

# Biochar-driven shifts in the soil bacterial community structure of degraded semiarid soils

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## ABSTRACT

• Low biochar rates (5 Mg ha<sup>-1</sup>) increased bacterial richness and diversity in degraded soil.

• Biochar origin controlled microbial assembly and niche specialization patterns.

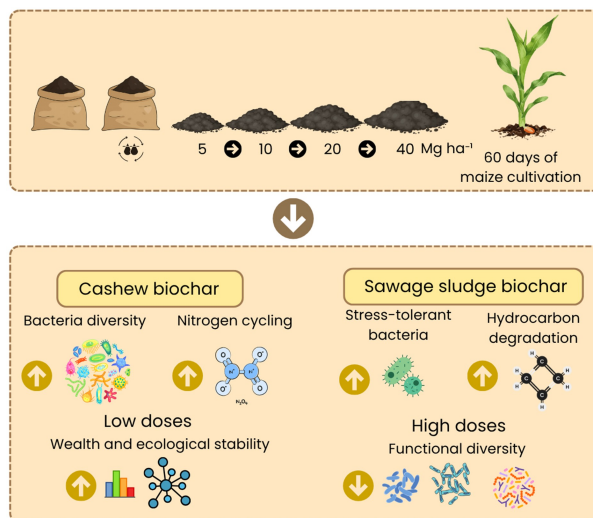
• Sewage-sludge biochar favored stress-tolerant and metal-adapted bacterial taxa.

• Cashew bagasse biochar enhanced nitrogen cycling and chemoheterotrophic functions.

• Network complexity peaked at intermediate biochar doses and declined at high rates.

Biochar has emerged as a nature-based solution to restore degraded drylands; however, its effects on soil microbiomes in semiarid and desertification-prone environments remain less understood. Here, we evaluated how biochar origin and application influence the structure of bacterial communities in a highly degraded Planosol from the Brazilian Caatinga biome. A controlled experiment was conducted using two biochar types, cashew bagasse-derived biochar (CB) and sewage-sludge biochar (SSB), applied at four doses (5, 10, 20, and 40 Mg ha<sup>-1</sup>), plus an unamended control (0 Mg ha<sup>-1</sup>), followed by 90 days of maize cultivation. Biochar changed bacterial community composition, with the emergence of distinct microbial assemblages depending on biochar type and dose. A single low dose (5 Mg ha<sup>-1</sup>) of biochar was sufficient to enhance bacterial richness and Shannon diversity, while SSB promoted the dominance of stress-tolerant taxa (e.g., *Bacillus*). Functional prediction and co-occurrence networks revealed that CB promoted functions related to nitrogen cycling and chemoheterotrophy, whereas SSB favored metal-tolerant and hydrocarbon-degrading taxa. Network complexity increased with biochar addition, especially at intermediate SSB doses. Overall, CB promoted more stable ecological responses, while sewage-sludge biochar caused stronger shifts. Our results demonstrate that biochar origin and application rate are critical to microbial restoration, i.e., moderate doses stimulate diversity, ecological specialization, and soil multifunctionality, whereas higher inputs, especially of nutrient- and metal-rich biochars, may compress functional traits. Plant-derived biochars, particularly CB, demonstrated potential for restoring soil bacterial structure and resilience in degraded semiarid landscapes when applied at appropriate rates.

**Keywords** bacterial diversity, dryland ecosystems, Caatinga, 16S rRNA sequencing, soil restoration, microbial ecology



## 1 Introduction

Drylands cover ~38% of the Earth's surface, supporting ~36% of the global population and providing numerous valuable ecosystem services (Wang et al., 2022). However, dryland areas face limitations such as water scarcity and low-quality soils (Mak-Mensah et al., 2023), typically characterized by low organic carbon, weak water retention, and reduced biological activity, which make them vulnerable to degradation, mainly exacerbated by climate variability and human activities, ultimately leading to degradation (United Nations, 1994).

Particularly in Brazil, the dryland ecosystem is mainly covered by the Caatinga biome, which represents ~11% of the Brazilian territory, being characterized by high temperatures, irregular rainfall, vulnerable soils, and significant economic vulnerability (Mattar et al., 2018; Alvalá et al., 2019). Importantly, the Caatinga biome and its biodiversity, support the livelihoods of ~28 million people through ecosystem services, food production, and natural-resource-based economic activities. However, the microbial diversity in Caatinga is yet poorly known, which brings concern about its conservation (de Araujo Pereira et al., 2021). Indeed, the microbial community in this biome is crucial to soil functioning due to its role in nutrient cycling and ecosystem services (Silva et al., 2024b). The Caatinga biome has been facing degradation during the last two decades, and efforts of restoration have been applied with positive effects on soil microbial communities and functionality (da Silva et al., 2017; Albuquerque et al., 2020; de Araujo Pereira et al., 2021, 2022; Araujo et al., 2024).

To assess soil microbial communities in the Caatinga soils, researchers have applied DNA sequencing to investigate their relationship with degradation and restoration (Araujo et al., 2023). Thus, previous studies have shown significant findings regarding microbial richness, diversity, composition, and functionality (Lacerda-Júnior et al., 2019; de Araujo Pereira et al., 2021, 2022; da Costa et al., 2022; Silva et al., 2024a, 2024c). However, these studies have assessed the effect of restoration strategies applied for decades, such as grazing exclusion, which focuses on avoiding grazing and allowing natural revegetation (de Araujo Pereira et al., 2022).

On the other hand, applied nature-based solutions, such as biochar, have emerged as a promising strategy to mitigate these effects faster than grazing exclusion (do Nascimento et al., 2023) since C-rich materials can improve soil quality, supply nutrients, and enhance biological activity (Lehmann and Joseph, 2015). Particularly, biochar, produced from the pyrolysis of organic materials, is rich in organic matter and presents high porosity that retains water and provides a

favorable habitat for microorganisms (Wong and Ogbonaya, 2021; Huang et al., 2023). Indeed, previous studies have shown that the application of biochar can positively alter soil microbial communities, promoting greater complexity and stability (Zhou et al., 2019; Nan et al., 2020). In the Caatinga soils under desertification and restoration, a previous study showed that biochar increased the microbial biomass and promoted higher enzymatic activity, consequently promoting organic matter decomposition by the microbiota (da Silva et al., 2021). Recently, Barbosa et al. (2024) showed key soil enzymes, including  $\beta$ -glucosidase, urease, and phosphatases, with high activity following biochar application (Barbosa et al., 2024). In addition, stoichiometric analyses indicated that biochar alleviated microbial carbon and nitrogen limitations but increased phosphorus limitations, suggesting that biochar amendments in highly degraded soils may require supplemental phosphorus to sustain optimal microbial function (Barbosa et al., 2024).

These biochars are produced from agro-industrial and sewage residues through pyrolysis, representing a waste-to-resource technological strategy that converts problematic organic by-products into stable soil amendments. However, the effects of biochars with contrasting physical and chemical properties on bacterial communities in desertified soils remain poorly resolved. In particular, biochars derived from plant residues and from sewage sludge differ markedly in nutrient load, metal content, and carbon stability, which may impose distinct environmental filters on bacterial communities. Therefore, we hypothesized that nutrient- and metal-rich sewage-sludge biochar will preferentially select stress-tolerant and specialist taxa through strong nutrient pulses and metal-associated stress, potentially leading to functional convergence and simplified interaction networks. In contrast, carbon-rich, plant-derived biochar from cashew bagasse is expected to promote generalist taxa and more stable, modular microbial networks by providing heterogeneous microhabitats and a slower-release carbon source. To test this hypothesis, we evaluated how biochar origin and dose influence bacterial community structure, diversity, functional potential, and co-occurrence patterns in a highly degraded semiarid soil using 16S rRNA gene sequencing combined with functional prediction and network analyses.

## 2 Materials and methods

### 2.1 Soil collection and characterization

The soil studied was collected from Aroeira Farm in Iruçuaba municipality, Ceará state, Brazil, with the following coordinates: 3°46'16.0" S and 39°49'49.7" W (Fig. S1). Iruçuaba is characterized by Bshw (Köppen's classification)

as a tropical hot semiarid climate. The region is affected by desertification due to intense anthropogenic activities combined with natural conditions, such as low precipitation levels and high-water evaporation rates (Araujo et al., 2024).

The studied soil was classified as Planosol according to the WRB/FAO classification system (Oliveira Filho et al., 2019). Before starting the experiment, a soil characterization analysis was carried out (Table 1). Afterwards, all analyses were conducted during the plant harvest (Table 2). Briefly, the soil pH was evaluated using a 1:2.5 soil-to-water extract. Soil organic carbon (SOC) was measured using the potassium dichromate digestion method in an acidic medium, followed by titration with ferrous ammonium sulfate (Yeomans and Bremner, 1988). Soil available phosphorus (P) and sodium ( $\text{Na}^+$ ) were determined through the Melich-1 extractor ( $0.05 \text{ mol L}^{-1} \text{ HCl} + 0.012 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ ) and quantified via colorimeter (P) and flame photometry ( $\text{Na}^+$ ) as proposed by Teixeira et al. (2017). The exchangeable cations, aluminum ( $\text{Al}^{3+}$ ), calcium ( $\text{Ca}^{2+}$ ), and magnesium ( $\text{Mg}^{2+}$ ) values were quantified by extraction with a potassium

chloride solution ( $1 \text{ M KCl}$ );  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  and quantified via atomic absorption spectrometry;  $\text{Al}^{3+}$  was quantified by titration with  $0.025 \text{ M NaOH}$  according to Teixeira et al. (2017). The determination of the semi-total contents of iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) in the soil that was the object of study was carried out according to method 3050B (USEPA, 1996).

