Treatments: VB = vineyard pruning biochar, TB = tomato biochar, CB = clove biochar, BB = banana biochar, each produced at 300 °C and 500 °C

Table 3 Effect of Biochar applications on bulk and rhizosphere soil pH and EC

Treatment	Soil pH				Soil EC			
	Growth period							
	I		II		I		II	
	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere
Control	8.31^{fg}	8.36^{b-g}	7.77^{i}	7.96^{f-h}	386.6^{de}	551.4^{b-d}	479.0^{cd}	411.6^{d}
VB300	8.27^{fg}	8.44^{a-d}	7.84^{ih}	7.99^{e-g}	396.0^{de}	642.2^{b-d}	647.6^{a-d}	431.2^{d}
TB300	8.31^{e-g}	8.45^{a-d}	8.01^{e-g}	8.07^{c-f}	611.0^{b-d}	592.6^{b-d}	866.8^{a-c}	610.2^{a-d}
CB300	8.36^{b-g}	8.43^{a-e}	8.17^{a-c}	8.15^{a-d}	396.6^{de}	729.4^{a-c}	591.4^{b-d}	526.6^{cd}
BB300	8.31^{fg}	8.46^{a-c}	8.00^{e-g}	8.02^{e-g}	524.8^{c-e}	595.4^{b-d}	1005.0^{a}	674.0^{a-d}
VB500	8.34^{c-g}	8.47^{ab}	8.04^{d-f}	8.11^{b-e}	273.6^{e}	424.6^{de}	499.2^{cd}	336.6^{d}
TB500	8.44^{a-d}	8.37^{a-f}	8.00^{fg}	8.03^{e-g}	398.2^{de}	946.8^{a}	955.8^{ab}	721.4^{a-d}
CB500	8.36^{b-g}	8.48^{a}	8.24^{a}	8.19^{ab}	394.2^{de}	679.2^{bc}	581.0^{b-d}	632.2^{a-d}
BB500	8.26^{g}	8.34^{d-g}	7.90^{gh}	7.99^{fg}	479.0^{c-e}	768.6^{ab}	845.8^{a-c}	744.6^{a-d}
ANOVA (One-way; LSD 5%)	4.176***		9.752***		4.890***		2.377**	

Means with different letters are significantly different at the 5% level of probability, (**) Significantly different at the 1% level of probability, (***) Significantly different at the 0.1% level of probability

in the second period, several bulk soil treatments (VB300, TB300, VB500, TB500, BB500) surpassed rhizosphere activity. β-Glucosidase activity was significantly enhanced in rhizosphere soils. In the first period, CB300 increased rhizosphere activity by 25.8% compared to bulk soil, while in the second period, rhizosphere activity surged by 21.4–26.3% (VB300 and CB500, respectively) (Table 4).

3.3 Biochar Modulates Microbial Metabolic Activity, Nitrogen Transformations, and Bacterial Abundance

Biochar significantly influenced dehydrogenase activity, nitrification, denitrification, and bacterial abundance, reflecting its broad impact on soil microbial function (Figs. 3 and 4, and 5; Tables 5 and 6, and 7). Dehydrogenase

