

## RESEARCH ARTICLE

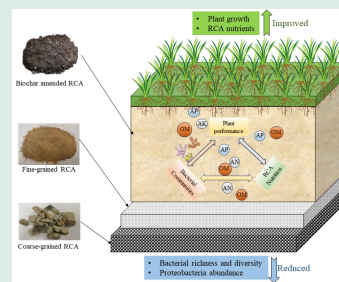
# Biochar effects on bacterial communities in recycled concrete aggregates

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## HIGHLIGHTS

- Biochar application enhanced the nutrients of recycled concrete aggregates.
- Higher salinity caused by biochar application inhibits urease and phosphatase activities.
- Biochar addition improves certain bacterial species that promote plant growth.



**ABSTRACT:** The substantial generation of building waste had precipitated significant environmental issues in China, attributable to urban expansion. Recycled concrete aggregate (RCA) produced from building waste had been used as an alternative material in green roofs as substrates. However, the biological dynamics of RCA are unknown. Thus, the column test was performed to investigate the effects of different biochar types and application ratios on the biochemical properties and bacterial communities of RCA. Results show that the nutrients of RCA have been improved by biochar addition regardless of biochar types and application ratios. This led to the increase in plant weight and height by at least 227% and 38% in biochar amended RCA. However, biochar application reduced bacterial richness and  $\alpha$ -diversity, while enhancing plant growth. This indicated that biochar application directly improves certain bacterial species that promote plant growth and productivity. Biochar application reduced the Proteobacteria abundance and enhanced the percentage of Acidobacteriota in RCA, indicating that biochar application shaped the bacterial communities into raw soil composition. The outcome of this research suggests that biochar application improved RCA biochemical performance as a substrate for green roofs, which provides a potential method to consume substantial RCA.

**KEYWORDS:** Recycled concrete aggregates, Biochar, Bacterial communities, Enzyme activities

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## 1 Introduction

One characteristic of sustainable green buildings is green roofs, which are established using soil and different types of plants on the roof (Berndtsson, 2010). Due to their ability to improve the urban environment by lowering rainfall runoff, lowering construction energy costs, absorbing toxic gases and particulate matter, fostering the growth of urban agriculture, etc., green roofs are becoming increasingly popular (Cao et al., 2014). Furthermore, enhancing green roof amenities can expand the availability of natural biological regions, improving urban biodiversity and creating new wildlife habitats (Oberndorfer et al., 2007). Substrate and plants were the two main components of green roof systems, and soil was typically the source of the substrate. Conventional green roofs possess the drawbacks of an extremely weighty substrate, restricted rainwater runoff interception, and frequently necessitate watering during dry season (Berndtsson et al., 2006). Identifying alternative materials to supplant conventional substrates in green roof systems is essential.

It has been known that the generation of a large amount of construction waste caused serious environmental problems in China, due to the development of cities (Yu, 2021). These concrete wastes are typically dumped in landfills, which poses a serious risk to human health and the environment (Safiuddin et al., 2013). Previous studies proposed an ecological treatment method for solid waste based on urease induced carbonate precipitation technology, finding that this method can convert heavy metal ions in solid waste into heavy metal carbonates and hydroxides, and generate calcium carbonate crystals (Bian et al., 2024a; 2024b; Chen et al., 2024a). In addition, these crystals can cement particles and improve the strength and hydraulic properties of solid waste, thereby impeding rainwater infiltration and providing nutrition environment for plant growth (Chen et al., 2021a; 2021b; 2024b; Guo et al., 2023; Liu et al., 2023; Wang et al., 2024). To improve the utilization of waste resources, the idea of replacing the soil of green roof substrate with recycled concrete aggregates (RCA) was proposed in the current study. Reports indicate that RCA exhibits greater porosity and water absorption, along with reduced strength relative to natural aggregates (Shi et al., 2016), rendering it a perfect material for green roof substrates as a substitute for soil. It has been reported that RCA has been used as a material substitute for natural materials instead of being disposed of in a landfill (Besklubova et al., 2023).