Bulk density (BD) was measured using the volumetric ring method, calculating the mass ratio of dry soil at  $105^\circ \text{C}$  and ring volume (Blake and Hartge, 1986). Particle size was measured using the pipette method (clay fraction), sieving (sand), and the silt fraction, considering the total mass of the soil sample used for the analysis minus the sum of the sand and clay fractions. Sodium hydroxide ( $1.0 \text{ mol L}^{-1} \text{ NaOH}$ ) was used for the chemical dispersion of particles (Gee and Bauder, 1986).

## 2.2 Biochar production and characterization

The biochars used in this experiment were produced

**Table 1** Soil properties (0–10 cm) of the highly degraded soil (Planosol) collected at Irauçuba, Ceará, Brazil.

| Chemical |                                                      |                                                     |                                                         |                                                         |                                                         |                                             |                             |                              | Physical                               |             |             |             |
|----------|------------------------------------------------------|-----------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------|---------------------------------------------|-----------------------------|------------------------------|----------------------------------------|-------------|-------------|-------------|
| pH       | $\text{Na}^+$<br>( $\text{cmol}_c \text{ kg}^{-1}$ ) | $\text{K}^+$<br>( $\text{cmol}_c \text{ kg}^{-1}$ ) | $\text{Ca}^{2+}$<br>( $\text{cmol}_c \text{ kg}^{-1}$ ) | $\text{Mg}^{2+}$<br>( $\text{cmol}_c \text{ kg}^{-1}$ ) | $\text{Al}^{3+}$<br>( $\text{cmol}_c \text{ kg}^{-1}$ ) | H+Al<br>( $\text{cmol}_c \text{ kg}^{-1}$ ) | C<br>( $\text{g kg}^{-1}$ ) | P<br>( $\text{mg kg}^{-1}$ ) | Soil density<br>( $\text{g cm}^{-3}$ ) | Sand<br>(%) | Silt<br>(%) | Clay<br>(%) |
| 5.1      | 0.08                                                 | 0.09                                                | 6.97                                                    | 0.46                                                    | 0.54                                                    | 2.52                                        | 6.07                        | 8.45                         | 1.60                                   | 76.0        | 17.0        | 7.0         |

Source: Barbosa et al., 2024.

**Table 2** Soil characterization (0–10 cm) of highly degraded soil (Planosol) collected in Irauçuba, Ceará state, Brazil, after completion the end of the experiment.

| Treatments | pH                | TOC ( $\text{g kg}^{-1}$ ) | TN ( $\text{mg kg}^{-1}$ ) | Fe ( $\text{mg kg}^{-1}$ ) | Cu ( $\text{mg kg}^{-1}$ ) | Mn ( $\text{mg kg}^{-1}$ ) | Zn ( $\text{mg kg}^{-1}$ ) | P ( $\text{mg kg}^{-1}$ ) | BD ( $\text{g cm}^{-3}$ ) |
|------------|-------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|---------------------------|
| Control    | $5.13 \pm 0.05$   | $8.28 \pm 0.85$            | $84.00 \pm 0.25$           | $84.17 \pm 7.38$           | $5.33 \pm 0.93$            | $17.84 \pm 0.44$           | $1.23 \pm 0.16$            | $5.72 \pm 0.10$           | $1.60 \pm 0.09$           |
| SSB 5      | $5.26 \pm 0.04$   | $14.05 \pm 0.80^*$         | $112.00 \pm 0.85^*$        | $83.83 \pm 2.59$           | $4.20 \pm 1.30$            | $18.70 \pm 0.86$           | $1.76 \pm 0.16^*$          | $6.72 \pm 0.28$           | $1.58 \pm 0.08$           |
| SSB 10     | $5.44 \pm 0.08^*$ | $7.14 \pm 0.51$            | $112.00 \pm 0.80^*$        | $79.08 \pm 2.28$           | $2.54 \pm 1.09^*$          | $18.93 \pm 0.27$           | $2.14 \pm 0.14^*$          | $10.63 \pm 0.57^*$        | $1.56 \pm 0.06$           |
| SSB 20     | $5.68 \pm 0.19^*$ | $8.97 \pm 0.91$            | $126.00 \pm 0.85^*$        | $69.32 \pm 0.75$           | $3.18 \pm 1.30^*$          | $15.05 \pm 2.79$           | $2.68 \pm 0.08^*$          | $14.93 \pm 0.93^*$        | $1.46 \pm 0.04$           |
| SSB 40     | $5.74 \pm 0.17^*$ | $12.54 \pm 0.62^*$         | $140.00 \pm 0.85^*$        | $55.21 \pm 3.99^*$         | $2.01 \pm 0.88^*$          | $10.98 \pm 2.00^*$         | $3.52 \pm 0.20^*$          | $22.92 \pm 0.32^*$        | $1.57 \pm 0.08$           |
| CB 5       | $5.12 \pm 0.04$   | $12.38 \pm 0.42^*$         | $84.00 \pm 0.39$           | $75.43 \pm 9.10$           | $4.35 \pm 0.46$            | $15.99 \pm 1.82$           | $1.06 \pm 0.15$            | $5.86 \pm 0.18$           | $1.54 \pm 0.07$           |
| CB 10      | $5.17 \pm 0.10$   | $9.52 \pm 0.25$            | $105.00 \pm 0.58^*$        | $69.85 \pm 2.34^*$         | $3.03 \pm 1.86^*$          | $17.27 \pm 0.96$           | $1.07 \pm 0.14$            | $5.48 \pm 0.19$           | $1.54 \pm 0.07$           |
| CB 20      | $5.48 \pm 0.04^*$ | $9.21 \pm 0.05$            | $133.00 \pm 0.71^*$        | $56.78 \pm 1.80^*$         | $3.63 \pm 0.52^*$          | $14.48 \pm 1.32^*$         | $0.97 \pm 0.09$            | $7.28 \pm 0.47^*$         | $1.59 \pm 0.05$           |
| CB 40      | $5.83 \pm 0.24^*$ | $12.78 \pm 0.15^*$         | $171.50 \pm 0.48^*$        | $62.40 \pm 7.95^*$         | $3.80 \pm 0.46^*$          | $13.42 \pm 0.85^*$         | $0.97 \pm 0.08$            | $7.90 \pm 0.39^*$         | $1.55 \pm 0.04$           |

Significance level

|                      |    |    |    |    |    |    |    |    |    |
|----------------------|----|----|----|----|----|----|----|----|----|
| A (Types biochar) ** | ** | ** | ** | ** | ns | ** | ** | ** | ns |
| B (Doses biochar) ** | ** | ** | ** | ** | ** | ** | ** | ** | ns |
| A × B                | ** | ** | ** | ** | ns | ** | ** | ** | ns |

SSB5 = Sewage sludge biochar dose  $5 \text{ Mg ha}^{-1}$ ; SSB 10 = Sewage sludge biochar dose  $10 \text{ Mg ha}^{-1}$ ; SSB 20 = Sewage sludge biochar dose  $20 \text{ Mg ha}^{-1}$ ; SSB 40 = Sewage sludge biochar dose  $40 \text{ Mg ha}^{-1}$ ; CB 5 = Cashew biochar dose  $5 \text{ Mg ha}^{-1}$ ; CB 10 = Cashew biochar dose  $10 \text{ Mg ha}^{-1}$ ; CB 20 = Cashew biochar dose  $20 \text{ Mg ha}^{-1}$ ; CB 40 = Cashew biochar dose  $40 \text{ Mg ha}^{-1}$ ; pH = Hydrogenionic potential; TOC = Total organic carbon; TN = Total nitrogen; Fe = Semitotal iron available in soil; Zn = Semi-total available zinc in soil; Cu = Semitotal available copper in soil; Mn = Semitotal available Mn in soil; P = Available phosphorus; SD = Soil density. \* represents the significant difference between the treatment mean and the control mean by the Tukey's test at 5% significance.  $\pm$  Standard deviation; ns = non significantly. A × B × C = Interaction between Types biochar (A) × Doses biochar (B) × Control (C); \*\* represents the significance of the interaction by Tukey's test at 5% and 1% ( $p < 0.05$  and  $p < 0.01$ ).

through pyrolysis of cashew (*Anacardium occidentale*) agro-industrial waste (pseudofruit) and sewage sludge, and their characterization is described in [Barbosa et al. \(2024\)](#). Briefly, cashew bagasse and a 1:1 mixture of sewage sludge with pruning residues were used as feedstock for biochar production. Cashew underwent pyrolysis at 500 °C for 3 hours and 10 minutes, while the 1:1 mixture of sewage sludge and pruning residues was pyrolyzed for 1 hour and 37 minutes ([Barbosa et al., 2024](#)). Cashew bagasse was selected due to its local abundance and waste management challenges in the cashew industry of Northeast Brazil.

Nutrient contents in the biochar were quantified using modified dry ash extraction, as suggested by [Enders and Lehmann \(2012\)](#). Phosphorus content was determined by the colorimetric molybdenum vanadate-phosphoric acid method and quantified using a spectrophotometer at 400 nm ([Teixeira et al., 2017](#)). Calcium, magnesium, aluminum, iron, manganese, and zinc levels were determined using inductively coupled plasma optical emission spectrometry (ICP-OES), while potassium and sodium levels were measured by flame photometry. Total nitrogen content was obtained via sulfuric acid digestion and determined using the Kjeldahl method ([Mendonça and Matos, 2017](#)). Chemical and physical characterization is presented in [Table 3](#).