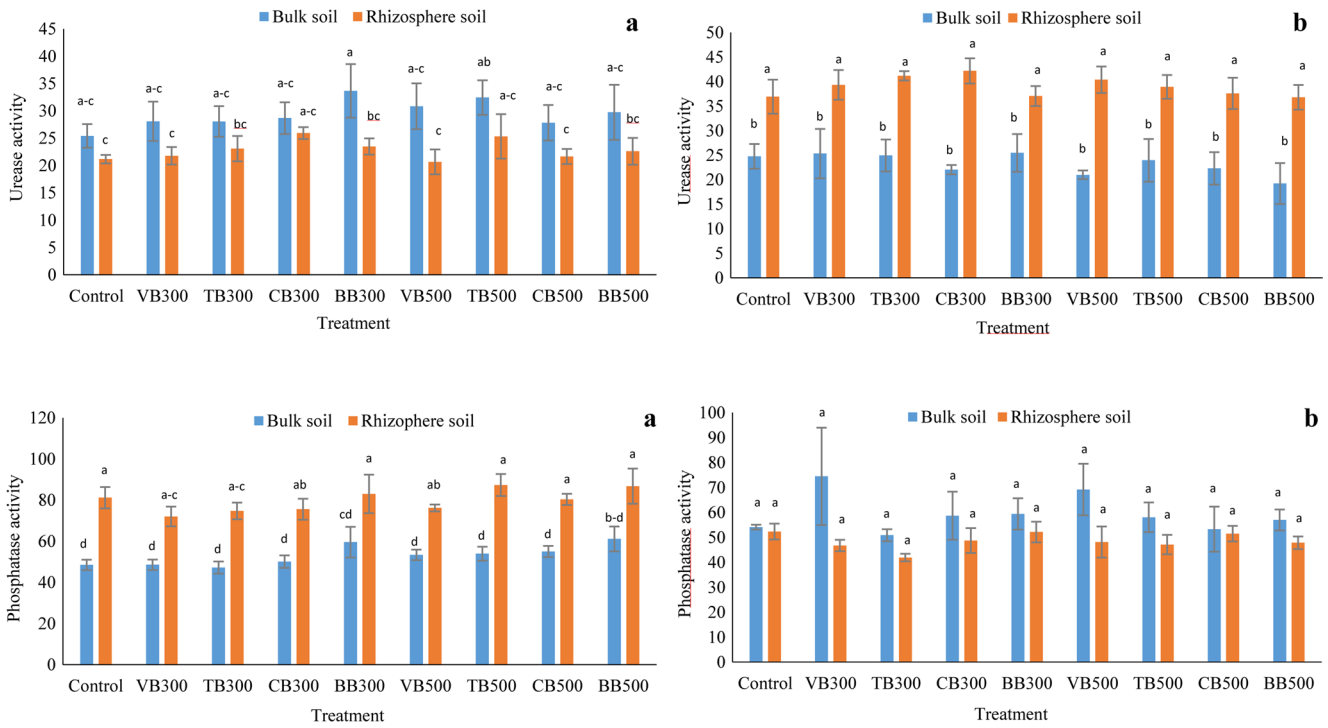


Fig. 2 Effects of biochar applications on bulk and rhizosphere soil urease (μg NH_4^+-N g^{-1} dry soil h^{-1}) and alkaline phosphatase (μg PNP g^{-1} dry soil h^{-1}) activities across two growing periods (A: first period, B: second period). Values represent means ± standard error (n = 3). Means with different letters are significantly different at the 5% level of probability. Treatments: VB = vineyard pruning biochar, TB = tomato biochar, CB = clove biochar, BB = banana biochar, each produced at 300 °C and 500 °C