Furthermore, RCA combined with conventional granite aggregates has diminished mechanical characteristics and bulk density (Yap et al., 2018), potentially making it acceptable for soil replacement in green roof substrates. The water permeability of RCA would diminish because of the self-cementing properties. This may reduce rainfall water penetration and enhance the resilience of RCA-constructed infrastructure to climate change (Wang et al., 2023a). Moreover, the water permeability of RCA was normally related to the particle size distribution, mixed RCA with fine aggregate contents is suitable for the capillary barrier of the green roofs (Chen et al., 2024c; 2025). However, the elevated pH of approximately 12 and the deficient nutrient levels in RCA render it inappropriate for plant growth (Liu et al., 2022). Li et al. (2018a) discovered that the incorporation of 4.4 kg/m<sup>3</sup> urea fertilizer in RCA increased plant growth. It is necessary to identify suitable materials to substitute for fertilizer in plant development within RCA.

Biochar is a carbon-rich, stable materials generated through pyrolysis with limited oxygen and carbonization of biomass (Pardo et al., 2019). It possesses a well-defined pore architecture and considerable adsorption capacity, which can enhance soil structure, moisture retention, nutrient availability, and organic matter content (Shinogi and Kanri, 2003). It has been documented that biochar possesses the capability to adsorb a variety of pollutants due to its unique physicochemical properties (Qiao et al., 2023). Thus, the application of biochar in RCA might enhance the chemical properties of RCA and make it suitable for plant growth. Biochar has consistently been utilized on vegetated soil to alter nutritional characteristics and enhance plant performance (Wang et al., 2018). Moreover, biochar improves soil water-holding capacity, especially for salinity soil (Wang et al., 2023b). This suggested that biochar might be suitable for improving water retention in RCA. Besides, biochar application reduces soil saturated permeability due to the filling of biochar particles in soil into-aggregate pores upon liquid water transport (Chen et al., 2023). In addition, biochar can be customized to possess distinct surface physicochemical characteristics by selecting different kinds of feedstock and the pyrolysis procedure (Guo et al., 2023). This also contributed to the enhanced capability of pollutant absorption (Fan et al., 2025). Various feedstocks affect the characteristics of biochar, such as pH, ash content, resident matter, specific surface areas, and element composition (Singh et al., 2022). The advantage of biochar effects on plant performance depends on feedstock types and application ratios, as extensively reported in

agricultural studies (Gao et al., 2021). It has been observed that peanut shell biochar applied at a 5% ratio enhances the nutrient content of RCA and lowers its pH, hence providing more favorable conditions for the growth of *Schefflera arboricola* (Guo et al., 2023). However, the existing studies on the biological characteristics of bacterial communities in RCA with biochar application are somewhat restricted.

Comprehending the biological dynamics of urban soil microbiomes is becoming vital as cities enhance their green infrastructure projects to improve and restore ecosystem functions (Tzoulas et al., 2007). Bacterial communities show unique responses to environmental gradients. For instance, Li et al. (2023) have found that the phylum level composition of bacterial communities was predominantly comprised of Proteobacteria, Acidobacteria, Bacteroidetes, and Actinobacteria across five distinct green infrastructures. The bacterial diversity and richness exhibit significant variation in response to alterations in environmental conditions (Cui et al., 2023). As for rooftop farms, Acidobacteriota and Proteobacteria were the most dominant phyla (Dai et al., 2023). The substrates of mixed-vegetation and Sedum green roofs exhibited unique bacterial and fungal communities ( $p < 0.0001$ ), with mixed-vegetation roofs demonstrating a higher mycorrhizal fungi abundance, whereas Sedum green roofs displayed elevated pathogen species (Hoch et al., 2019). Biochar has often been used in green roofs to improve their nutrients, moisture content, and microbial community responses. Chen et al. (2018) indicated that the application of 10%–15% sludge biochar over the green roof resulted in the most substantial impact on microbial biomass, ranging from 63.9% to 89.6%.

Since RCA and biochar were applied for substrates in current study, it is critical that we better understand how stress (i.e., higher pH) and amendments jointly shape bacterial communities. Concurrently, we conducted 24 green roof systems with raw soil, RCA, or RCA amended with different biochar types and ratios as substrates. *Miscanthus* was selected for planting in those substrates due to its suitability for saline-alkaline land. The properties of substrates, plant performance, enzyme activities, and bacterial communities were analyzed. Furthermore, canonical correlation analysis was performed to identify the relationships between substrates properties and bacterial communities. Molecular ecological networks were used to examine how the biochar application ratios and types affects the complexity of bacterial communities in green roofs. Finally, the relationships between soil properties with bacterial communities and metabolites were determined.