### 2.3 Experimental design

A completely randomized design was used, consisting of a  $2 \times 4$  factorial arrangement with two biochar sources, i.e., sewage-sludge biochar (SSB) and cashew-bagasse-derived biochar (CB), and four application doses (5, 10, 20, and 40 Mg ha<sup>-1</sup>), plus an additional unamended control (0 Mg ha<sup>-1</sup>). Each treatment had four replicates, totaling 36 experimental units. The biochar doses were selected based on a maximum dosage of 2% (w/w), considered the limit for soil biochar application ([Novotny et al., 2015](#)). Additionally, the biochar application rates were defined based on previous research demonstrating consistent agronomic and environmental benefits. Several studies have reported increases in soil quality and crop productivity when biochar is applied at rates ranging from 5 to 50 Mg ha<sup>-1</sup> ([Major, 2010](#); [Jeffery et al., 2011](#); [Liu et al., 2013](#)). Thus, the dose of 5 Mg ha<sup>-1</sup> was used, progressively doubling to 10, 20, and 40 Mg ha<sup>-1</sup>, keeping the doses below the upper limit of 50 Mg ha<sup>-1</sup> mentioned in the literature.

The experiment was conducted in a greenhouse under controlled temperature and humidity conditions, with a temperature maintained at 27 °C and a humidity level of approximately 85%. PVC columns with a diameter of 20 cm and a height of 50 cm were filled with degraded soil. Biochar was incorporated into the soil and incubated for 30 days. Maize (*Zea mays* L., variety BRS 2022) was cultivated in this experiment. Soil fertilization was applied to each column

as follows: urea (837 mg), simple superphosphate (4433.6 mg), and KCl (418.6 mg) at 25 days before plant emergence ([Fernandes et al., 1993](#); [Barbosa et al., 2024](#)). Furthermore, additional fertilization with urea (837 mg) and KCl (209.3 mg) was made at 25 and 45 days after emergence, respectively ([Fernandes et al., 1993](#); [Barbosa et al., 2024](#)).

Each column was equipped with tensiometers with mercury manometers at a depth of 0.2 m to measure matric potential. Readings were taken twice daily (early morning and afternoon). Matric potential values were converted into moisture levels using the soil water retention curve (SWRC) specific to each treatment. Irrigation was based on available water capacity (AWC), calculated as the difference between soil moisture at field capacity (FC) and permanent wilting point (PWP) ( $AWC = FC - PWP$ ). Distilled water irrigation was initiated whenever 30% of the AWC was depleted, as indicated by soil moisture readings. When needed, the amount of water required to raise soil moisture to FC was calculated using the SWRC. The experiment lasted 90 days.

### 2.4 Soil sampling and analysis

Soil samples were collected from a depth of 0–10 cm. Subsamples (100 g) were air-dried, sieved (2 mm), and homogenized for soil chemical analysis, which were conducted according to the characterization procedures described in subsection 2.1. Other subsamples were stored at –20 °C for DNA-based analysis.

#### 2.4.1 Next-generation sequencing

Total soil DNA was extracted from 0.5 g of the soil sample using a DNeasy PowerSoil kit (Qiagen®) following the manufacturer's protocol. DNA quality was verified by spectrophotometry (Nanodrop ND-1000). The V4 region of the 16S rRNA gene was amplified using the 2X Kapa HiFi Hot Start Ready Mix (Roche, Pleasanton, CA, USA) with the primer set 515F-Y (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVHHHTWTCTAAT-3') ([Caporaso et al., 2011](#)), with the following program: 95 °C for 3 min, followed by 35 cycles at 98 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s, and 72 °C for 5 min. The second indexing PCR was performed using the Nextera XT v2 set A index kit (Illumina, San Diego, CA, USA). PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) and quantified by Qubit fluorometer with the DNA BR Assay kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Additionally, the libraries were sequenced using the Illumina MiSeq Reagent Kit v2 (300 cycles,  $2 \times 150$  bp) at the Genomics and Bioinformatics Center (CeGenBio) of the Federal University of Ceará, Brazil.

### 2.4.2 Statistical and bioinformatics analysis

Before ANOVA, data were checked for normality and homoscedasticity using Shapiro–Wilk and Levene’s tests, respectively. Raw sequences were first trimmed using cutadapt (Martin, 2011; Bandara et al., 2022) to remove primers and low-quality bases, after which reads were processed using DADA2 pipeline (Callahan et al., 2016) implemented in QIIME 2 environment (v2025.4, available at the website of qiime2.org) using default parameters. This workflow included quality filtering, error modeling, denoising, paired-end read merging, and chimera removal. A total of 31 million quality reads were obtained at a median frequency of 861838 reads per sample from 28189 unique amplicon sequence variants (ASVs). Taxonomic assignment was performed using a naïve Bayes classifier trained on the above primer pair to classify ASVs’ taxonomies using the Silva 138 database (Quast et al., 2013), enabling high-resolution identification of bacterial lineages.

Relative abundance profiles of the bacterial community were assessed at the phylum and class levels based on ASVs generated by the DADA2 pipeline. Taxonomic assignments, and relative abundances were calculated by normalizing ASV counts to the total number of sequences per sample. Only taxa with a mean relative abundance  $\geq 1\%$  across samples were individually displayed, while less abundant groups were pooled into an “Others” category. Stacked bar plots were generated to visualize shifts in bacterial community composition across biochar types and application rates, allowing comparative assessment of dominant bacterial groups under sewage-sludge biochar (SSB) and cashew bagasse-derived biochar treatments. Alpha diversity was assessed using Observed ASVs (richness) and the Shannon diversity index, calculated in QIIME 2 from the ASV feature table. Differences in bacterial community structure among treatments were evaluated using non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity matrices calculated from ASV relative abundances. Statistical differences in community structure were tested by two-way permutational multivariate analysis of variance (PERMANOVA), considering biochar source and application rate as fixed factors (Ramette, 2007). In addition to the global ordination including all treatments, separate NMDS analyses were generated for sewage sludge biochar and cashew biochar treatments to better visualize treatment-specific community patterns. Pairwise correlations between soil physical and chemical properties, microbial biomass indicators, enzyme activities, and the relative abundance of dominant bacterial taxa were assessed separately for each biochar type. Correlation analyses were performed at both phylum and class levels, using the relative abundance data of taxa retained after quality filtering. Correlation coefficients were calculated using Spearman’s rank correlation, given

**Table 3** Chemical and physical characterization of biochar from cashew bagasse residues and the 1:1 mixture of sewage sludge and pruning residue.

| Attributes | Unit                | Cashew | Sewage sludge |
|------------|---------------------|--------|---------------|
| pH         | H <sub>2</sub> O    | 9.6    | 9.1           |
| C          | g kg <sup>-1</sup>  | 480.10 | 348.00        |
| N          | g kg <sup>-1</sup>  | 27.09  | 24.45         |
| C/N        | –                   | 17.72  | 14.20         |
| P          | g kg <sup>-1</sup>  | 11.62  | 17.70         |
| Na         | g kg <sup>-1</sup>  | 0.35   | 4.09          |
| K          | g kg <sup>-1</sup>  | 7.71   | 6.10          |
| Ca         | g kg <sup>-1</sup>  | 1.95   | 19.30         |
| Mg         | g kg <sup>-1</sup>  | 4.54   | 7.30          |
| Cu         | mg kg <sup>-1</sup> | 51.0   | 170.0         |
| Fe         | mg kg <sup>-1</sup> | 768    | 15300         |
| Mn         | mg kg <sup>-1</sup> | 45.0   | 390.0         |
| Zn         | mg kg <sup>-1</sup> | 59.0   | 1390          |
| Cd         | mg kg <sup>-1</sup> | nd     | 1.0           |
| Cr         | mg kg <sup>-1</sup> | 2.0    | 40.0          |
| Mo         | mg kg <sup>-1</sup> | 1.0    | 10.0          |
| Ni         | mg kg <sup>-1</sup> | 4.0    | 23.0          |
| Pb         | mg kg <sup>-1</sup> | 1.0    | 16.0          |
| Al         | g kg <sup>-1</sup>  | 1.35   | 26.8          |
| Density    | kg m <sup>-3</sup>  | 546.7  | 277.1         |

Source: Adapted from Barbosa et al., 2024. nd = Below the limit of quantification.

the non-normal distribution of microbial community data. Only statistically significant correlations ( $p < 0.05$ ) are highlighted in the heatmaps. Visualization was performed using a color gradient ranging from negative (red) to positive (blue) correlations.

Niche specialization patterns of bacterial taxa were evaluated using the CLAM (Classification Method) approach, which classifies taxa into habitat specialists, generalists, or rare taxa based on their relative abundance and frequency across contrasting environments. In this case, we compared bacterial community specialization between the control soil and each biochar treatment, separately for sewage-sludge biochar (SSB) and cashew bagasse-derived biochar (CB) at increasing application rates (5, 10, 20, and 40 Mg ha<sup>-1</sup>). Taxa were classified as specialists, generalists, or rare using the CLAM method with the default likelihood threshold of 0.8. Taxa were categorized as rare when their total abundance and occurrence frequency across samples were below the method detection boundary defined by the CLAM algorithm, indicating insufficient information for reliable habitat preference classification. The relative proportions of each ecological category were then used to assess how biochar type and dose influenced bacterial niche breadth and

community assembly patterns. This analysis was conducted in R using the vegan package implementation of the CLAM test (Chazdon et al., 2011).