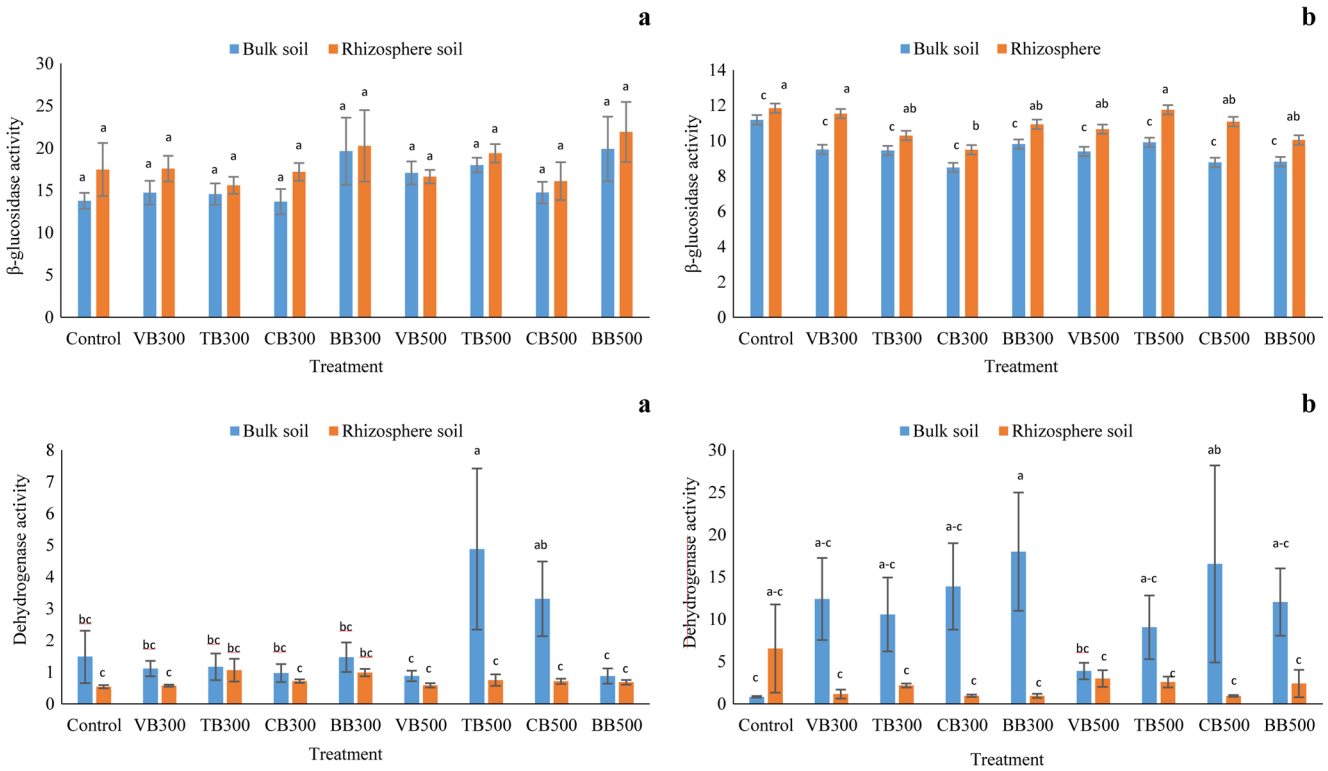


Fig. 3 Effects of biochar applications on bulk and rhizosphere soils β-glucosidase (μg PNG g^{-1} dry soil h^{-1}) and dehydrogenase (μg TPF g^{-1} dry soil) activities across two growing periods (A: first period, B: second period). Values represent means ± standard error (n = 3). Means with different letters are significantly different at the 5% level of probability. Treatments: VB = vineyard pruning biochar, TB = tomato biochar, CB = clove biochar, BB = banana biochar, each produced at 300 °C and 500 °C

Table 4 Effect of biochar applications on bulk and rhizosphere soil urease and β-glucosidase activities

Treatment	Urease activity (μg $NH4^+$-*N* g^{-1} dry soil h^{-1})				β-glucosidase activity (μg PNG g^{-1} dry soil h^{-1})			
	Growth period							
	I		II		I		II	
	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere
Control	25.42^{a-c}	21.17^{c}	24.75^{b}	36.93^{a}	13.76^{a}	17.46^{a}	11.18^{c}	11.84^{a}
VB300	28.09^{a-c}	21.78^{c}	25.33^{b}	39.32^{a}	14.72^{a}	17.57^{a}	9.50^{c}	11.53^{a}
TB300	28.06^{a-c}	23.08^{bc}	24.95^{b}	41.18^{a}	14.56^{a}	15.60^{a}	9.44^{c}	10.29^{ab}
CB300	28.67^{a-c}	25.93^{a-c}	22.06^{b}	42.17^{a}	13.66^{a}	17.19^{a}	8.48^{c}	9.49^{b}
BB300	33.65^{a}	23.48^{bc}	25.47^{b}	37.07^{a}	19.63^{a}	20.25^{a}	9.80^{c}	10.93^{ab}
VB500	30.83^{a-c}	20.66^{c}	21.00^{b}	40.38^{a}	17.05^{a}	16.62^{a}	9.39^{c}	10.65^{ab}
TB500	32.44^{ab}	25.33^{a-c}	23.95^{b}	38.93^{a}	17.99^{a}	19.38^{a}	9.90^{c}	11.75^{a}
CB500	27.83^{a-c}	21.65^{c}	22.31^{b}	37.59^{a}	14.736^{a}	16.09^{a}	8.77^{c}	11.08^{ab}
BB500	29.74^{a-c}	22.60^{bc}	19.23^{b}	36.79^{a}	19.90^{a}	21.90^{a}	8.81^{c}	10.04^{ab}
ANOVA (One-way; LSD 5%)	1.767*		7.422***		NS		54.740***	

Means with different letters are significantly different at the 5% level of probability, (*) Significantly different at the 5% level of probability, (***) significantly different at the 0.1% level of probability, (NS) Not significantly different at 5% level of probability

Table 5 Effect of biochar applications on bulk and rhizosphere soils alkaline phosphatase and dehydrogenase activities

