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Microbial nutrient limitation and carbon use efficiency in saline-alkali soil amended with biochar: insights from ecoenzymatic C:N:P stoichiometry

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Abstract

Biochar application is known to improve soil quality, enhance nutrient bioavailability, and increase carbon retention. However, its effects on ecoenzymatic activities and stoichiometric relationships in saline-alkali soils remain poorly understood. This study examines how biochar amendments influence microbial stoichiometry, nutrient limitations, and carbon use efficiency in saline-alkali soils. We compared two types of biochar—acid-modified biochar (pH 2.3) and alkaline biochar (pH 8.8)—at application rates of 1%, 2%, and 5% in soils planted with *Medicago sativa* L. Our results demonstrated that alkaline biochar increased enzymatic C:N stoichiometry at higher rates, while acid-modified biochar decreased it at lower rates. Both biochar types reduced enzymatic C:P and N:P stoichiometry; alkaline biochar shifted microbial metabolism from nitrogen to phosphorus limitation, while acid-modified biochar alleviated nitrogen limitation at rates of 2% and 5%. Furthermore, alkaline biochar at rates of 2% and 5% reduced microbial carbon limitation and enhanced carbon use efficiency, whereas acid-modified biochar did not. Microbial carbon use efficiency was consistently higher with alkaline biochar than with acid-modified biochar at equivalent application rates. These findings highlight that the impact of biochar on soil microbial processes depends on the biochar feedstock type, with differences in surface adsorption properties, nutrient supply, pH, and liming effects driving changes in soil properties, microbial community dynamics, and plant growth. Our study offers insights into optimizing nutrient cycling and carbon sequestration in saline-alkali soils, demonstrating the potential of biochar in sustainable soil management.

Highlights

- Alkaline biochar enhanced C/P-acquiring enzymes; acid-modified biochar reduced N-acquiring enzymes.
- Alkaline biochar shifted microbial limitation from N to P; acid-modified biochar alleviated N limitation at 2% and 5%.
- Alkaline biochar reduced microbial C limitation and boosted CUE at 1% and 2%; acid-modified biochar did not.

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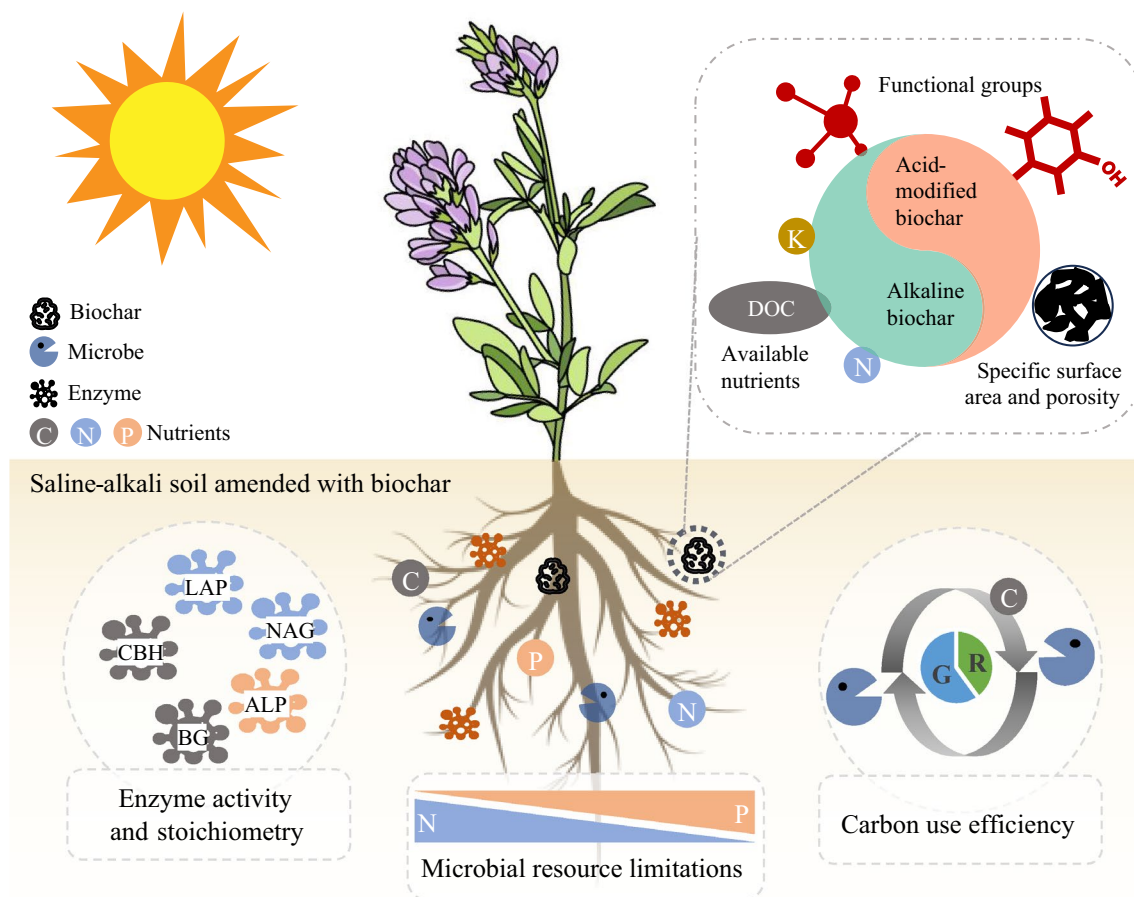
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Keywords Biochar amendment, Saline-alkali soil, Eoenzymatic stoichiometry, Microbial nutrient limitation, Carbon use efficiency

Graphical Abstract



1 Introduction

Soil salinization represents a significant global challenge, particularly in arid and semi-arid regions where irrigation is a prevalent agricultural practice (Hussain Shah et al. 2022). Affecting an estimated 831 to 1125 million hectares of arable land globally, soil salinization leads to substantial crop yield reductions, ranging from 18 to 43% (Hussain Shah et al. 2022; Ma et al. 2023). Salinization exacerbates soil fertility by increasing salt concentration and raising soil pH, which decreases nutrient availability, disrupts soil structure, and induces osmotic stress (Elmajdoub and Marschner 2013). The excessive accumulation of sodium ions in saline soil competes with

essential nutrients, such as phosphorus, calcium, and potassium, hindering nutrient uptake and reducing plant growth (Li et al. 2022). Additionally, soil salinization negatively impacts organic matter accumulation and soil carbon cycling—key processes for maintaining soil health (Xing et al. 2024). The detrimental effects of salinity extend to enzymatic activities vital for nutrient cycling, further limiting the availability of plant-accessible nutrients (Ma et al. 2023).

In response to these challenges, biochar, a carbon-rich material produced by the pyrolysis of organic feedstocks, has emerged as a promising amendment for improving soil conditions, particularly in saline-alkali environments

(Liu et al. 2023; Yin et al. 2024). Studies have highlighted the potential of biochar to enhance soil structure, increase water retention, reduce pH, and improve nutrient availability in degraded soil (Schmidt et al. 2021; Chen et al. 2022; Meng et al. 2023). The effectiveness of biochar largely depends on its physicochemical properties, such as specific surface area, porosity, and the presence of functional groups, which facilitate nutrient retention. Specifically, the high cation exchange capacity of biochar, coupled with functional groups, like carboxyl and phenolic groups, creates binding sites for essential nutrients while reducing the bioavailability of harmful ions (Lehmann et al. 2011; Pokharel and Chang 2023). These properties not only promote plant growth but also support microbial colonization, which is crucial for organic matter decomposition and nutrient cycling, thereby improving soil fertility and carbon use efficiency (Lehmann et al. 2011; Liu et al. 2020).