The functional potential of bacterial communities was inferred using the FAPROTAX (Functional Annotation of Prokaryotic Taxa) database, which assigns putative ecological functions to bacterial taxa based on well-established links between taxonomy and metabolic capabilities described in cultured representatives. Taxonomic profiles at the class level were used as input for FAPROTAX annotation. The resulting functional categories include processes related to carbon cycling (e.g., fermentation, hydrocarbon degradation, photoautotrophy), nitrogen cycling (e.g., nitrogen fixation, nitrate reduction, and respiration), sulfur and iron metabolism, and trophic strategies (e.g., chemoheterotrophy, phototrophy, predatory or parasitic lifestyles). Functional profiles were subsequently normalized and visualized to compare patterns across biochar sources and application rates (Louca et al., 2016). Also, statistical differences between treatments were evaluated using the Kruskal–Wallis test followed by Dunn's post hoc test with Benjamini–Hochberg correction. Pairwise comparisons between each dose and the control were additionally assessed using Wilcoxon rank-sum tests. Functions selected for visualization were based on statistical significance. Boxplots were used to represent the distribution of functional abundances treatments.

Microbial co-occurrence networks were constructed to evaluate how biochar source and application rate influenced the structure, complexity, and interaction patterns of soil bacterial communities. Separate networks were generated for the control and for each biochar dose (5, 10, 20, and 40 Mg ha<sup>-1</sup>), considering cashew bagasse-derived biochar and sewage-sludge biochar independently. Pairwise correlations among ASVs were calculated using Spearman's rank correlation coefficients. Only robust and statistically significant correlations were retained ( $|p| \geq 0.6$  and  $p < 0.01$ ), following false discovery rate (FDR) correction to control for multiple comparisons. Positive and negative correlations were interpreted as potential cooperative and competitive interactions, respectively. The resulting adjacency matrices were used to construct undirected networks. Network visualization and layout optimization were performed using the Fruchterman–Reingold algorithm, which spatially organizes nodes based on their connectivity patterns, facilitating the identification of densely connected modules and dominant interaction clusters. Network complexity was named based on multiple topological properties, including average degree ( $K$ ), edge density, clustering coefficient, and network centralization metrics (degree, betweenness, and closeness), which together describe the connectivity, cohesion, and hierarchical organization of microbial co-occurrence networks

(Table S1). Co-occurrence networks were visualized and analyzed using Gephi software (Bastian et al., 2009).

### 3 Results

Biochar application promoted changes in several soil chemical parameters compared to the unamended soil, with some variables already responding at lower doses and stronger shifts observed at intermediate and higher doses (Table 2). The application of sewage-sludge biochar (SSB) resulted in higher soil Zn and P contents (2.68 and 14.93 mg kg<sup>-1</sup>, respectively) as compared with the cashew-bagasse biochar (CB). At the 40 Mg ha<sup>-1</sup> dose, SSB differed from CB by promoting higher soil P content (22.92 mg kg<sup>-1</sup>), whereas Cu and Mn content (2.01 and 10.98 mg kg<sup>-1</sup>, respectively) were lower than those obtained with CB application (3.80 and 13.42 mg kg<sup>-1</sup>, respectively).

The bacterial community composition changed in response to the application of biochar (Fig. 1). At the phylum level, Firmicutes, Proteobacteriota, Actinobacteriota, Acidobacteriota, and Chloroflexi dominated across all treatments, accounting for more than 75% of the total bacterial community (Fig. 1A). The relative abundance of Proteobacteriota and Firmicutes increased slightly, whereas Acidobacteriota and Actinobacteriota showed a reduction, with higher rates of biochar. Lesser-represented groups such as Planctomycetota, Verrucomicrobiota, and Gemmatimonadota showed subtle yet consistent variations depending on the biochar type. At the class level, Alphaproteobacteria, Gammaproteobacteria, and Bacilli were the most abundant classes, followed by Acidobacteriae, Actinobacteria, and Ktedonobacteria (Fig. 1B). The relative enrichment of Bacilli was more pronounced in soils receiving biochar at 10 Mg ha<sup>-1</sup>, particularly from sewage sludge biochar. In contrast, Acidobacteriae decreased progressively with biochar addition.

The alpha diversity metrics showed distinct responses of soil bacterial communities to biochar type and application rate (Fig. 2). The number of observed species significantly increased in the lower dose (5 Mg ha<sup>-1</sup>) of both biochar (Fig. 2A). In contrast, intermediate doses (20 Mg ha<sup>-1</sup>) led to a decline in richness, while the highest rate (40 Mg ha<sup>-1</sup>) promoted a strong recovery in diversity, surpassing the control. The Shannon index showed a more stable pattern, with moderate variations among treatments (Fig. 2B).

Non-metric multidimensional scaling (NMDS) showed clear distinctions in bacterial community composition among treatments (Fig. 3). The ordination stress value (0.129) indicated a reliable two-dimensional representation of community dissimilarities. According to the two-way PERMANOVA, both biochar source ( $F = 1.984$ ,  $p = 0.0248$ ) and dose ( $F =$

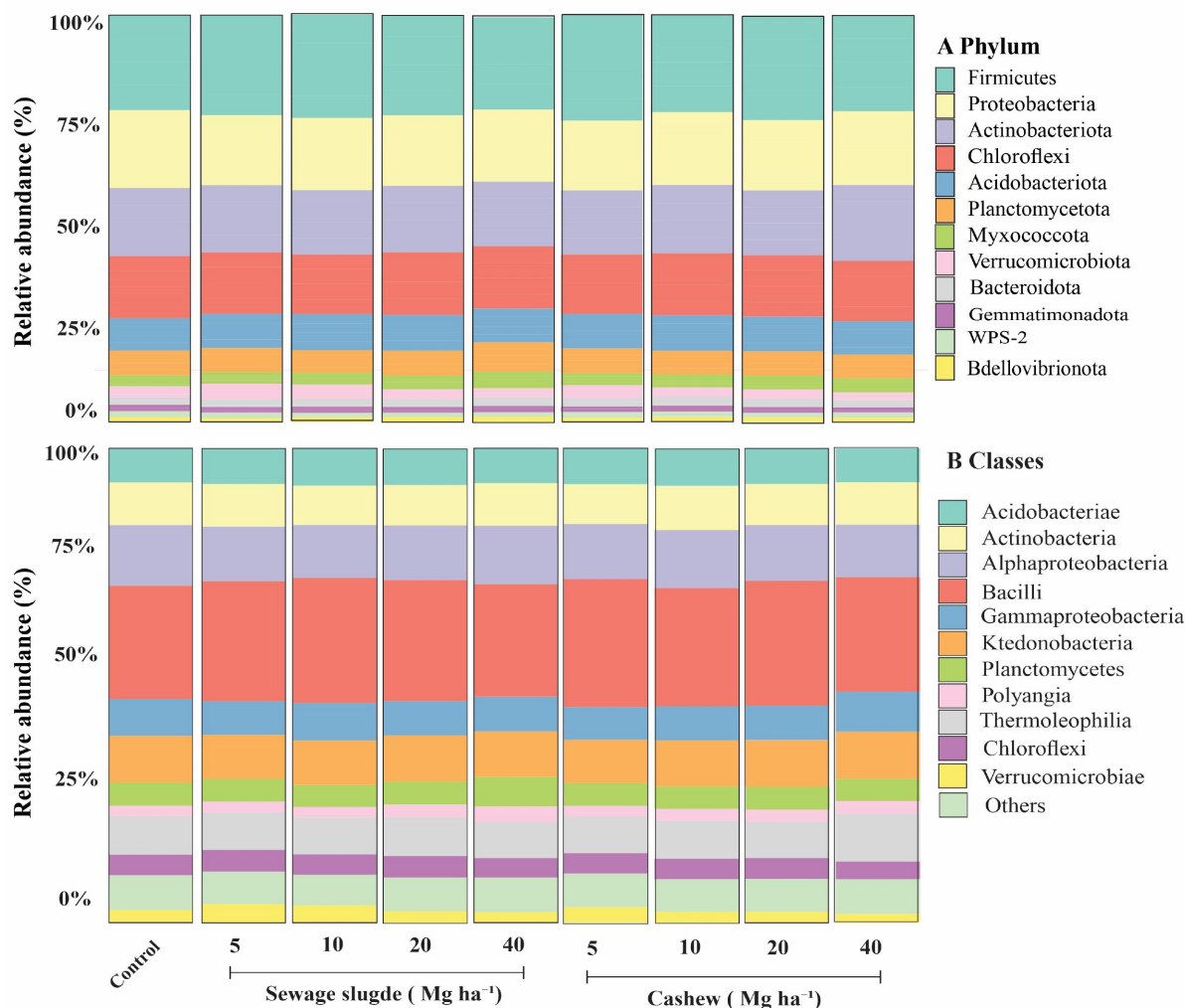


**Fig. 1** Relative abundance of dominant bacterial (A) phyla and (B) classes in soils amended with increasing doses of sewage sludge or cashew bagasse-derived biochar. The 10 most abundant taxa are shown, with minor groups grouped as “Others.” Bars represent the mean relative abundance across replicates for each treatment.

2.972,  $p = 0.001$ ) significantly influenced bacterial community structure. The control samples clustered distinctly from the amended soils, demonstrating the strong impact of biochar incorporation on bacterial assembly. Increasing doses of sewage sludge biochar promoted more pronounced community shifts, with the highest dose ( $40 \text{ Mg ha}^{-1}$ ) forming a separate cluster. In contrast, cashew biochar treatments were more dispersed, suggesting greater compositional heterogeneity and a milder influence on community restructuring.