Treatment	Alkaline phosphatase activity (μg PNP g^{-1} dry soil h^{-1})				Dehydrogenase activity (μg TPF g^{-1} dry soil h^{-1})			
	Growth period							
	I		II		I		II	
	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere
Control	48.48^{d}	81.16^{a}	54.10^{a}	52.33^{a}	1.47^{bc}	0.54^{c}	0.82^{c}	6.53^{a-c}
VB300	48.56^{d}	72.02^{a-c}	74.48^{a}	46.74^{a}	1.11^{bc}	0.57^{c}	12.39^{a-c}	1.16^{c}
TB300	47.20^{d}	74.73^{a-c}	50.88^{a}	41.92^{a}	1.17^{bc}	1.06^{bc}	10.56^{a-c}	2.17^{c}
CB300	50.05^{d}	75.57^{ab}	58.70^{a}	48.71^{a}	0.97^{bc}	0.72^{c}	13.87^{a-c}	0.95^{c}
BB300	59.58^{cd}	83.00^{a}	59.41^{a}	52.16^{a}	1.47^{bc}	0.99^{bc}	17.97^{a}	0.94^{c}
VB500	53.34^{d}	76.19^{ab}	69.18^{a}	48.12^{a}	0.88^{c}	0.58^{c}	3.87^{bc}	2.99^{c}
TB500	53.95^{d}	87.31^{a}	58.06^{a}	47.13^{a}	4.88^{a}	0.75^{c}	9.04^{a-c}	2.57^{c}
CB500	52.96^{d}	77.81^{a}	53.30^{a}	51.51^{a}	3.31^{ab}	0.71^{c}	16.53^{ab}	0.94^{c}
BB500	61.12^{bcd}	86.76^{a}	57.00^{a}	47.84^{a}	0.87^{c}	0.68^{c}	12.04^{a-c}	2.40^{c}
ANOVA (One-way; LSD 5%)	8.439***		NS		2.330**		2.009*	

Means with different letters are significantly different at the 5% level of probability, (*) Significantly different at the 5% level of probability, (**) Significantly different at the 1% level of probability, (***) significantly different at the 0.1% level, (NS) Not significantly different at 5% level of probability

activity was higher in bulk soils during both periods. Increases ranged from 51.7% to 550.7% in the first period, with CB500 producing the largest increase. By the second period, activity surged further (251.7–1811.7%), indicating long-term stimulation of microbial metabolism, particularly in biochars with high-temperature pyrolysis and stable carbon structures (Table 5). Nitrification activity was significantly enhanced by biochar, especially in bulk soils. In the first period, VB500 bulk soil exhibited a 45.1% increase relative to the rhizosphere (Table 6). During the second period, bulk soils continued to show higher activity (81.7–258.3%), whereas rhizosphere soils remained largely unchanged, suggesting that biochar's effect on ammonia-oxidizing microbes is more pronounced in bulk soil over time. Denitrification activity showed contrasting trends. Initially, rhizosphere soils displayed higher activity, with BB500 exhibiting the largest increase. However, in the second period, bulk soils generally exceeded rhizosphere activity across treatments, although biochar did not consistently enhance denitrification beyond control levels. Bacterial abundance was markedly higher in rhizosphere soils during the first period, with increases from 163.6% (VB300) to 3150.0% (TB300), highlighting the stimulatory effect of labile carbon from biochar and root exudates (Table 7). In contrast, bulk soil bacterial populations generally declined, except for under CB500, which increased counts by 83.3%. By the second period, bacterial abundance stabilized across treatments, with BB500 maintaining a 490.9% increase in rhizosphere soil (Table 7).