## 2 Materials and methods

### 2.1 Biochar, RCA, and raw soil

Three distinct feedstocks were chosen for producing biochar, including wood, dairy manure, and perishable garbage. The feedstocks were placed to drying in an oven at 100 °C for 24 h until a consistent weight was attained. Subsequently, they were sieved through a 4.75-mm mesh for a uniform biochar particle size. The pyrolysis processes were conducted using a tube furnace, with the pyrolysis temperature maintained at 500 °C for 1 h (José et al., 2019). The pH and electrical conductivity (EC) of biochar were measured by pH meter and EC meter (ASTM, 2013). The properties of biochar were presented in Table S1. The RCA was generated from deconstructed concrete debris sourced from structures over 35 years old. In the current study, river sand and cement constituted the principal components of the RCA. The RCA was classified as clayey sand according to the Unified Soil Classification System (ASTM, 2011). The pH of RCA was 10, and the specific gravity was 2.45 g/cm<sup>3</sup>. The maximum dry density was 1670 kg/m<sup>3</sup>, which was obtained based on ASTM standard D698 (ASTM, 2012). A completely decomposed granite (CDG) was one type of soil that has been commonly used in Guangdong province (Ng et al., 2022). Thus, it has been selected for use in the control test.

### 2.2 Experimental design and sampling

A series of one-dimensional soil column studies were conducted outdoor to simulate the installation of green roofs on flat surfaces. Eight test conditions were established: RCA, CDG, RCA combined with 5% (w/w) and 10% wood biochar (WB5 and WB10), RCA supplemented with 5% and 10% dairy manure biochar (DMB5 and DMB10), and RCA enhanced with 5% and 10% perishable waste biochar (PWB5 and PWB10). The selection of biochar application ratio was referred to Guo et al. (2023). Three replicates were established for each test series, resulting in a total of 24 columns in this investigation. A drum mixer was employed to combine the RCA and biochar in the laboratory. To ensure the quality of RCA saturation, pressurized water spray was used. The drum mixer operated at a speed of 30 revolutions per minute and was mixed for approximately 2 h. The mixed samples were subsequently compressed within the column. The structure of column was presented in Fig. 1. Coarse-grained RCA (particle size approximately 5 mm) was

used at the bottom of the column to guarantee drainage efficiency. A 100 mm layer of fine-grained recycled concrete aggregate (particle size approximately 2.5 mm) was placed atop the coarse-grained recycled concrete aggregate. This ensures that less water may travel through. A mixed RCA with biochar was subsequently compacted in the column above the fine-grained RCA layer to a depth of 600 mm to ensure adequate space for plant root development. The mixed samples have been compacted in 100 mm thick layers to regulate compaction quality (Guo et al., 2023). Subsequently, *Miscanthus* with a root depth of  $150 \pm 5$  mm was transplanted into each column. *Miscanthus* was a promising species for phytoremediation and was particularly good for saline-alkaline soils (Xu et al., 2021), rendering it an ideal plant species for this study. The initial height of *Miscanthus* was around 350 mm, and any heights over 350 mm were pruned to that same height. After transplantation, all columns were watered with 2 L of deionized water every 48–72 h to maintain adequate moisture in the root zone up to 150 mm.

### 2.3 Test procedures

The substrate samples were obtained from the surface of the column at 100 mm depth. The samples were combined with water in a 1:10 ratio to ascertain the pH and EC using pH and EC meters (ASTM, 2013). The organic matter (OM) was quantified using the potassium dichromate oxidation technique with external heating (Tabatabai, 1996). The available nitrogen (AN) was measured by alkaline diffusion method (Mulvaney and Khan 2001). The available phosphorus (AP) was extracted by sodium bicarbonate solution and measured by molybdenum antimony colorimetric method (Yuan and Lavkulich, 1995). The available potassium (AK) was measured by ammonium acetate extraction flame photometer (Salomon and Smith, 1957). The dehydrogenases activity was measured by the Lenhard

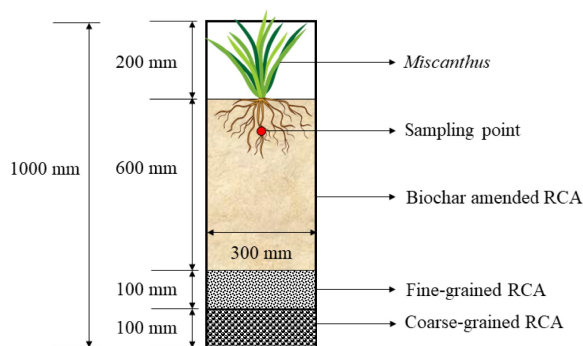
method, which has been modified by Casida et al. (1964). The activity of urease was assessed using the Gorin and Ching Chang method (Gorin and Chang 1966). The alkaline phosphatase activity was measured using the method from (Tabatabai and Bremner, 1969).