To improve visualization of treatment-specific patterns, separate NMDS analyses were performed for sewage sludge and cashew biochar treatments (Fig. S2). In both cases, clear shifts in bacterial community structure were observed across application rates relative to the control. These patterns were supported by PERMANOVA, which indicated significant differences among treatments for sewage sludge biochar ( $R^2 = 0.34$ ,  $p = 0.001$ ) and cashew

biochar ( $R^2 = 0.39$ ,  $p = 0.001$ ). In brief, the cashew biochar treatment, samples tended to separate along NMDS1 with increasing application rates. In contrast, sewage sludge biochar showed a more heterogeneous distribution.

Spearman correlation analyses showed distinct associations between bacterial taxa and soil physicochemical and biochemical attributes depending on the biochar source (Fig. 4). Under sludge-derived biochar, members of Firmicutes, Actinobacteriota, and Verrucomicrobiota showed significant positive correlations with nutrient-related parameters, such as total N, available P, and microbial biomass carbon (Fig. 4A, 4C). Conversely, Acidobacteriota and Chloroflexi were negatively associated with pH and nutrient indicators. At the class level, Bacilli, Gammaproteobacteria, and Verrucomicrobiae were positively correlated with enzyme activities (acid phosphatase, urease, and  $\beta$ -glucosidase). In contrast, soil treated with cashew-derived biochar exhibited a weaker overall correlation pattern, with fewer significant

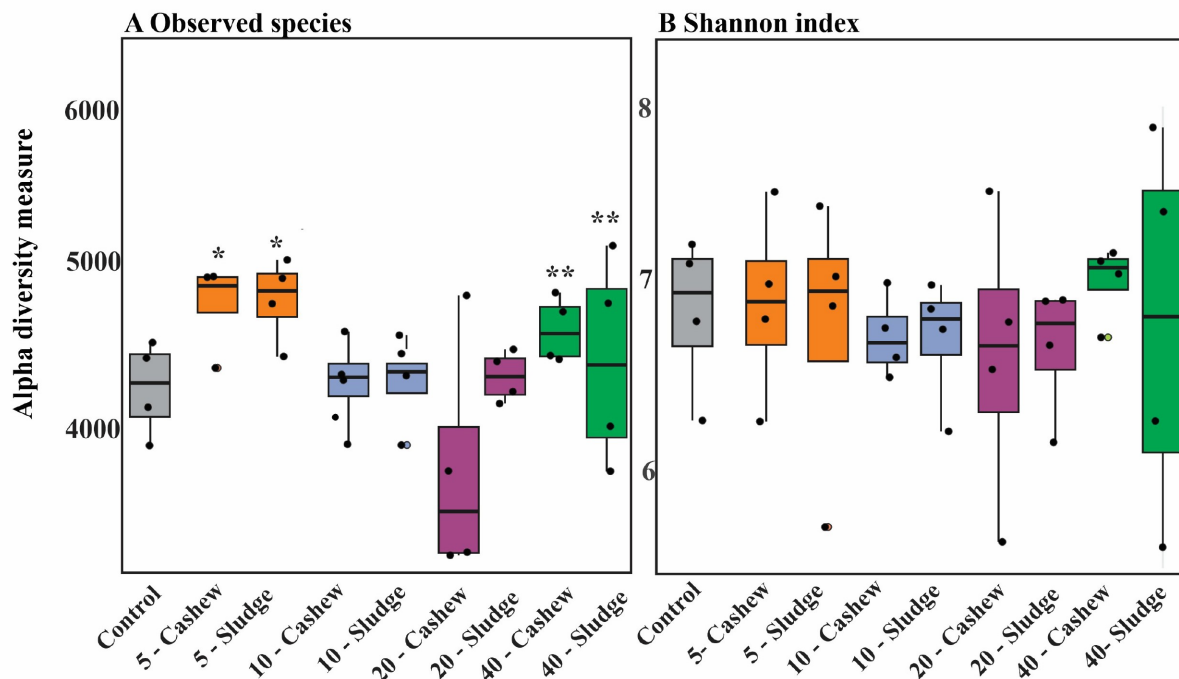


**Fig. 2** Alpha diversity responses of soil bacterial communities under different doses of sewage sludge (Sludge) and cashew bagasse-derived (Cashew) biochar. (A) Observed species richness (number of ASVs) and (B) Shannon diversity index across all treatments. Boxplots represent median, interquartile range, and outliers for each treatment and significant differences compared to the control indicated by asterisks (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).

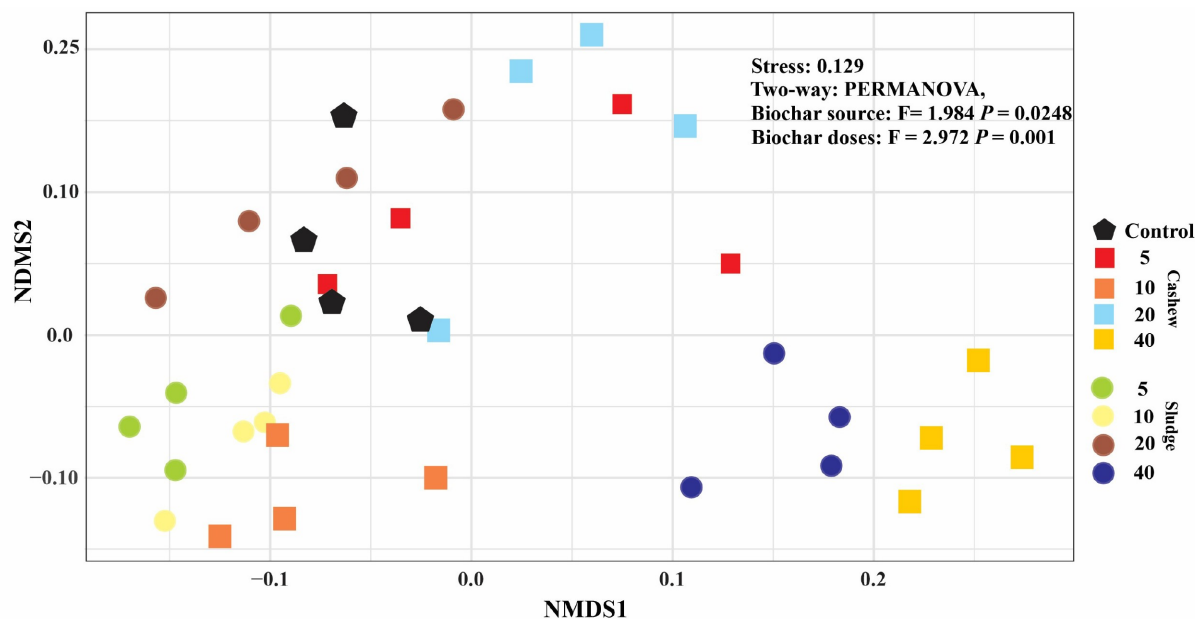


**Fig. 3** Non-metric multidimensional scaling (NMDS) ordination based on Bray–Curtis dissimilarity under different doses and sources of biochar derived from sewage sludge and cashew bagasse. Each point represents one biological replicate, with colors indicating biochar dose and shape denoting biochar type or control. Ellipses represent 95% confidence intervals for the 40 Mg ha<sup>-1</sup> treatments. The stress value (0.129) indicates a good ordination fit. Two-way PERMANOVA revealed that both biochar source ( $F = 1.984$ ,  $P = 0.0248$ ) and biochar dose ( $F = 2.972$ ,  $P = 0.001$ ) significantly influenced bacterial community structure.

associations (Fig. 4B, 4D). Nonetheless, Bacilli and Alphaproteobacteria maintained positive relationships with carbon and nitrogen fractions, while Thermolephilia and Acidobacteriae were negatively associated with most

biochemical parameters.

The CLAM analysis revealed consistent shifts in ecological specialization patterns across treatments, indicating that biochar addition altered the balance between generalist,

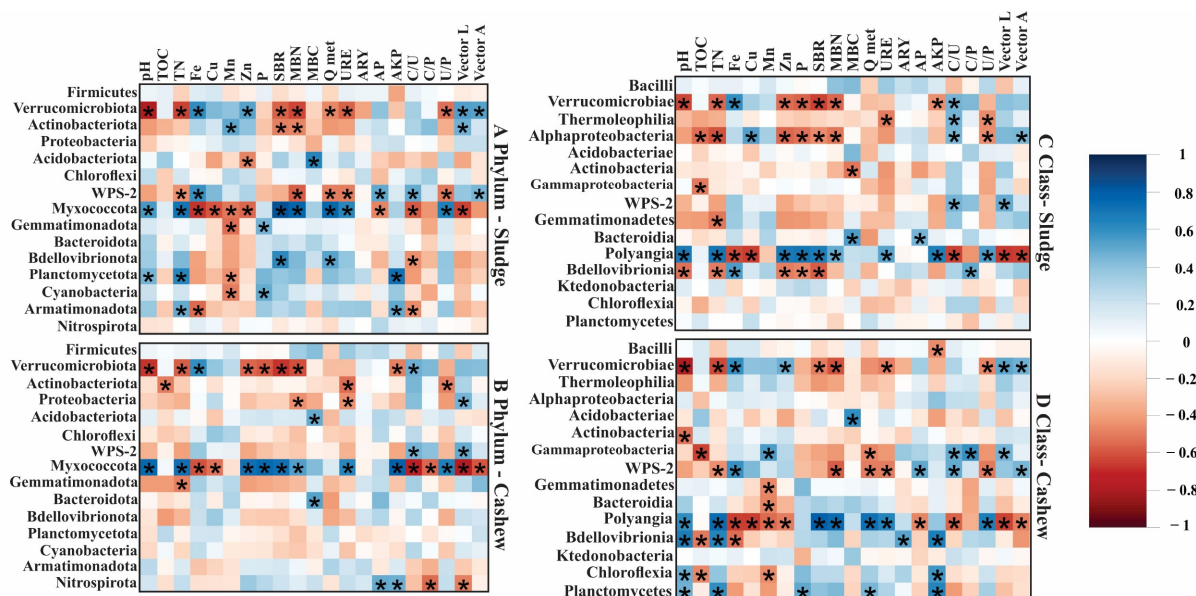


**Fig. 4** Spearman correlation heatmaps between soil bacterial taxa (at the phylum and class levels) and physicochemical and biochemical soil attributes under different biochar sources. Panels (A) and (B) show correlations for phyla in soils amended with sludge and cashew-derived biochar, respectively; panels (C) and (D) represent correlations at the class level for the same treatments. Blue and red colors indicate positive and negative correlations, respectively, with the intensity proportional to the correlation coefficient. Asterisks denote significance levels (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).