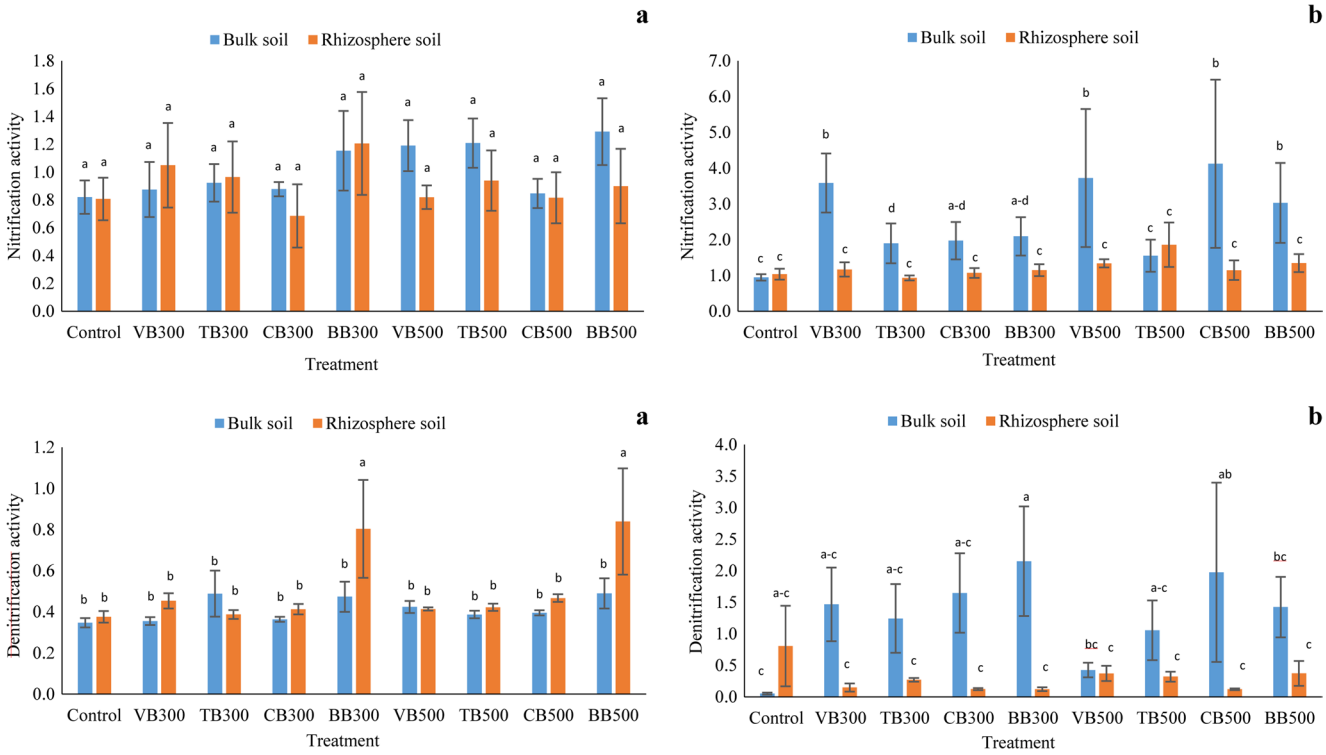


Fig. 4 Effects of biochar applications on bulk and rhizosphere soil nitrification (μg NO_2^--N g^{-1} dry soil h^{-1}) and denitrification (μg NO_2^--N g^{-1} dry soil h^{-1}) activities across two growing periods (A: first period, B: second period). Values represent means ± standard error (n = 3). Means with different letters are significantly different at the 5% level of probability. Treatments: VB = vineyard pruning biochar, TB = tomato biochar, CB = clove biochar, BB = banana biochar, each produced at 300 °C and 500 °C

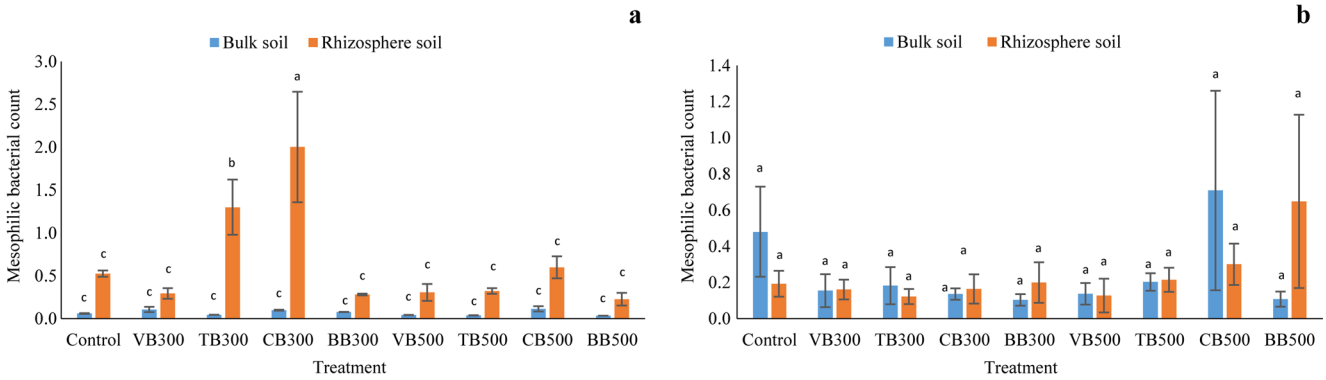


Fig. 5 Effects of biochar applications on bulk and rhizosphere soil mesophilic bacterial count (x10^5 CFU g^{-1} dry soil) across two growing periods (A: first period, B: second period). Values represent means ± standard error (n = 3). Means with different letters are significantly different at the 5% level of probability. Treatments: VB = vineyard pruning biochar, TB = tomato biochar, CB = clove biochar, BB = banana biochar, each produced at 300 °C and 500 °C