Despite the promising benefits of biochar, its effects on soil microbial processes, especially in saline-alkali soils, remain complex and variable. Factors such as the pH, pyrolysis temperature, and feedstock type of biochar can significantly influence its impact on microbial activity and soil health (Dai et al. 2021; Joseph et al. 2021; Liao et al. 2022). For instance, a meta-analysis indicated that biochar produced at temperatures below 500 °C enhanced the activity of soil enzymes involved in carbon, nitrogen, and phosphorus acquisition, whereas biochar produced at higher temperatures (≥ 500 °C) showed negligible effects on these enzymatic activities (Liao et al. 2022). Additionally, acidic carbon-rich biochar tends to favor carbon sequestration but has a limited impact on nutrient availability, while neutral mineral-rich biochar enhances nutrient sorption capacities (Qi et al. 2017). Furthermore, biochar has been shown to stimulate and inhibit the activity of extracellular enzymes involved in microbial processes such as carbon degradation, nitrogen mineralization, and phosphorus solubilization (Dai et al. 2021; Liao et al. 2022). Some studies reported increased enzyme activity (e.g., β -glucosidase, β -D-cellobiosidase, β -1,4-*N*-acetylglucosaminidase, and phosphatase) in biochar-amended soils (Lasota et al. 2022; Pokharel and Chang 2023; Shang et al. 2023), while others reported a reduction in enzyme activity (e.g., β -glucosidase and phosphatase) (Lamirato et al. 2011; Zhang et al. 2019). A global meta-analysis found that biochar generally has no significant effect on carbon and phosphorus acquisition enzymes, but it can influence nitrogen acquisition enzymes (Pokharel et al. 2020). These contrasting outcomes underscore the need for further investigation into the mechanisms through which

biochar affects enzyme activities and nutrient cycling across different soil environments.

One promising approach to unraveling these complexities is ecoenzymatic stoichiometry, which explores the balance of carbon (C), nitrogen (N), and phosphorus (P) in soil organic matter to gain insights into microbial nutrient limitation and energy status (Moorhead et al. 2016; Cui et al. 2020; Lasota et al. 2022). Vector analysis, a key method within this framework, can be employed to assess microbial carbon limitation and the relative availability of nitrogen and phosphorus (Fanin et al. 2016; Cui et al. 2022). Additionally, microbial carbon use efficiency (CUE)—the ratio of carbon incorporated into microbial biomass to the total carbon assimilated by microbes (Sinsabaugh et al. 2016; Schimel et al. 2022)—is intricately linked to ecoenzymatic stoichiometry and microbial metabolism. Variations in C:N:P ratios can influence microbial metabolism, enzymatic activity, and CUE (Moorhead et al. 2016; Cui et al. 2020; Lasota et al. 2022). By integrating ecoenzymatic stoichiometry with microbial community data, researchers can explore how microbial community composition and diversity influence nutrient cycling and enzymatic activity (Boyle et al. 2008; Orland et al. 2019). This combined approach will provide a more comprehensive understanding of how biochar, through its effects on soil pH and nutrient availability, modulates microbial metabolic strategies and nutrient cycling (Bastida et al. 2021; Philippot et al. 2024). Ultimately, examining enzyme activity, stoichiometry, and microbial community dynamics together will offer valuable insights into how biochar application can enhance microbial processes, improve soil health, and optimize carbon cycling efficiency in saline-alkali soils.

While numerous studies have investigated the benefits of biochar (Zhang et al. 2019; Pokharel and Chang 2023), the specific interactions between biochar properties (such as pH and application rate), microbial community dynamics, nutrient limitations, carbon cycling processes in saline-alkali soils remain poorly understood (Gul et al. 2015; Khadem and Raesi 2017). This knowledge gap is especially critical in saline-alkali environments, where high salt concentrations and extreme pH values significantly constrain nutrient availability and microbial activity. To address these gaps, the present study investigates the effects of biochar derived from two distinct feedstocks—one acidic modified and one alkaline—at three different application rates on saline-alkali soils. Specifically, the study aims to: (1) investigate the influence of biochar feedstock type and application rate on soil extracellular enzymatic activities and stoichiometry; (2) examine shifts in microbial nutrient limitations and carbon use efficiency using vector analysis

and partial least squares path modeling; (3) assess the role of microbial community dynamics in modulating enzyme activities, stoichiometry, and nutrient limitations in biochar-amended soils. We hypothesized that acid-modified biochar will outperform alkaline biochar in improving soil conditions, enhancing microbial activity, and promoting nutrient cycling by neutralizing soil pH and alleviating salinity stress. This research will provide crucial insights into how biochar can optimize microbial processes and enhance soil fertility in challenging saline-alkali environments.

2 Materials and methods

2.1 Experimental materials and design

The experimental saline-alkali soil was collected in June 2022 from a salinized grassland at the Linze Grassland Agricultural Experiment Station of Lanzhou University (100°06'E, 39°11'N), Gansu Province, China. The soil was classified as millet calcareous soil and exhibited a salinity of 1.21% and a pH of 8.6. Soil samples were collected from ten randomly chosen points (5 m×5 m) along a 500 m long and 100 m wide field of salinized grassland, with the top 0–20 cm layer being sampled after removing dead plants and stones. The collected soil was air-dried and sieved through a 2 mm mesh, then evenly mixed to obtain a homogeneous sample. The pretreated soil was transported to the laboratory and stored in a glasshouse at room temperature (approximately 24 °C) until used in subsequent pot experiments.

Two types of biochar were employed in this experiment: an alkaline biochar derived from corn straw (pH 8.8, AL-biochar) and an acid-modified biochar (pH 2.3, AC-biochar). Detailed properties and elemental compositions of the biochars, as well as experimental saline-alkali soil, are provided in Table S1 and Fig. S1. The biochars were pyrolyzed at 400 °C and then added to 1.0 kg of sieved saline-alkali soil at the rates of 0%, 1%, 2%, and 5% (m/m). The application rates were selected based on a review of published literature (Joseph et al. 2021; Chagas et al. 2022) and practical applications, where biochar rates are typically classified into low ($\leq 1\%$), medium (1–2%), and high (2–5%) ranges. As the upper limit of biochar addition is not commonly used in practical applications due to cost and other limitations, we selected the rates of 1%, 2%, and 5% for this study.

A total of seven experimental treatments were established, including a control group (0% biochar), with each treatment replicated twelve times. The biochar-amended soils were equilibrated for two weeks in a greenhouse before planting. Experimental pots (diameter: 13 cm, height: 15 cm) were used for the pot experiment and placed in a greenhouse under controlled

conditions (24 °C, 50% relative humidity, 16 h of light per day). Plants were watered every two days to maintain consistent soil moisture levels. *Medicago sativa* L. (Zhonglan 1), known for its high salt tolerance, was selected as the test plant species due to its suitability for saline-alkali conditions. Salt tolerance was confirmed through a germination assay showing a 96% germination rate at 0 mmol L⁻¹ NaCl and NaHCO₃, and 58% at 60 mmol L⁻¹. The plants were cultivated for ninety days, after which the plant and soil samples were collected for further analysis.

2.2 Analysis of soil biogeochemical properties

Soil pH was measured using a pH meter with a glass electrode in a 1:5 soil-to-water suspension. Soil salinity was measured by the mass method. Soil organic carbon (SOC) was determined via the K₂Cr₂O₇ oxidation method (Walkley and Black 1934). Total nitrogen (TN) was quantified using the Kjeldahl method (Sparks et al. 1996), and total phosphorus (TP) via the Olsen method (Olsen 1954). Available phosphorus was assessed using the sodium hydrogen carbonate solution-Mo-Sb antimonyl method (Hieltjes and Lijklema 1980). Soil microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) were measured using the chloroform fumigation–extraction method as previously described (Zhang et al. 2023). Soil ammonium (NH₄⁺) was analyzed using a modified indophenol method, while nitrate (NO₃⁻) and nitrite (NO₂⁻) were determined using the VCl₃/Griess method (Hood-Nowotny et al. 2010).

2.3 Assay of plant functional traits

At harvest, plant root and shoot biomass and root functional traits were measured. Roots and shoots were initially heated at 105 °C for 30 min, then dried at 65 °C to a constant weight for dry biomass determination. Roots were washed with distilled water and scanned using a root analyzer (WinRhizo Pro LA2400, Regent) to determine morphological traits such as length, projected area, surface area, average diameter, volume, length per volume, and number of tips. Plant traits and soil properties results are shown in Table S2. These results indicated that both AL- and AC- biochar additions reduced the soil salinity and pH, as well as improved soil nutrition and plant growth.

2.4 Assays of soil ecoenzymatic activities

Activities of nine soil extracellular enzymes were measured, including carbon-acquiring enzymes (sucrase, β -1,4-glucosidase [BG], cellobiohydrolase [CBH]), nitrogen-acquiring enzymes (urease, β -1,4-N-acetylglucosaminidase [NAG], L-leucine aminopeptidase

[LAP]), a phosphorus-acquiring enzyme (alkaline phosphatase [ALP]), and oxidation-associated enzymes (polyphenol oxidase [PPO], peroxidase [PER]) (Foster et al. 2016; Moorhead et al. 2016).