specialist, and rare bacterial taxa (Fig. 5). In control treatments, the majority of ASVs were classified as generalists, representing over 55% of the total bacterial community. The proportion of specialist taxa increased progressively with biochar application, particularly under higher doses of sewage sludge-derived biochar, reaching up to 24.8% at 20 Mg ha<sup>-1</sup>. Sludge biochar tended to favor the emergence of specialist groups. In contrast, cashew biochar maintained a higher proportion of generalists and a relatively stable share of rare taxa.

Functional annotation based on FAPROTAX indicated that the predicted metabolic potential of bacterial communities was modulated by both biochar type and application rate (Fig. 6; Figs. S3–S4). In the control treatment, functions related to chemoheterotrophy, aerobic chemoheterotrophy, and nitrogen cycling were predominant. The application of sewage sludge biochar resulted in moderate shifts in functional profiles, with a general tendency toward reduced chemoheterotrophic functions at intermediate doses (10–20 Mg ha<sup>-1</sup>), followed by partial recovery at the highest dose (40 Mg ha<sup>-1</sup>). Functions associated with aromatic compound degradation showed a decreasing trend with increasing dose, although differences among treatments were not consistently significant. In contrast, cashew biochar induced more pronounced functional changes. Core metabolic functions such as chemoheterotrophy and aerobic chemoheterotrophy were reduced at intermediate doses, particularly at 20 Mg ha<sup>-1</sup>, and recovered at the highest dose. Nitrogen fixation followed a similar pattern, with lower values at intermediate doses compared to the control. Additionally,

fermentation increased markedly at 40 Mg ha<sup>-1</sup>. In general, several functions exhibited non-linear responses to biochar addition, with reductions at intermediate doses and partial recovery at higher doses.

Co-occurrence network analysis showed strong structural reorganization of the bacterial community under biochar amendment (Fig. 7). The control network showed moderate connectivity (2167 total edges), dominated by positive associations (1778 edges), reflecting balanced interactions among microbial taxa. With the application of sewage sludge biochar, network complexity initially increased, reaching a maximum at 20 Mg ha<sup>-1</sup> (3781 total edges), characterized by a predominance of positive correlations (> 90%). However, at the highest dose (40 Mg ha<sup>-1</sup>), the network became less connected, with fewer edges and a reduction in clustering. In contrast, cashew biochar generated smaller and more modular networks across all doses, with the highest connectivity observed at 20 Mg ha<sup>-1</sup> (3205 edges). Although positive interactions dominated, a relatively greater proportion of negative correlations was detected compared to sludge biochar.

To provide a more comprehensive assessment of network complexity, additional topological properties were evaluated (Table S1). Consistent with edge-based patterns, sewage sludge biochar at 20 Mg ha<sup>-1</sup> exhibited the highest average degree (37.81) and edge density (0.19). In contrast, the reduction in these metrics at 40 Mg ha<sup>-1</sup> supports a threshold effect, where excessive inputs constrain network connectivity. Cashew-derived biochar promoted more moderate increases in connectivity while maintaining higher modularity.

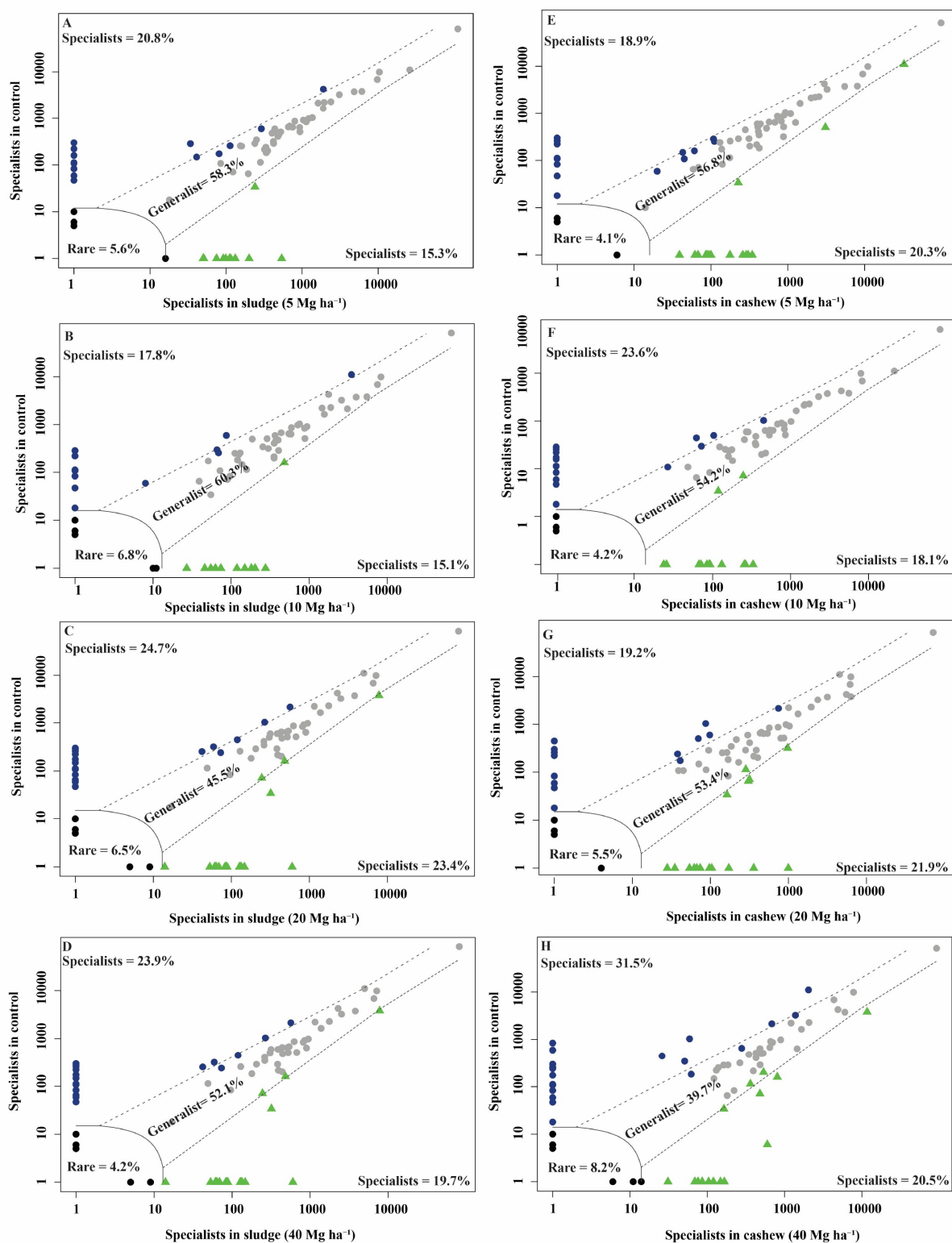


**Fig. 5** CLAM classification of bacterial ASVs comparing the unamended control and each biochar-amended treatment (SSB and CB at 5, 10, 20, and 40 Mg ha<sup>-1</sup>). Each point represents an ASV, plotted by its relative abundance (or frequency) in the control (x-axis) versus the biochar treatment (y-axis). ASVs are categorized as control specialists (points enriched toward the x-axis), biochar specialists (points enriched toward the y-axis), generalists (points distributed near the 1:1 diagonal), and rare/unclassified taxa (points with insufficient abundance/occurrence for reliable classification under the CLAM threshold). Symbols and/or fill styles in the plot correspond uniquely to these four categories.