3.4 Biochar-Mediated Microbial Activity Correlates with Plant Growth

Concerning plant growth parameters (previously reported by Galadima et al. (2020) and rhizosphere microbial activities in the first growing period (Tables S1 and S2, see supplemental data), significant positive correlations were found between alkaline phosphatase and β-glucosidase (r = 0.67, p < 0.05), nitrification and β-glucosidase (r = 0.40, p < 0.05), and nitrification and alkaline phosphatase (r = 0.48, p < 0.05). Denitrification also correlated with β-glucosidase (r = 0.34, p < 0.05). Plant traits such as root length were positively related to soil pH (r = 0.35, p < 0.05), while root diameter correlated positively with denitrification (r = 0.28, p < 0.05). Leaf number and length both correlated with stem diameter (r = 0.30, p < 0.05), and dry weight correlated positively with stem diameter (r = 0.51, p < 0.001) and leaf number (r = 0.32, p < 0.05). In the second period, strong positive

Table 6 Effect of biochar applications on bulk and rhizosphere soils nitrification and denitrification activities

Treatment	Nitrification activity (μg NO_2^- -*N* g^{-1} dry soil h^{-1})				Denitrification activity (μg NO_2^- -*N* g^{-1} dry soil h^{-1})			
	Growth period							
	I		II		I		II	
	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere
Control	0.82^{a}	0.81^{a}	0.95^{c}	1.03^{c}	0.35^{b}	0.38^{b}	0.50^{c}	0.80^{a-c}
VB300	0.87^{a}	1.05^{a}	3.58^{b}	1.17^{c}	0.35^{b}	0.45^{b}	1.46^{a-c}	0.15^{c}
TB300	0.92^{a}	0.96^{a}	1.89^{d}	0.93^{c}	0.49^{b}	0.39^{b}	1.24^{a-c}	0.27^{c}
CB300	0.88^{a}	0.69^{a}	1.97^{a-d}	1.07^{c}	0.36^{b}	0.41^{b}	1.65^{a-c}	0.12^{c}
BB300	1.15^{a}	1.20^{a}	2.09^{a-d}	1.15^{c}	0.47^{b}	0.80^{a}	2.15^{a}	0.12^{c}
VB500	1.19^{a}	0.82^{a}	3.72^{b}	1.34^{c}	0.42^{b}	0.41^{b}	0.42^{bc}	0.37^{c}
TB500	1.21^{a}	0.94^{a}	1.55^{c}	1.86^{c}	0.39^{b}	0.42^{b}	1.05^{a-c}	0.32^{c}
CB500	0.85^{a}	0.82^{a}	4.12^{b}	1.15^{c}	0.39^{b}	0.47^{b}	1.97^{ab}	0.12^{c}
BB500	1.29^{a}	0.90^{a}	3.03^{b}	1.34^{c}	0.49^{b}	0.84^{a}	$1.42a^{bc}$	0.37^{c}
ANOVA (One-way; LSD 5%)	NS		10.94**		2.276**		1.872*	

Means with different letters are significantly different at the 5% level of probability, (*) Significantly different at 5% level of probability, (**) Significantly different at 0.1% level of probability, (NS) Not significantly different at 5% level of probability

Table 7 Effect of biochar applications on bulk and rhizosphere soils bacterial count (x10^5 cfu g^{-1} dry soil)

Treatment	Growth period			
	I		II	
	Bulk	Rhizosphere	Bulk	Rhizosphere
Control	0.06^{c}	0.52^{c}	0.48	0.19
VB300	0.11^{c}	0.29^{c}	0.15	0.16
TB300	0.04^{c}	1.30^{b}	0.18	0.12
CB300	0.10^{c}	2.00^{a}	0.14	0.16
BB300	0.08^{c}	0.28^{c}	0.10	0.20
VB500	0.04^{c}	0.30^{c}	0.14	0.13
TB500	0.04^{c}	0.32^{c}	0.20	0.21
CB500	0.11^{c}	0.60^{c}	0.71	0.30
BB500	0.03^{c}	0.23^{c}	0.11	0.65
ANOVA (One-way; LSD 5%)	4.176***		NS	

Means with different letters are significantly different at the 5% level of probability, (***) Significantly different at the 0.1% level of probability, (NS) Not significantly different at the 5% level of probability

correlations emerged between denitrification and dehydrogenase ($r=0.99$, $p<0.001$), and between leaf number and root diameter ($r=0.62$, $p<0.001$). Plant dry weight correlated positively with urease ($r=0.51$, $p<0.001$), root diameter ($r=0.40$, $p<0.05$), and leaf number ($r=0.56$, $p<0.001$). Chlorophyll content was positively correlated with urease ($r=0.33$, $p<0.05$), stem diameter ($r=0.10$, $p<0.05$), leaf number ($r=0.32$, $p<0.05$), and dry weight ($r=0.31$, $p<0.05$).