For β -1,4-glucosidase, cellobiohydrolase, β -1,4-N-acetylglucosaminidase, L-leucine aminopeptidase, alkaline phosphatase, polyphenol oxidase, and peroxidase, 2.00 g of fresh soil was mixed with 60 mL of 50 mM Tris buffer (pH 8), shaken at 25 °C and 150 rpm for one hour. The suspension (200 μ L) was combined with 50 μ L of enzyme–substrate solution in a black microplate, with Tris buffer as a control. Assays were conducted in triplicate, incubated at 25 °C, and absorbance was measured at specific wavelengths (Table S3) (Peng and Wang 2016). Enzyme activity was expressed as μ mol substrate hydrolyzed per gram of dry soil per hour (μ mol g⁻¹ h⁻¹).

For urease activity, 5.00 g of fresh soil was combined with 1 mL of toluene, 20 mL of citrate buffer (pH 6.7), and 10 mL of 10% urea solution, incubated at 37 °C for 24 h, and filtered. Filtrate (1 mL) was mixed with water, phenol-sodium solution, and sodium hypochlorite solution, reacted for 20 min, diluted with water, and analyzed at 578 nm (Kandeler and Gerber 1988). Urease activity was quantified as mg N g⁻¹ 24 h⁻¹.

For sucrase activity, 2.00 g of fresh soil was combined with toluene, phosphate buffer (pH 5.5), and sucrose solution, incubated at 37 °C for 24 h and filtered. Filtrate (0.1 mL) was mixed with 3,5-dinitrosalicylic acid solution, heated, cooled, diluted with water, and analyzed at 508 nm (Guan et al. 1986). Sucrase activity was expressed as mg glucose g⁻¹ 24 h⁻¹.

2.5 Vector analysis of coenzymatic stoichiometry

Vector analysis was employed to assess soil microbial resource limitations in response to biochar amendment (Moorhead et al. 2016). Vector length [Eq. (1)] represents microbial carbon limitation, while vector angle [Eq. (2)] quantifies relative phosphorus versus nitrogen acquisition. Vector length and vector angle were calculated as follows:

$$\text{Length} = \sqrt{(x^2 + y^2)} \quad (1)$$

$$\text{Angle } (^{\circ}) = \text{Degrees}(\text{Atan2}(x, y)) \quad (2)$$

where x represents natural log-transformed ratios [$\ln(\text{BG} + \text{CBH}) / \ln(\text{BG} + \text{CBH} + \text{NAG} + \text{LAP})$] and y represents natural log-transformed ratios [$\ln(\text{BG} + \text{CBH}) / \ln(\text{BG} + \text{CBH} + \text{ALP})$].

Vector angle > 45° indicates phosphorus limitation, while angle < 45° indicates nitrogen limitation. Increasing vector angle signifies stronger phosphorus limitation, while decreasing angle indicates stronger nitrogen limitation. Microbial carbon limitation increases with vector length.

2.6 Microbial carbon use efficiency

Microbial carbon use efficiency was estimated using stoichiometry theory (CUE_{ST}) as per Eqs. (3)–(5) (Sinsabaugh et al. 2016; Schimel et al. 2022). CUE_{ST} evaluates microbial shifts in carbon and energy use among available polymeric sources in response to nutrient stoichiometry.

$$\text{CUE}_{\text{ST}} = \text{CUE}_{\text{max}} \times \left(\frac{S_{\text{C:N}} \times S_{\text{C:P}}}{[(K_{\text{C:N}} + S_{\text{C:N}}) \times (K_{\text{C:P}} + S_{\text{C:P}})]} \right)^{0.5} \quad (3)$$

$$S_{\text{C:N}} = \frac{B_{\text{C:N}}}{L_{\text{C:N}}} \times \frac{1}{\text{EEAr}_{\text{C:N}}} \quad (4)$$

$$S_{\text{C:P}} = \frac{B_{\text{C:P}}}{L_{\text{C:P}}} \times \frac{1}{\text{EEAr}_{\text{C:P}}} \quad (5)$$

where half-saturation constants $K_{\text{C:N}}$ and $K_{\text{C:P}}$ were set to 0.5, and CUE_{max} was 0.6 (Sinsabaugh et al. 2016). Ratios of microbial biomass C:N or C:P ($B_{\text{C:X}}$), substrate C:N or C:P availabilities ($L_{\text{C:X}}$), and coenzymatic activities for C and nutrients (X) ($\text{EEAr}_{\text{C:X}}$) were incorporated into the model. $L_{\text{C:N}}$ and $L_{\text{C:P}}$ were estimated as SOC:TN and SOC:TP, respectively. $\text{EEAr}_{\text{C:N}}$ was calculated as $(\text{BG} + \text{CBH})/(\text{NAG} + \text{LAP})$, and $\text{EEAr}_{\text{C:P}}$ was calculated as $(\text{BG} + \text{CBH})/\text{ALP}$.

2.7 Microbial community analysis

Microbial community analyses were conducted following Liu et al. (2024). Total genomic DNA was extracted from 0.5 g soil samples using the FastDNA SPIN kit (MP Biomedicals, Solon, OH, USA). The V3-V4 region of bacterial 16S rRNA genes was amplified using primers 338F and 806R, and sequenced on an Illumina NovaSeq 6000 platform (PE250, Biomarker Technologies, Qingdao, China). Raw sequences were processed using the Qiime2 platform (<https://qiime2.org/>), with taxonomic annotation based on the Ribosomal Database Project database (version 11.5). Alpha-diversity metrics (Shannon, Simpson, Richness, Chao1) were calculated using the “vegan” package in R.

2.8 Statistical analysis

Pearson’s correlation analysis was performed using the “psych” package in R, with p -values corrected using the FDR method. Regression random forest analysis was conducted to identify the importance of predictors for vector length, vector angle, and CUE using “randomForest” and “rfPermute” packages. The importance of each predictor was estimated by the percentage increase in mean squared error (MSE), and the significance of each predictor was evaluated using a

bootstrap aggregation algorithm with 1000 permutation replicates. Redundancy analysis (RDA) and variation partition analysis explored the effects of biochar and induced changes on soil enzyme activities using the “MASS” package. Partial least squares path modeling discerned contributions of biochar and induced changes to variations in vector length, vector angle, and CUE, with a bootstrap test (1000 permutations) comparing path coefficients between biochar treatments. Significant differences were calculated using two-way analysis of variance (two-way ANOVA), least significant difference (LSD), and Wilcoxon tests, with all values expressed as mean $\pm$ standard error. All data analyses were performed using R version 4.1.2.

3 Results

3.1 Soil extracellular ecoenzyme activities and stoichiometry

The addition of AC- and AL-biochar had distinct effects on soil enzyme activities (Fig. 1 and Table S4). Specifically, all three AC-biochar addition rates significantly suppressed the activities of carbon-acquiring enzymes, such as cellobiohydrolase, with the lowest value of $0.934 \pm 0.050 \mu\text{mol g}^{-1} \text{h}^{-1}$ at the 2% application rate ($p < 0.05$, LSD test). In contrast, AL-biochar reduced cellobiohydrolase activity only at the 1% application rate ($p < 0.05$, LSD test, Fig. 1). Conversely, β -1,4-glucosidase exhibited the highest value at the 5% application rate of AL-biochar. Overall, all AC- and AL- biochar increased sucrase activity except for the 5% addition rates of AL-biochar ($p < 0.05$, LSD test).

For nitrogen-acquiring enzymes, all AC-biochar treatments decreased L-leucine aminopeptidase and β -1,4-N-acetylglucosaminidase activities with the lowest values at the 2% application rate, while increasing urease activity, which peaked at the 2% application rate ($p < 0.05$, LSD test). AL-biochar, on the other hand, decreased L-leucine aminopeptidase and urease activities with the lowest values at the 5% application rate but increased β -1,4-N-acetylglucosaminidase activity, which reached $4.745 \pm 0.524 \mu\text{mol g}^{-1} \text{h}^{-1}$ at the 5% application rate. Notably, β -1,4-N-acetylglucosaminidase and urease showed opposing responses to AC- and AL-biochar additions.

For phosphorus-acquiring enzymes, AL-biochar significantly elevated alkaline phosphatase activity with increasing addition rates ($p < 0.05$, LSD test) with the highest value of $46.356 \pm 6.416 \mu\text{mol g}^{-1} \text{h}^{-1}$ at the application rate of 5%. In contrast, AC-biochar did not significantly affect alkaline phosphatase activity ($p > 0.05$, LSD test). Additionally, both AC- and AL- biochar reduced the activities of oxidative enzymes, specifically peroxidase and polyphenol oxidase, with the lowest

enzyme activities observed at the 5% biochar application rate ($p < 0.05$, LSD test, Fig. 1). This reduction in enzyme activity may be attributed to the adsorption of phenolic compounds or the scavenging of reactive oxygen species by biochar, which are normally involved in the activation of oxidative enzymes (Wu et al. 2020).