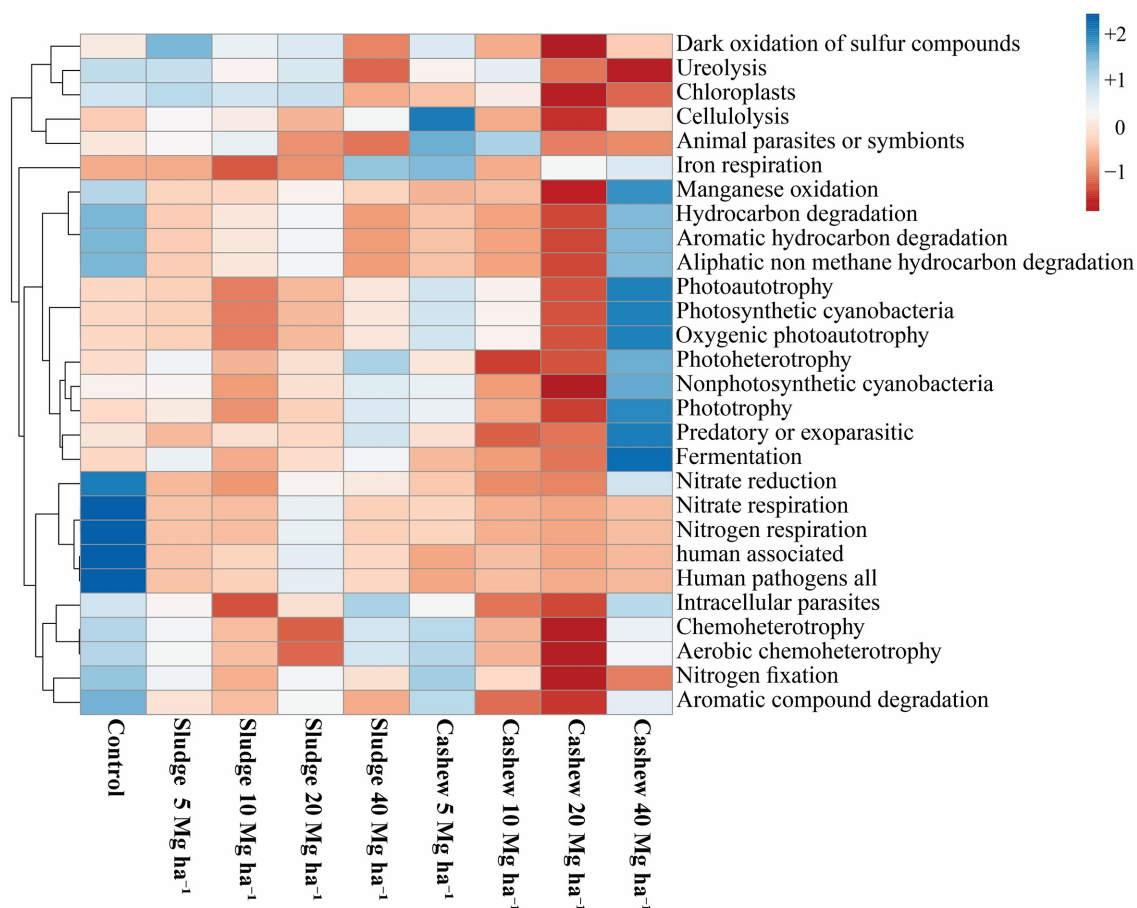


**Fig. 6** Functional prediction of bacterial communities based on FAPROTAX analysis under increasing doses of sewage sludge and cashew-derived biochar. The heatmap displays the relative abundance of predicted functional groups related to key microbial processes involved in nutrient cycling, respiration, and organic compound degradation. Colors indicate functional enrichment levels (blue: higher; red: lower) relative to the control, illustrating distinct metabolic potentials associated with biochar type and application rate.

Moreover, the higher centralization observed under sewage sludge biochar indicates a more hierarchical structure, potentially driven by a subset of highly connected nodes, whereas cashew biochar maintained a more distributed network topology.

## 4 Discussion

This study demonstrated that biochar source and dose act as distinct environmental filters shaping bacterial community assembly in degraded semiarid soils. By directly contrasting two biochars with different physical and chemical properties, we showed that nutrient- and metal-rich sewage-sludge biochar and carbon-rich cashew bagasse biochar drive divergent microbial assembly pathways. Sewage-sludge biochar imposed chemical filtering, favoring stress-tolerant and specialist taxa and promoting sharper community restructuring, whereas cashew-derived biochar generated milder, resource-heterogeneous conditions that supported

generalist taxa, higher modularity, and more stable interaction networks. These contrasting responses support our hypothesis that biochar origin determines not only the magnitude but also the mechanism of bacterial community reorganization.

Our results showed that a 5 Mg ha<sup>-1</sup> biochar dose, regardless of the source, significantly enhanced bacterial diversity and richness, as indicated by the Shannon index and observed species. This enhancement results from several attributes of biochar, including the provision of carbon sources, which can serve as both energy reservoirs and microhabitats for microbial colonization (Ali et al., 2025). A previous study showed the ability of biochar to act as a microbial spot, mitigating environmental stresses, such as moisture fluctuations, high temperatures, and nutrient limitations, and consequently promoting coexistence and community stability (Mikiciuk et al., 2024).

The application of SSB (20 Mg ha<sup>-1</sup>) promoted an increase in the dominance of stress-tolerant taxa such as *Bacilli* (Belykh et al., 2024). This indicates that biochar

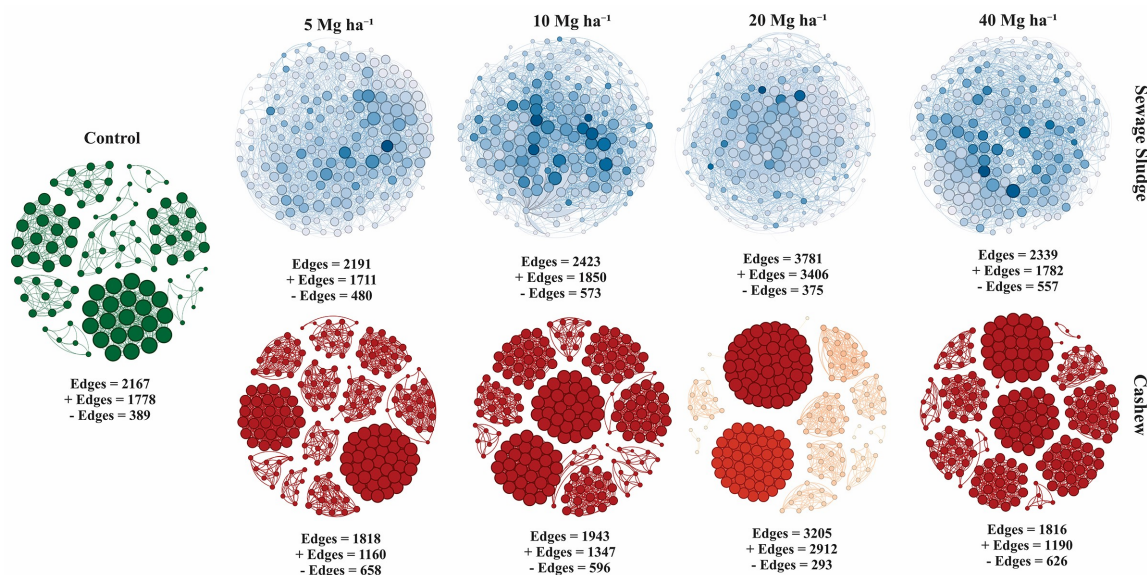


**Fig. 7** Co-occurrence networks of bacterial communities under increasing doses of sewage sludge and cashew biochar. Each node represents a bacterial ASV, and edges indicate significant positive (blue) or negative (red) correlations. Network complexity and connectivity varied with biochar type and dose, as shown by the number of total, positive, and negative edges. Larger node size denotes higher degree centrality, indicating the relative importance of taxa within the microbial network.

amendments showed a threshold effect, beyond which excessive nutrient and trace element inputs can create stressful or inhibitory conditions, leading to ecological filtering. Such filtering reduces evenness, compresses functional niches, and favors copiotroph or opportunistic taxa capable of tolerating elevated concentrations of metals and labile nutrients (Natasha et al., 2022).

Importantly, both biochars increased soil carbon contents and elevated pH through the addition of recalcitrant carbon and alkaline ash constituents (Table 2), thereby ameliorating key edaphic constraints on microbial colonization and activity (Singh et al., 2022). Accordingly, diversity and richness were positively associated with TOC and pH, indicating that these biochar-mediated shifts enhance microbial establishment, promote stable coexistence, and support greater functional redundancy (Guo et al., 2023). Conversely, trace metals and semi-total forms of Fe and Cu were negatively correlated with diversity and with the abundance of sensitive taxa, highlighting the importance of chemical moderation in biochar application to avoid detrimental effects on soil microbial communities (da Silva et al., 2021).

The slight increases in Proteobacteriota and Firmicutes with higher amendment rates, together with the small reductions in Acidobacteriota and Actinobacteriota, are consistent with a modest shift toward copiotrophic microbial strategies. Proteobacteriota and many Firmicutes are broadly regarded as copiotrophic, responding positively to enhanced nutrient availability and carbon inputs, whereas Acidobacteriota and several Actinobacteriota include predominantly oligotrophic groups that tend to decline under enriched resource conditions (Fierer et al., 2007; Leff et al., 2015).

The predominance of Firmicutes, particularly under SSB, suggests strong selection for copiotroph taxa able to utilize recalcitrant carbon and tolerate chemical stressors (Yadav et al., 2024). The observed increases in Actinobacteria and Proteobacteria at intermediate doses indicate that moderate biochar inputs can stimulate microbial taxa associated with organic matter decomposition and nutrient cycling, contributing to enhanced ecosystem functioning (Hu et al., 2024). Conversely, the decline of Chloroflexi under high SSB doses suggests the vulnerability of oligotrophic or stress-sensitive taxa to chemical imbalances and potentially toxic elements accumulation. The maintenance of *Conexibacter* and *Sphingomonas* under CB treatments highlights the role of biochar in supporting taxa involved in complex organic matter degradation, which is crucial for restoring soil biochemical processes and promoting long-term fertility (Wu et al., 2025).