Concerning plant growth parameters (previously reported by Galadima et al. (2020) and bulk soil microbial activities in the first period (Tables S3 and S4, see supplemental data), strong positive correlations were observed between β-glucosidase and alkaline phosphatase ($r=0.80$, $p<0.001$), urease and β-glucosidase ($r=0.57$, $p<0.001$), and nitrification with β-glucosidase ($r=0.74$, $p<0.001$). Denitrification correlated positively with nitrification ($r=0.65$, $p<0.001$). Among plant traits, leaf number correlated with stem diameter ($r=0.30$, $p<0.05$) and leaf length ($r=0.30$, $p<0.05$). Dry weight correlated with stem diameter ($r=0.51$, $p<0.001$) and leaf number ($r=0.32$, $p<0.05$). In the second period, β-glucosidase correlated positively with urease ($r=0.36$, $p<0.05$), nitrification with bacterial number ($r=0.46$, $p<0.01$), and denitrification with dehydrogenase ($r=0.99$, $p<0.001$). Root length was positively correlated with nitrification ($r=0.37$, $p<0.05$) and denitrification ($r=0.38$, $p<0.05$). Root diameter correlated positively with bacterial number ($r=0.42$, $p<0.01$). Leaf number correlated with root diameter ($r=0.62$, $p<0.001$), while dry weight correlated negatively with urease ($r=-0.63$, $p<0.001$) but positively with root diameter ($r=0.39$, $p<0.05$) and leaf number ($r=0.56$, $p<0.001$).

4 Discussion

The results of this study demonstrate that biochar significantly alters soil microbial activity, enzyme dynamics, and nutrient cycling, with effects being spatially heterogeneous between the rhizosphere and bulk soil. Rhizosphere soils consistently exhibited higher microbial enzyme activities and bacterial abundance than bulk soils, supporting the hypothesis that biochar's influence is amplified by root-associated processes (Kuzyakov and Blagodatskaya 2015; Wu et al. 2025). Root exudates likely provided labile carbon and other substrates that synergistically interacted with biochar, creating microbial hotspots that enhanced urease, β-glucosidase, and alkaline phosphatase activities (Deng et al. 2023; Liu et al. 2022). These findings align with recent studies showing that rhizosphere–biochar interactions amplify microbial-mediated nutrient cycling, which

is critical for plant nutrient acquisition (Wu et al. 2025; Deng et al. 2023; Ahmad et al. 2023). Biochar pyrolysis temperature emerged as a key determinant of its effects on microbial processes. Low-temperature biochars (300 °C) were particularly effective at stimulating microbial activity, likely due to their higher content of labile carbon fractions that serve as immediate energy sources for microorganisms (Abel et al. 2013; Ghorbani et al. 2022). These biochars promoted nitrogen mineralization and carbon turnover, evidenced by elevated urease and β-glucosidase activities in rhizosphere soils (Biswal et al., 2025; Liu et al. 2022). In contrast, high-temperature biochars (500 °C) primarily influenced physicochemical properties, notably pH and electrical conductivity, which indirectly shaped microbial communities and soil metabolic activity (Zhong et al.2025; Zhang et al. 2025). High-temperature biochars improved pH buffering and nutrient retention, creating favorable conditions for processes such as nitrification and dehydrogenase activity (Ghorbani et al. 2022; Biswal et al., 2025). This dual mode of action—direct microbial stimulation via labile carbon versus indirect chemical modification—highlights the need to match biochar characteristics to specific soil functions (Ghorbani et al. 2022; Biswal et al., 2025). The interplay between biochar-induced changes in soil chemistry and microbial activity was evident in nitrogen cycling. Nitrification was consistently enhanced, particularly under high-temperature biochars, reflecting the combined effects of improved pH, microhabitat provision, and ammonium availability (Hale et al. 2023; Xue et al. 2023). Denitrification initially increased in rhizosphere soils, likely due to labile carbon from root exudates, but declined over time as biochar improved aeration and reduced anaerobic microsites (Zhong et al. 2022, 2025). These patterns suggest that biochar can promote nitrogen retention while potentially mitigating N_2O emissions, consistent with recent evidence on biochar's role in sustainable nitrogen management (Zhang et al. 2023; Shyam et al. 2025; Zhong et al. 2025). Bacterial abundance patterns further supported these mechanisms: low-temperature biochars increased rhizosphere microbial populations (Wu et al. 2025), whereas high-temperature biochars fostered selective microbial communities in bulk soil through improved habitat stability and water retention (Xue et al. 2023; Deshoux et al. 2023).