The enzyme C:N ratio exhibited a gradual increase with AL-biochar addition ($p < 0.05$, LSD test, Fig. 2), whereas AC-biochar initially reduced the C:N ratio at the 1% and 2% addition rates before returning to baseline levels at a 5% addition rate. At the 2% and 5% addition rates, the disparity in enzyme C:N ratios between AC- and AL-biochar treatments was significant ($p < 0.01$, LSD test). Both AC- and AL-biochar decreased enzyme C:P and N:P ratios ($p < 0.05$, LSD test, Fig. 2), with no significant differences observed at 2% and 5% addition rates.

3.2 Microbial resource limitations and carbon use efficiency

The vector angle in soil enzymatic stoichiometry reflects the nutrient limitations faced by microbes. As shown in Fig. 3, the enzyme vector angle was less than 45° in control samples ($19.6^\circ \pm 7.6^\circ$), but increased and remained above 45° (67.5° – 69.8°) with AL-biochar addition ($\geq 1\%$), indicating a shift from nitrogen to phosphorus limitation. AC-biochar at 2% and 5% also increased the vector angle slightly above 45° (45.6° – 46.1°), suggesting a reduction in nitrogen limitation and a trend toward phosphorus limitation.

Biochar addition had a lesser impact on enzyme vector length compared to the vector angle (Fig. 3A and B). The 1% and 2% rates of AL-biochar significantly decreased enzyme vector length by 47.7% and 36.0%, respectively, compared to the control, indicating alleviated microbial carbon limitation at these addition levels ($p < 0.05$, LSD test). No significant changes in enzyme vector length were observed with AC-biochar addition. Enzyme vector length was negatively correlated with vector angle in both AL- (Pearson's $r = -0.49$, $p < 0.001$) and AC- (Pearson's $r = -0.36$, $p < 0.05$) biochar treatments (Fig. 3C).

Microbial carbon use efficiency can evaluate the ability of microbes to utilize carbon substrates (Sinsabaugh et al. 2016; Schimel et al. 2022), significantly increased with AL-biochar at the 1% and 2% addition rates ($p < 0.05$, LSD test), whereas AC-biochar had no significant effect ($p > 0.05$, LSD test, Fig. 4A). Microbial carbon use efficiency was higher under AL-biochar treatment compared to AC-biochar at the same addition rate ($p < 0.05$, LSD test). The best microbial carbon use efficiency (0.597) was observed at the 1% and 2% addition rates of AL-biochar. Additionally, Microbial carbon use efficiency was negatively correlated with enzyme vector length (AL: Pearson's $r = -0.45$, $p < 0.01$; AC: Pearson's $r = -0.39$, $p < 0.01$).

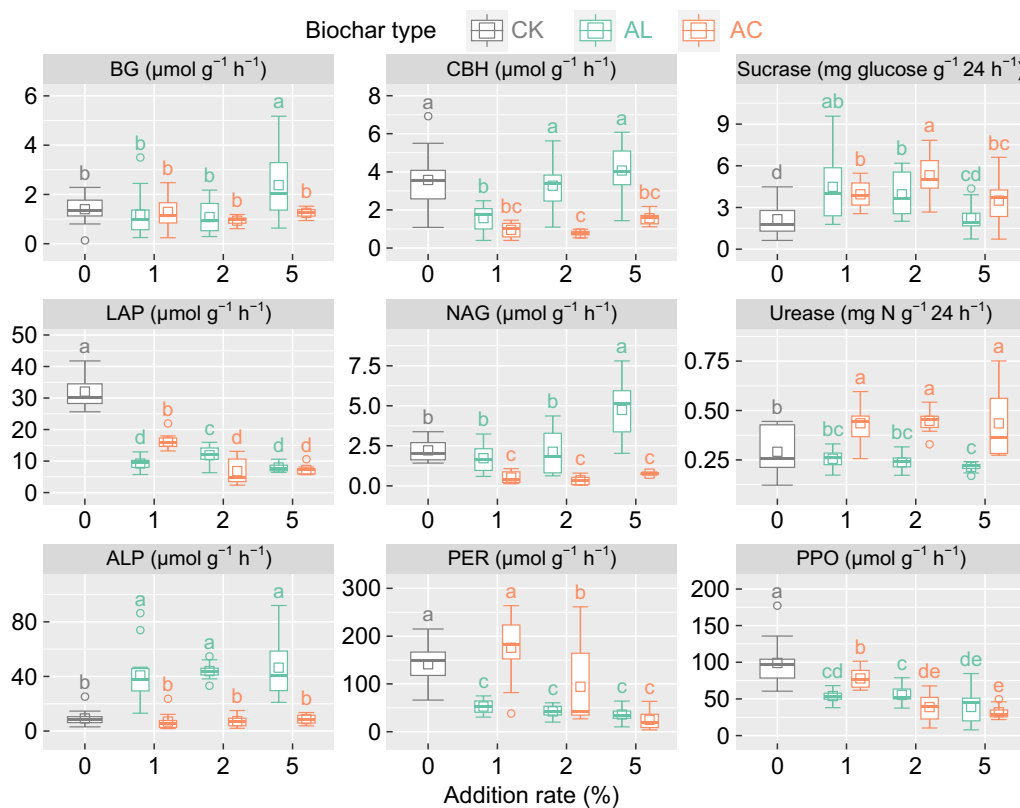


Fig. 1 Effects of AL- and AC-biochar amendments on soil extracellular enzyme activities. Enzymes measured include β -1,4-glucosidase (BG), cellobiohydrolase (CBH), sucrase, β -1,4-N-acetylglucosaminidase (NAG), L-leucine aminopeptidase (LAP), urease, alkaline phosphatase (ALP), polyphenol oxidase (PPO), and peroxidase (PER). CK denotes the control group. Distinct lowercase letters indicate statistically significant differences at the 0.05 level (LSD test) among the treatments

and positively correlated with enzyme vector angle in AL-biochar treatments (AL: Pearson's $r=0.38$, $p<0.01$; AC: Pearson's $r<0.01$, $p>0.05$, Fig. 4B). The increase in microbial carbon use efficiency with biochar application suggests a greater incorporation of assimilated carbon into microbial biomass rather than CO_2 release (Lehmann et al. 2011).

3.3 Drivers of microbial resource limitation and carbon use efficiency

Redundancy analysis revealed that the first two axes explained 75.6% of the variance in AL-biochar treatments and 65.2% in AC-biochar treatments (AL: $F=2.23$, $p<0.001$; AC: $F=2.01$, $p<0.001$, Fig. 5A). AL- and AC-biochar had distinct effects on soil enzymatic activities. For AL-biochar, the addition rate was the primary factor affecting soil enzymatic activities, accounting for 8.83% of the variance, followed by water content (7.64%), pH (7.57%), and ammonia nitrogen (7.57%, Figs. 5B and S2). In AC-biochar treatments, available phosphorus (11.59%) and pH (11.23%) were the main factors, followed by ammonia nitrogen (9.52%) and salinity (8.58%).

Variance partitioning analysis indicated that biochar addition primarily influenced soil enzymatic activities through plant-soil interactions (Fig. 5C). The impact of plant-soil interactions induced by AL-biochar (23.2%) on soil enzymatic activities was greater than that induced by AC-biochar (15.7%). Conversely, AL-biochar had a lesser effect on soil property changes (14.5%) compared to AC-biochar (20.5%).