At higher taxonomic resolution, Firmicutes and Proteobacteria showed positive associations with pH, TOC, and available phosphorus, while Chloroflexi declined with increasing SSB doses, showing functional replacement by copiotroph taxa under enriched conditions. *Bacilli* is considered stress-tolerant but also copiotrophic in many soils, responded positively to P and pH gradients, thriving in more fertile microhabitats created by SSB additions (Fierer et al., 2007). In contrast, Alphaproteobacteria and Actinobacteria exhibited a clear dependence on biochar type, performing better under moderate CB applications. This is consistent with their preference for chemically stable with moderate nutrient-enrichment environments that support their specialized oligotrophic strategies (Fierer et al., 2007; Yan et al., 2025).

Niche breadth and overlap analyses revealed that mode-

rate biochar applications expanded niche breadth and reduced overlap among dominant taxa, suggesting enhanced resource partitioning, microhabitat diversification, and reduced interspecific competition (Brtnicky et al., 2021; Sharma et al., 2025). In contrast, high SSB doses reduced niche breadth and increased overlap, indicative of functional compression. This pattern may arise from multiple mechanisms, including increased soil microporosity that homogenizes microbial habitats and reduces niche diversity (Premalatha et al., 2023; Deshoux et al., 2023), ultimately favoring the dominance of a limited number of tolerant taxa (Tian et al., 2025).

These observations were consistent with multivariate ordination analyses (NMDS), where high-dose SSB communities diverged significantly from the control and low-dose CB treatments, while intermediate CB doses clustered intermediate ordination positions, reflecting recovery of functional potential without disruption of community structure. The PERMANOVA results further confirmed significant effects of biochar type, dose, and their interaction on bacterial composition, reinforcing the notion that biochar effects are both dose- and origin-dependent (Chen et al., 2020).

The correlation analysis showed that both bacterial diversity and richness were positively associated with TOC and pH, suggesting that biochar-induced amelioration of edaphic conditions enhances microbial establishment, coexistence, and functional redundancy (Guo et al., 2023). Conversely, trace metals and semi-total forms of Fe and Cu were negatively correlated with bacterial diversity and with the abundance of sensitive taxa, highlighting the importance of chemical moderation in biochar application (da Silva et al., 2021). At higher taxonomic resolution, Firmicutes and Proteobacteria showed positive associations with pH, TOC, and available phosphorus, while Chloroflexi declined with increasing SSB doses, suggesting functional replacement by copiotroph taxa under enriched chemical conditions. Specific bacterial taxa, such as *Bacillus* and *Alicyclobacillus*, responded to P and pH gradients, thriving in more fertile microhabitats, whereas *Sphingomonas* and *Conexibacter* were more dependent on biochar type, performing better under moderate CB applications (Yan et al., 2025).

These results emphasize the critical importance of biochar source and dosage in shaping soil bacterial communities. Moderate biochar inputs can promote microbial diversity, maintain functional redundancy, and support ecosystem resilience, whereas excessive inputs, particularly of nutrient- and metal-rich biochars such as SSB, may impose chemical stresses that reduce community evenness and functional diversity (Zhao et al., 2022). Thus, these effects can be attributed to a combination of changes in carbon availability, nutrient dynamics, pH modulation, and metal load, all of which act as environmental filters governing microbial colonization and competitive interactions (Philippot et al., 2024).

The network analysis showed not just a numerical change, but a functional reorganization of the soil bacterial community driven by biochar addition. The stronger connectivity and predominance of positive interactions across treatments indicated a shift toward more synergistic microbial assemblages, which is consistent with the broader understanding that biochar improves microbial habitat quality and nutrient availability in degraded soils (Zhang et al., 2023). The non-linear response of network complexity under sewage-sludge biochar suggested a threshold in the balance between stimulation and stress. At intermediate doses (particularly 20 Mg ha<sup>-1</sup>), sludge-derived biochar likely increases the availability of labile nutrients and raises pH and this resource-driven release from limitation can increase niche opportunities and promote cross-feeding, leading to a denser network dominated by positive associations. In contrast, the decline in connectivity at 40 Mg ha<sup>-1</sup> is consistent with stronger chemical filtering under excessive inputs. High sludge-biochar additions increase nutrient and ash load and may intensify exposure to potentially toxic elements, which can reduce community evenness and exclude stress-sensitive taxa. Such exclusion tends to simplify the interaction landscape by concentrating the community around a smaller set of tolerant, fast-growing taxa, reducing the number of viable co-occurrences and weakening overall clustering (Zhang et al., 2023).

Cashew-residue biochar appears to enhance cohesion in the networks. Also, modular networks are often associated with increased ecological stability and resilience, suggesting that inputs derived from native plant residues may foster microbial niches more compatible with the existing soil microbiota than sewage-sludge biochar (Siles et al., 2021). Importantly, higher modularity indicated stronger compartmentalization of the community into sub-networks (modules) that may reflect microhabitat heterogeneity and resource partitioning promoted by plant-derived biochar (Siles et al., 2021). Biochar particles can create spatially structured niches (e.g., pores, surfaces, and gradients in moisture and carbon availability), allowing groups of taxa to interact more strongly within localized microenvironments than across the entire community. From an ecological perspective, modularity is often interpreted as a stabilizing feature because disturbances may remain partially confined within modules, reducing the probability of cascading interaction failures across the whole network.

Increases in average degree and edge density at intermediate biochar doses indicate enhanced network connectivity, which likely reflects greater niche availability and resource heterogeneity, facilitating microbial co-occurrence and interaction strength (Duan et al., 2023). This pattern is consistent with the alleviation of carbon and nutrient limitations observed in amended soils, promoting more interactive and functionally integrated communities. In contrast, the decline

in connectivity and clustering at the highest sewage sludge biochar dose suggests a shift toward stronger environmental filtering, where excessive nutrient and metal inputs constrain microbial coexistence and reduce the number of viable interactions (Yang et al., 2025). The higher centralization observed under sewage sludge biochar further indicates a more hierarchical network structure, potentially driven by a reduced number of highly connected taxa that dominate interaction patterns under chemically enriched conditions. Such configurations are often associated with lower functional redundancy and increased vulnerability to disturbance (Efthymiou et al., 2023). Conversely, the higher modularity and lower centralization observed under cashew-derived biochar suggest a more compartmentalized network organization, which may enhance ecological stability by buffering disturbances within modules and promoting niche differentiation (Xiao et al., 2024). This indicates that plant-derived biochar supports a more distributed and resilient interaction network, in contrast to the more centralized and potentially constrained networks observed under sewage sludge biochar at higher doses.

The functional profiling of microbial communities further supports the idea that biochar amendments promote a reorganization of bacterial functional potential in degraded soils. Under cashew biochar, core metabolic functions related to chemoheterotrophy and nitrogen cycling exhibited non-linear responses, with reductions at intermediate doses followed by recovery at the highest application rate (Li et al., 2026). This pattern suggests a transient shift in microbial resource use strategies, potentially reflecting adjustments to increased carbon availability and changes in substrate accessibility (Li et al., 2026). In contrast, sewage sludge biochar resulted in more moderate functional shifts, with a tendency toward reduced chemoheterotrophic activity at intermediate doses and relatively stable functional profiles across treatments. The overall response indicates that this biochar type may favor microbial communities with greater tolerance to nutrient and metal inputs, leading to less pronounced functional reconfiguration (Wu et al., 2025). Importantly, these functional patterns are consistent with the network analysis, where intermediate doses were associated with increased network complexity and reorganization, rather than simple functional enhancement (Wu et al., 2025). In general, these results highlight that biochar effects on microbial functioning are strongly dose-dependent and influenced by feedstock characteristics.

From a practical perspective, these findings have direct implications for the restoration of degraded soils. Biochar selection should consider not only the carbon content but also the source, nutrient load, and potential for potentially toxic elements accumulation (Joseph et al., 2021). Moderate application rates, especially biochars derived from plant residues such as cashew, appear optimal for promoting

microbial-mediated recovery, enhancing organic matter decomposition, and improving nutrient cycling, ultimately contributing to long-term soil fertility and ecosystem functioning. Furthermore, integrating microbial response monitoring with soil chemical and physical assessments can guide sustainable biochar-based soil management strategies, ensuring that amendments restore both microbial diversity and functional potential without inducing deleterious chemical stress (Luo et al., 2025).

## 5 Conclusions

The present study demonstrated that biochar application significantly influences the diversity, structure, and ecological functions of bacterial communities in highly degraded semiarid soils of the Brazilian Caatinga. Moderate doses of both cashew bagasse biochar (CB) and sewage sludge biochar (SSB) enhanced bacterial diversity, richness, and niche breadth, favoring the establishment of taxa associated with organic matter decomposition and nutrient cycling. However, high doses of SSB reduced richness and promoted the dominance of stress-tolerant groups, highlighting the risks of excessive inputs of nutrient- and metal-rich biochars. In contrast, CB showed more stable and consistent benefits across doses, reinforcing its potential as a sustainable alternative for microbial recovery in degraded soils. Overall, these results emphasize that both the origin and dosage of biochar are critical determinants of its ecological outcomes. When properly managed, biochar can restore microbial diversity, improve soil quality, and promote ecosystem resilience in semiarid regions. From a practical perspective, moderate application rates, particularly of agro-industrial-derived biochars such as cashew bagasse, represent a promising strategy to rehabilitate degraded soils and ensure long-term agricultural sustainability in the Brazilian semiarid. Looking forward, integrating microbial indicators with soil physicochemical and functional metrics may provide valuable early-warning tools for optimizing biochar-based restoration practices. Future studies should evaluate long-term field dynamics, interactions with vegetation, and the combined effects of biochar with other regenerative practices to fully realize the potential of biochar in dryland ecosystem recovery.

## Electronic supplementary material

Supplementary material is available in the online version of this article at <https://doi.org/10.1007/s42832-026-0461-0> and is accessible for authorized users.

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