The correlations between microbial activities and plant growth parameters highlight the functional importance of biochar-induced changes. In the rhizosphere, enzyme activities were positively linked with plant biomass, leaf number, root length, and chlorophyll content, showing that biochar-driven nutrient cycling directly enhanced plant performance (Schulz and Glaser 2012; Fan et al. 2025). These links were more evident during the second growing season, reflecting the time needed for microbial communities to adjust to the biochar-amended soil environment (Kuzyakov and Blagodatskaya 2015; Ahmad et al. 2023). In bulk soil, some negative correlations, such as between urease and plant dry weight, suggest that excessive or unbalanced nutrient cycling can decrease efficiency, emphasizing the need to target biochar application to specific soil zones for the best results (Xue et al. 2023; Zhong et al. 2025). Collectively, these findings show that biochar functions as a system-level soil amendment, altering chemical, biological, and microbial parameters in an interconnected way (Lehmann and Joseph 2015; Jeffery et al. 2017). The strongest effects are seen in the rhizosphere, where biochar interacts with root exudates to boost enzyme activities, bacterial populations, and nutrient cycling (Kuzyakov and Blagodatskaya 2015; Wu et al. 2025). Low-temperature biochars quickly stimulate microbes through labile carbon, while high-temperature biochars support long-term soil fertility by improving pH, ionic content, and microbial habitats (Xue et al. 2023; Biswal et al., 2025). The spatial and temporal dynamics of these interactions suggest that biochar management should consider soil zone, feedstock type, and pyrolysis temperature to optimize microbial nutrient cycling and plant growth (Zhong et al. 2025; Shyam et al. 2025). Importantly, the study highlights broader implications for sustainable soil management and agriculture. By boosting microbial-driven nutrient cycling in the rhizosphere, biochar can improve nutrient use efficiency, lower nitrogen losses, and support plant growth under different soil conditions (Schulz and Glaser 2012; Guo et al. 2022). The findings also point to climate-smart uses, such as reducing N_2O emissions by improving nitrogen retention and microbial regulation (Shyam et al. 2025; Zhong et al. 2025). These insights offer practical guidance for customizing biochar amendments to achieve specific soil management goals, reinforcing its role as a versatile tool for improving soil health and ecosystem function (Lehmann and Joseph 2015; Wu et al. 2025).

While this study provides clear evidence that biochar alters microbial activity and nitrogen cycling in a spatially explicit manner, several limitations should be acknowledged. The results are based on controlled experimental conditions and a limited number of cropping seasons, which may not fully capture long-term biochar aging effects or variability under contrasting climatic conditions. In addition, microbial activity was inferred from enzyme activities, abundance, and process rates rather than direct molecular characterization of functional microbial groups, which may limit mechanistic resolution. Despite these constraints, the findings have important practical implications: they demonstrate that tailoring biochar feedstock and pyrolysis

temperature to specific soil zones (rhizosphere vs. bulk soil) can enhance nutrient use efficiency, promote nitrogen retention, and potentially reduce environmentally harmful nitrogen losses. Future studies integrating long-term field trials, greenhouse gas measurements, and microbial community profiling will be essential to validate these outcomes across agroecological zones and to refine biochar-based strategies for sustainable and climate-smart soil management.

5 Conclusion

This study provides mechanistic evidence that biochar enhances microbial activity more strongly in the rhizosphere than in bulk soil, supporting the hypothesis that root–microbe–biochar interactions drive spatially heterogeneous effects on soil enzymatic processes and bacterial abundance. Low-temperature biochars (300 °C) increased labile carbon availability, stimulating urease and β-glucosidase activities, while high-temperature biochars (500 °C) primarily modified soil pH and electrical conductivity, indirectly shaping microbial functions and nitrogen transformations. The positive correlations between rhizosphere microbial activity and plant growth demonstrate that biochar-mediated enhancements in nutrient cycling translate into measurable plant productivity benefits.

These findings have broader implications for soil management and sustainable agriculture. Strategically selecting biochar feedstock and pyrolysis conditions, and applying it to the rhizosphere zone, can optimize microbial-mediated nutrient cycling, improve soil fertility, and reduce nutrient losses. Beyond crop productivity, these results inform climate-smart soil strategies by potentially mitigating N_2O emissions through modulation of nitrogen pathways. Future research should focus on long-term microbial community dynamics and biochar persistence to evaluate the sustainability and ecosystem-wide impacts of these interventions.

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Data Availability The data supporting this study's findings are available upon reasonable request.

Code Availability Not applicable.

Declarations

Consent for Publication Not applicable.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Conflicts of interest/Competing Interests On behalf of all authors, the corresponding author states that there is no conflict of interest.

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