Partial least squares path modeling and random regression forest analysis further demonstrated differential effects of AL- and AC-biochar on the microbial nutrient limitation and CUE within the plant-soil-microbe system (Figs. 6 and S3). AL-biochar exerted significant direct (Standardized effect: 0.693) and indirect (Standardized effect: -0.121) effects on vector angle, explaining 50.5% of the variance in microbial phosphorus limitation (Figs. 6A and S4). It also had significant indirect effects on vector length through edaphic factors (Standardized effect: 0.408), explaining 50.5% of the variance in microbial carbon limitation (Fig. 6B). Although AL-biochar did not significantly affect microbial CUE directly or indirectly, microbial properties significantly influenced CUE (Standardized effect: -0.893 , Fig. 6C). In contrast,

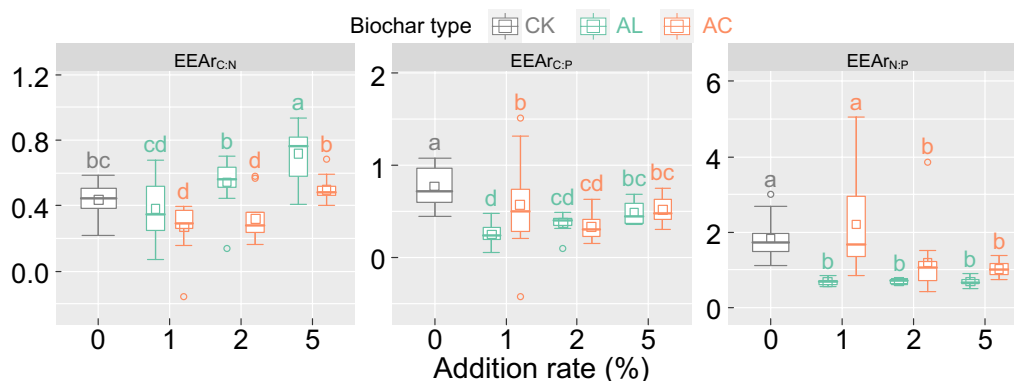


Fig. 2 Effects of AL- and AC-biochar amendments on the ratio of ecoenzymatic activities (EEAr). Distinct lowercase letters indicate statistically significant differences at the 0.05 level (LSD test) among the treatments

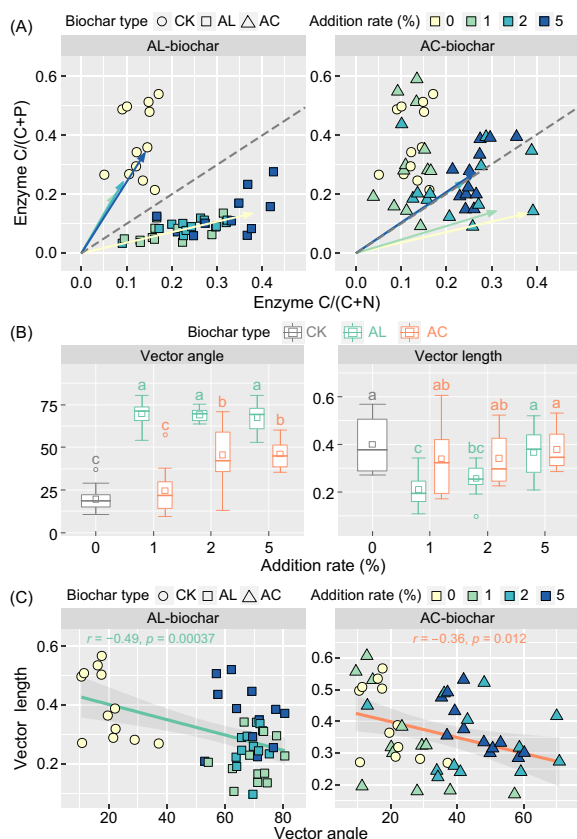


Fig. 3 (A) Ecoenzyme stoichiometry of the relative proportions of carbon to nitrogen acquisition versus carbon to phosphorus acquisition. Enzyme C activity was calculated as the sum of β -1,4-glucosidase and cellobiohydrolase, enzyme N as the sum of β -1,4-N-acetylglucosaminidase and L-leucine aminopeptidase, and enzyme P as alkaline phosphatase activity. (B) Effects of AL- and AC-biochar amendments on vector angle and vector length. (C) Correlation of vector angle and vector length in AL- and AC-biochar amendments. Distinct lowercase letters indicate statistically significant differences at the 0.05 level (LSD test) among the treatments

AC-biochar mainly produced indirect effects on vector angle (Standardized effect: 0.413), vector length (Standardized effect: -0.383), and CUE (Standardized effect: -0.152) through edaphic factors and microbial properties (Fig. 6D–F).

3.4 Correlations of soil ecoenzymatic stoichiometry and soil microbial community

AL-biochar addition increased bacterial diversity (Pearson's $r = 0.52$ – 0.74 , $p < 0.01$), whereas AC-biochar decreased it (Pearson's $r = -0.50$ to -0.81 , $p < 0.05$) in saline-alkali soil improvement, impacting C/N/P-acquiring and oxidative enzyme activities as well as their stoichiometry (Fig. S5). Changes in bacterial phyla, such as *Acidobacteria*, *Armatimonadetes*, *Chloroflexi*, *Elusimicrobia*, *Euryarchaeota*, *Gemmatimonadetes*, *Microgenomates*, *Parcubacteria*, and *Proteobacteria*, induced by biochar additions contributed to variations in enzyme activities and stoichiometry (Figs. 7 and S6).

For instance, the relative abundance of *Chloroflexi* decreased with increasing AL-biochar rates (Pearson's $r = -0.75$, $p < 0.001$), which positively correlated with L-leucine aminopeptidase, peroxidase, and polyphenol oxidase activities (Pearson's $r = 0.46$ – 0.56 , $p < 0.05$) and negatively with vector angle (Pearson's $r = -0.66$, $p < 0.01$). Conversely, *Chloroflexi* abundance increased with AC-biochar addition (Pearson's $r = 0.54$, $p < 0.01$), negatively correlating with L-leucine aminopeptidase, peroxidase, and polyphenol oxidase activities (Pearson's $r = -0.51$ to -0.57 , $p < 0.05$) and positively with vector angle (Pearson's $r = 0.56$, $p < 0.01$) and vector length (Pearson's $r = 0.44$, $p < 0.05$). Additionally, the phylum *Microgenomates* showed opposite relationships with CUE under AL- and AC-biochar treatments (AL: Pearson's $r = -0.37$, AC: Pearson's $r = -0.48$, $p < 0.05$).

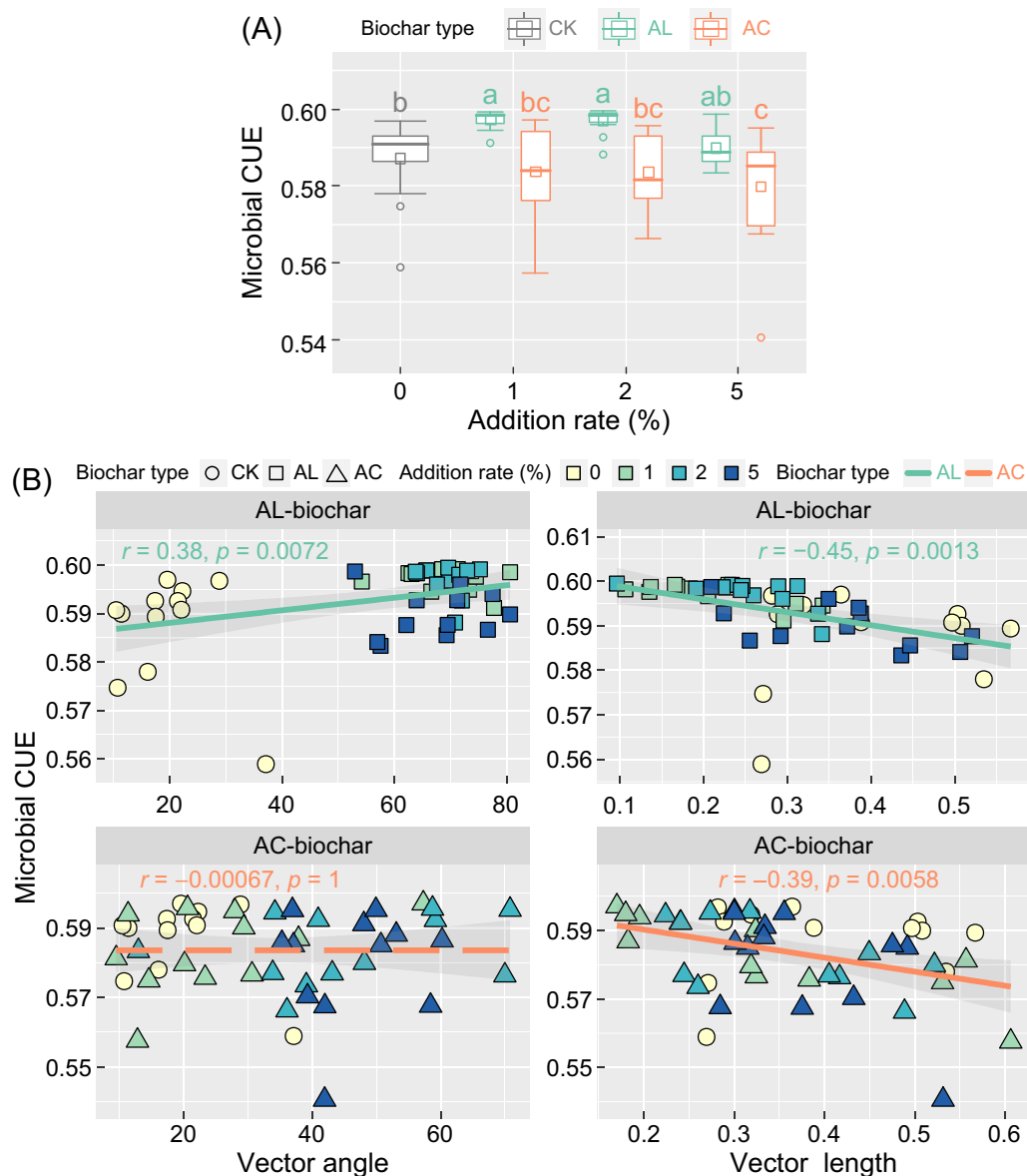


Fig. 4 (A) Effects of AL- and AC-biochar amendments on microbial carbon use efficiency (CUE). (B) Correlation of microbial CUE with vector angle and vector length in AL- and AC-biochar amendments. Distinct lowercase letters indicate statistically significant differences at the 0.05 level (LSD test) among the treatments

4 Discussion

4.1 Modulation of soil extracellular enzyme activities and stoichiometry by biochar amendments

Soil enzymatic activities are essential for nutrient dynamics and cycling, yet they are often inhibited by salt stress in saline soils. Our results indicate that both AL- and AC-biochar amendments variably influence the activities of key enzymes, depending on the type and amount of biochar applied (Fig. 1). Biochar serves as a complex source of carbon and nutrients, which can support microbial growth and enzymatic processes (Hammer et al. 2014;

Palansooriya et al. 2019). Our data showed that AL-biochar, which is characterized by higher dissolved organic carbon, higher O:C and H:C ratios, and lower C:N ratio (Table S1), promotes microbial activity through its more labile carbon content, including available nitrate (NO_3^-) (Domingues et al. 2017). The relatively higher nitrogen content in AL-biochar likely enhances microbial nitrogen demand, supporting the observed increases in enzymatic activities, such as those involved in cellulose degradation (β -1,4-glucosidase, sucrase) and phosphoric acid esters and anhydrides decomposition (alkaline phosphatase).

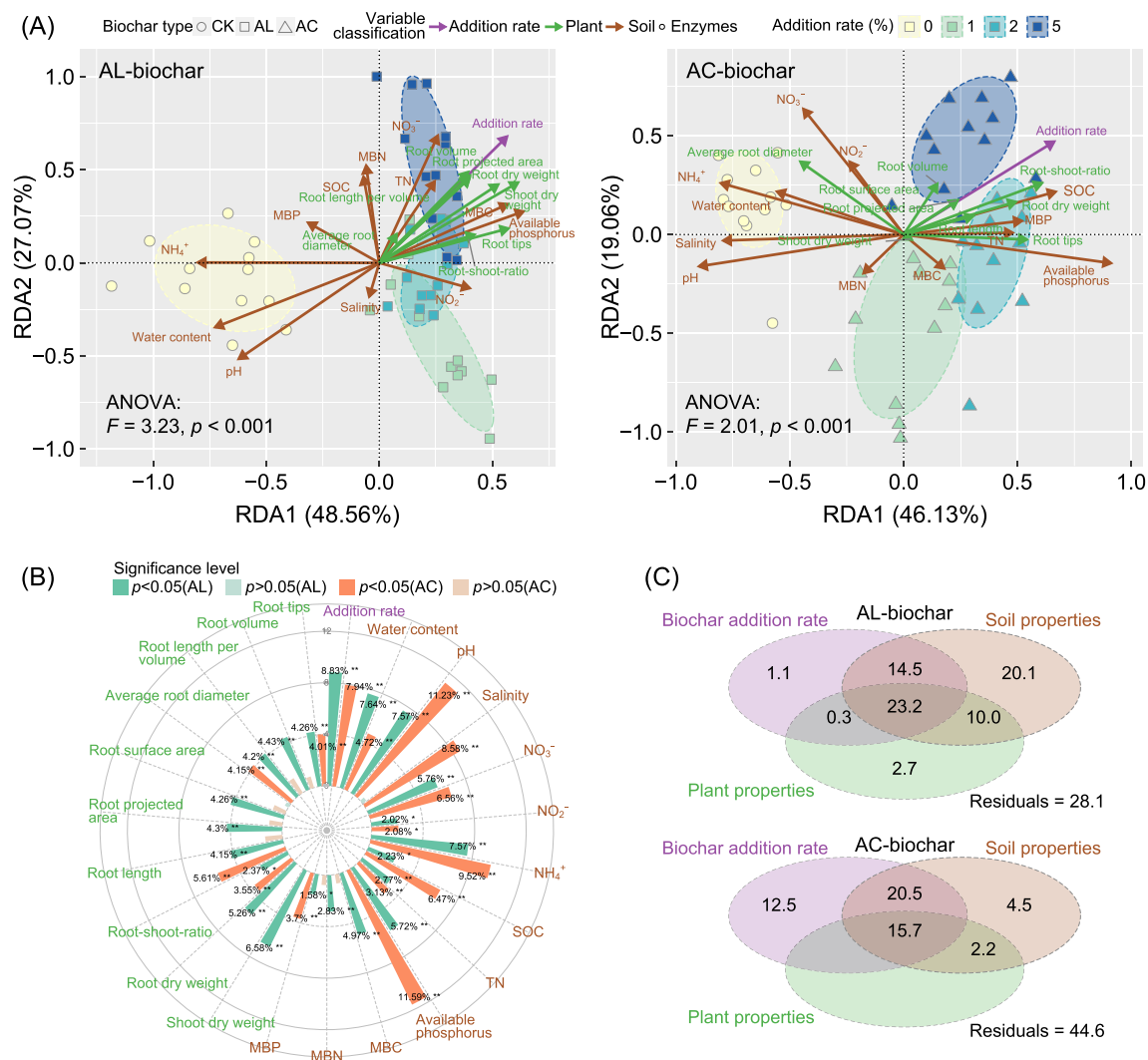


Fig. 5 (A) Redundancy analysis and (C) variation partition analysis performed to unravel the effects of biochar addition-induced soil properties and plant traits on soil extracellular enzyme activities. (B) Explanation of soil and plant traits' contributions to soil extracellular enzyme activities based on redundancy analysis. Asterisks indicate significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

These findings align with a recent study where biochar-derived carbon can enhance microbial demand for nitrogen and phosphorus, which in turn stimulates β -1,4-N-acetylglucosaminidase and alkaline phosphatase activities (He et al. 2023).

In contrast, AC-biochar with a higher C:N ratio and low H:C and O:C ratios (Table S1), contains a greater proportion of more recalcitrant organic matter, such as aromatic carbon and polycyclic aromatic hydrocarbons, which are known to resist microbial decomposition (Zhang et al. 2018). These chemical properties contribute to reduced microbial growth and enzyme production. Specifically, we observed a significant reduction in the activities of enzymes like cellobiohydrolase and

β -1,4-N-acetylglucosaminidase in soils amended with AC-biochar, which may be attributable to the biochar's ability to sequester enzymes and limit substrate availability (Al-Wabel et al. 2013). The inhibitory effects of AC-biochar may also stem from bactericidal or fungicidal compounds, including phenols, which can suppress microbial activity and enzyme production (Zhu et al. 2017; Jaiswal et al. 2018). Furthermore, the high specific surface area and porosity of AC-biochar can facilitate enzyme adsorption onto its surface, potentially leading to enzyme denaturation and reducing substrate-enzyme interactions, further impairing enzymatic function (Foster et al. 2016).

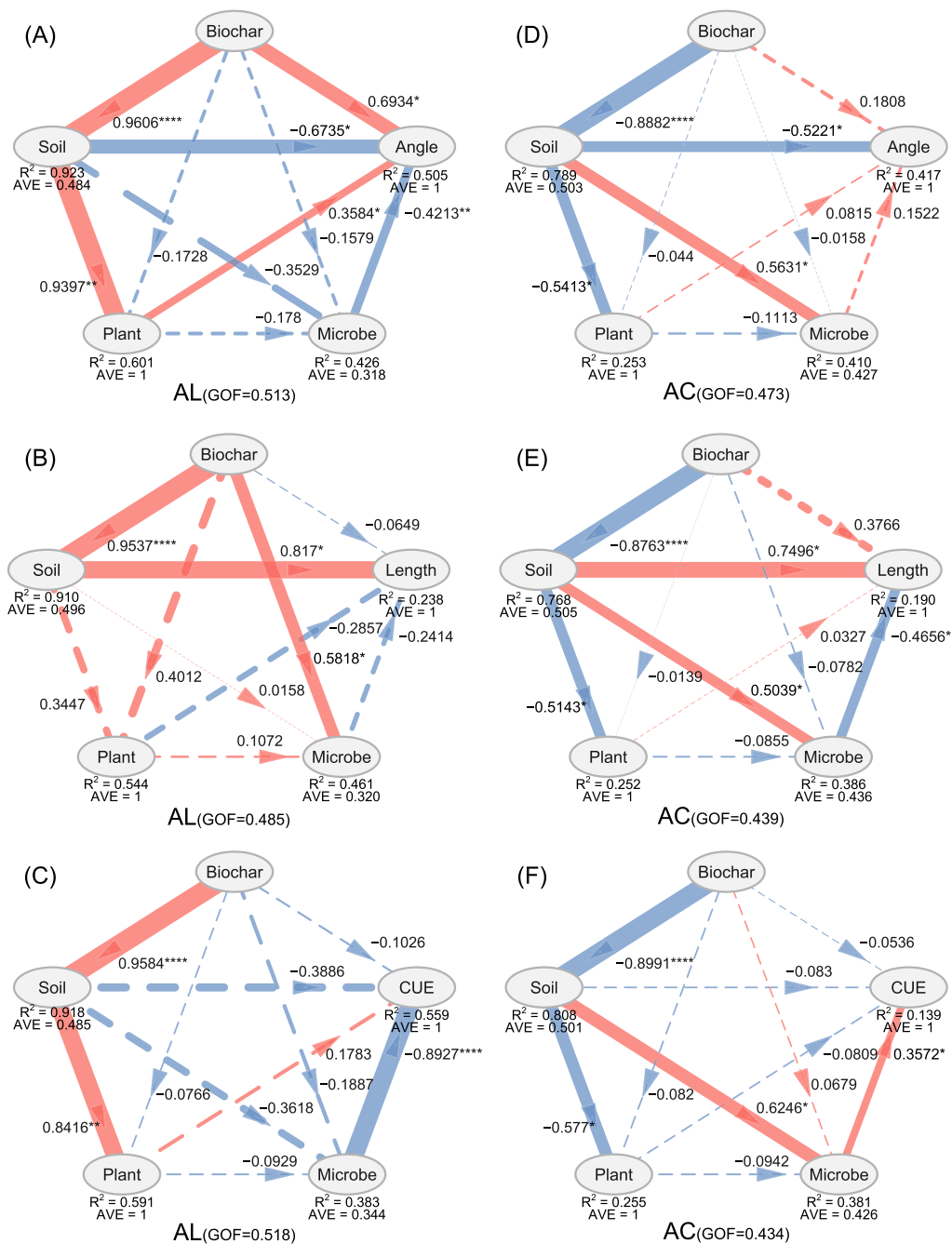


Fig. 6 Partial least squares path modeling illustrating the direct and indirect effects of AL- and AC-biochar amendments on vector angle (AL: A, AC: D), vector length (AL: B, AC: E), and microbial carbon use efficiency (AL: C, AC: F). GOF: goodness of fit. AVE: average variance extracted. The red solid line indicates a statistically significant positive relationship, while the blue solid line indicates a statistically significant negative relationship. The thickness of the solid line and the numerical values displayed in the arrows represent the standard path coefficients. The asterisks indicate the level of significance: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. The dashed line indicates non-significant paths ($p > 0.05$). The R^2 value close to the variables indicates the proportion of variance explained by the model for each dependent variable

In addition, the functional groups present in biochar, particularly phenolic compounds, carboxyl, and hydroxyl groups, can bind both substrates and extracellular

enzymes, potentially hindering enzyme–substrate interactions (Gul et al. 2015; Liao et al. 2022). This effect is particularly pronounced in AC-biochar, where the

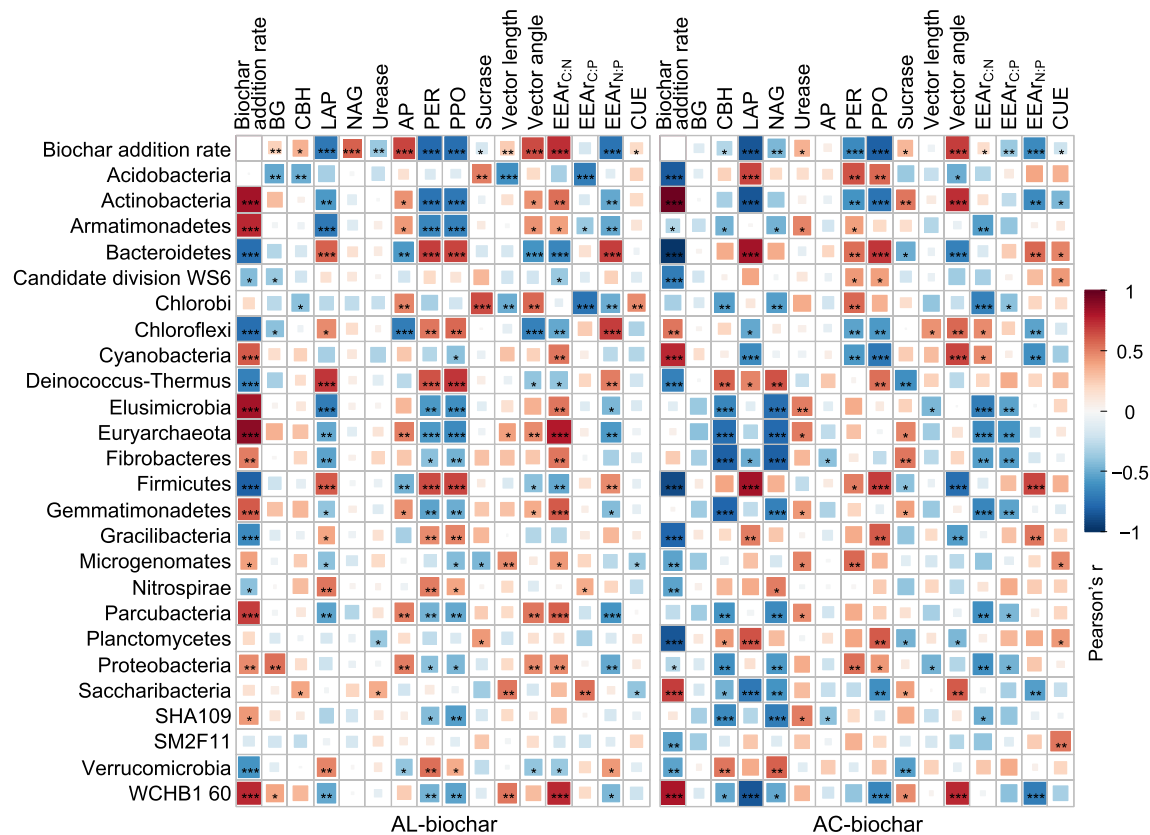


Fig. 7 Correlation of bacterial phyla with enzymatic activities, their stoichiometry, and carbon use efficiency. Asterisks indicate the significance level of Pearson's correlation: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

strong surface interactions with enzymes may hinder substrate diffusion to enzyme active sites. Interestingly, we observed that the degree of biochar-induced enzyme suppression is contingent on the type of biochar applied and its specific chemical characteristics, suggesting that different biochars affect microbial enzymatic activities through distinct mechanisms. These results contribute valuable insights into how biochar types and their chemical properties can shape soil microbial processes, but further studies are required to fully elucidate the underlying mechanisms driving enzyme sorption and inhibition. The variability in enzyme activities observed in our study underscores the need for more detailed investigations into how biochar amendments influence microbial nutrient dynamics and the broader soil biochemical environment.

4.2 Biochar-induced changes in microbial nutrient limitations and metabolic dynamics

Microbial enzyme production and activity are tightly coupled with nutrient availability, as microbes allocate resources toward enzyme synthesis under conditions of nutrient scarcity. In saline soils, which are often

characterized by nutrient deficiencies, microbial communities typically respond by ramping up enzyme production to mineralize and cycle limiting nutrients, thereby enhancing their availability for plant uptake (Qi et al. 2023; Shang et al. 2023). In this study, enzyme stoichiometry effectively reflected how biochar amendments modulate the allocation of carbon, nitrogen, and phosphorus by soil microbes in saline soils (Moorhead et al. 2016; Zhou et al. 2020).

We observed that the enzyme C:N ratio increased, while enzyme C:P and N:P ratios decreased with AL-biochar addition (Fig. 2). This suggests that biochar amendments under alkaline conditions shift microbial investment toward phosphorus-acquisition enzymes, likely in response to an increased microbial demand for phosphorus relative to carbon and nitrogen. This observation is consistent with the increase in soil N:P following AL-biochar application and the vector angle > 45° in enzyme stoichiometry plots (Fig. 3), which suggests that AL-biochar increased nitrogen bioavailability in soil, thus alleviating nitrogen limitation. However, it also appears to exacerbate phosphorus limitation for microbial metabolism. The elevated pH

in saline-alkaline soils amended with AL-biochar could have reduced phosphorus solubility, potentially forming insoluble calcium-phosphorus complexes that limit phosphorus availability to microbes (Cui et al. 2020; Ghodszad et al. 2022). Furthermore, the enhanced growth of the legume *Medicago sativa* L. (a nitrogen-fixing plant) in soils amended with AL-biochar may have intensified phosphorus limitation by further increasing nitrogen availability without concurrent phosphorus supplementation (Liu et al. 2018). These results highlight the importance of carefully considering nutrient availability, particularly phosphorus when applying AL-biochar to saline soils.

In contrast, the changes in enzyme C:N ratio followed an unimodal pattern with increasing AC-biochar addition rates, indicating a higher microbial demand for nitrogen compared to carbon and phosphorus. This was supported by the observed decrease in soil N:P following AC-biochar addition and the vector angle $< 45^\circ$ in enzyme stoichiometry plots under the 1% addition rate (Fig. 3). The higher C:N ratio of AC-biochar likely reduced nitrogen bioavailability compared to AL-biochar, which led to a lower microbial phosphorus demand and higher phosphorus mineralization potential (Guo et al. 2019). As the AC-biochar addition rate increased to 5%, the overall microbial nutrient limitations were alleviated, and a relative balance between nitrogen and phosphorus availability was achieved.

4.3 Enhancing microbial carbon use efficiency with AL-biochar amendment

Our study demonstrates that AL-biochar significantly alleviated microbial carbon limitation in saline-alkali soils, particularly at the 1% and 2% addition rates, as evidenced by the decreased vector length in C:N:P stoichiometry plots compared to the control (Fig. 3). This suggests that AL-biochar promotes microbial CUE, outperforming AC-biochar in this regard. This outcome deviates from our initial hypothesis, which posited that AC-biochar would better ameliorate saline-alkali soils by neutralizing soil pH and enhancing microbial activity. Instead, our data indicate that AL-biochar improved microbial CUE through increased availability of labile soil organic carbon, particularly from its aliphatic C–H, phenolic C–O, and polysaccharides components, which serve as energy sources for microbes to resist saline stress (Xu et al. 2023). In contrast, AC-biochar, which contains a higher proportion of recalcitrant carbon, provided fewer labile substrates for microbial metabolism, limiting microbial growth and reducing CUE. As the biochar addition rates increased, microbial biomass and respiration also increased, leading to higher carbon consumption and CO₂ loss which exacerbated carbon limitation, particularly in AL-biochar-amended soils (Keeler

et al. 2009). These findings highlight the importance of considering biochar addition rates to optimize microbial CUE and minimize carbon loss in saline soils.

Biochar amendments can also induce changes in the microbial community composition and functions, which in turn influence CUE (Khan and Joergensen 2019). Variations in microbial CUE are strongly influenced by factors such as the type and bioavailability of carbon substrate and the physiological and taxonomic characteristics of microbial communities (Dijkstra et al. 2011; Jones et al. 2018). For instance, microbial community shifts from r-strategists (which exhibit higher respiration and lower CUE, indicative of copiotrophic traits) to K-strategists (which show lower respiration and higher CUE, indicative of oligotrophic traits) can contribute to changes in microbial carbon limitation and CUE (Nannipieri et al. 2018; Yang et al. 2023).

Under saline stress, microbes expend considerable energy to synthesize osmoregulatory compounds to withstand saline stress, which limits their growth, reduces CUE, and impedes organic carbon sequestration (Elmajdoub and Marschner 2013; Khan et al. 2016). The observed increase in CUE under AL-biochar amendments indicates that microbes are utilizing available substrates more efficiently for net growth, investing more carbon into biomass production. This improvement in CUE is likely driven by the reduced environmental stress on microbes, as AL-biochar improves water and nutrient retention, modulates soil pH, and provides more favorable habitats for microbial communities (Pei et al. 2021; Kalu et al. 2024). Consequently, the appropriate biochar addition can enhance organic carbon storage and microbial CUE, alleviating carbon limitation in saline soils.

In this study, we found that microbial CUE was higher in AL-biochar-amended soils compared to AC-biochar-amended soils (Fig. 4). AL-biochar promotes the release of low molecular weight organic compounds, which reduce native soil organic matter decomposition and increase microbial CUE and potential carbon storage in saline soils compared to AC-biochar (Cheng et al. 2017; Giagnoni and Renella 2022). The higher CUE observed in AL-biochar-amended soils is likely due to the formation of more hydrophilic carbon pools, which are more favorable for microbial mineralization compared to the hydrophobic carbon found in AC-biochar amendments (Zheng et al. 2021).

From the thermodynamic perspective, the decomposition of recalcitrant carbon in AC-biochar-amended soils requires more energy from microbes. This energy is directed toward enzyme production for the breakdown of resistant carbon forms, rather than growth (Blagodatskaya and Kuzyakov 2008; Berggren

et al. 2010). These high metabolic costs associated with oxidative catabolic processes reduce microbial CUE in AC-biochar-amended saline soils. As a result, lower microbial CUE in these soils contributes to higher rates of CO₂ release through microbial respiration, which exacerbates carbon limitation (Manzoni et al. 2010). In contrast, AL-biochar, with its more labile carbon content, supports microbial growth and more efficient carbon use, reducing the need for excess energy expenditure on enzyme production and promoting higher CUE. Our results revealed a negative correlation between microbial CUE and carbon limitation, suggesting that improving CUE can help alleviate carbon constraints in saline soils. Additionally, microbial CUE was positively correlated with microbial phosphorus and nitrogen limitation under AL-biochar amendment, suggesting that AL-biochar supports a more balanced internal nutrient metabolism in soil microbes during carbon and nutrient acquisition (Liu et al. 2018).

5 Conclusion

This study elucidates the differential impacts of AL- and AC-biochar on soil extracellular enzyme activities, microbial nutrient limitations, and microbial CUE in saline-alkali soils. While both biochar types influence microbial nutrient dynamics, AL-biochar outperformed AC-biochar in enhancing microbial CUE, despite our initial hypothesis suggesting that AC-biochar would be more effective due to its potential for soil pH neutralization and salinity stress alleviation. AL-biochar shifted microbial nutrient metabolism from nitrogen to phosphorus limitation, while AC-biochar mitigated nitrogen limitation at higher application rates. AL-biochar's superior performance can be attributed to its higher labile carbon content and low surface adsorption capacity, which alleviated microbial carbon limitation and enhanced nutrient cycling. Additionally, biochar modulated soil biochemical responses through its indirect effects on soil properties, microbial communities, and plant traits. These findings underscore the importance of biochar physicochemical properties in determining its effectiveness for soil amendment, highlighting the need for targeted biochar selection to optimize nutrient cycling and carbon sequestration in saline-alkali soils.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s42773-025-00458-y>.

Supplementary file 1.

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Author contributions

Guorui Zhang: investigation, methodology, writing—original draft. Lan Zhang: investigation, data curation, methodology. Ziyue Shi: investigation. Yunfeng Yang: supervision, writing—review and editing, validation. Jie Liu: supervision, conceptualization, formal analysis, visualization, writing—original draft, funding acquisition.

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Data availability

The sequencing data were submitted to the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation (CNCB-NGDC) with accession number CRA027234 under BioProject PRJCA026895.

Declarations

Competing interests

The authors declare no competing financial interest.

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