### 2.4 DNA extraction and sequencing

Soil total genomic DNA extraction was used by E.Z.N.A. Mag-Bind Soil DNA Kit from 0.5 g soil. The purity and concentration of DNA were evaluated using electrophoresis and NanoDrop 2000, respectively. Using genomic DNA as the template, PCR amplification was performed according to the selected sequencing regions, employing specific primers bearing barcodes and Premix Taq. The 16S V4 Region Primers (515F and 806R) were used for bacterial diversity identification. Electrophoresis of PCR products was conducted on a 1% agarose gel to assess the fragment length and concentration for further analysis. All PCR products were purified using Agencourt AMPure XP beads, reconstituted in Elution Buffer, and subsequently labeled to complete library assembly. The Agilent 2100 Bioanalyzer was utilized to assess library size and concentration. Qualified libraries were sequenced on the HiSeq platform based on their insert size. Raw data were filtered to yield high-quality clean data, which were subsequently combined into tags and further grouped into operational taxonomic units (OTUs) based on overlapping clean reads. The dilution curve flattened suggests that the diversity of samples remain unchanged with the increase of data volume (Fig. S1).  $\alpha$ -diversity,  $\beta$ -diversity, network analysis, and model prediction were conducted using the OTU profile table and taxonomic annotation data (Ng et al., 2023). Chao1 index was used for evaluating bacterial richness and Simpson index was used for assessing fungal diversity.

### 2.5 Statistical analysis

The bacterial richness and diversity were calculated based on Mothur (version v.1.30.1). Before conducting the ANOVA test, the data was assessed for normal distribution by Z-scores for skewness and kurtosis. If the Z-scores of skewness and kurtosis are smaller than 1.96, then the data was considered normal (Kim, 2013). One-way ANOVA followed by a Tukey comparison test was conducted in IBM SPSS Statistics 27. The Non-metric multidimensional scaling (NMDS) was used to analyze the soil bacterial  $\beta$ -diversity, which was performed in MAGIGENE. The canonical correspondence analysis (CCA) was performed by Wekemo Bioincloud to analyze the relationship



**Fig. 1** Profile view of the test setup.

between bacterial phylum and soil properties. The OTU of soil bacterial communities was used for network analysis. The parameters of the network structure, encompassing the quantity of nodes (i.e., species in a network) and linkages (i.e., interspecies relationships), were analyzed using the Molecular Ecological Network Analysis Pipeline (Wekemo Bioincloud). Subsequently, Gephi software was used to visualize the network and the properties of modules (i.e., the characteristics of functional units in an ecosystem).

### 3 Results

#### 3.1 Effects of biochar on RCA properties and plant performance

The addition of biochar to RCA chemical properties depended on the types and application ratio, as indicated in Table 1. Specifically, the pH of WB5 and PWB5 was significantly ( $p < 0.05$ ) higher than that of RCA. Biochar application enhanced the EC at least by 33% of RCA, especially in PWB10. It has been noted that any biochar application could enhance the OM of RCA. Additionally, the OM of DMB10 was 651% higher than that of RCA. A higher amount of biochar application causes higher OM. For example, the OM in WB10, DMB10, and PWB10 were higher than in WB5, DMB5, and PWB5, respectively. However, minimal changes were observed in the levels of AN when different biochar application ratios were applied. The CDG soil has the highest AN content. As for AP, the results were contrary. Biochar application enhanced AP in RCA regardless of biochar types and application ratios. The minimum AP was found in CDG, which was  $6.0 \pm 1.4$  mg/kg. There was a significant increase of AP in DMB10 compared with that in RCA. The highest AK was observed in DMB10, around  $1196.7 \pm 259.3$  mg/kg. Biochar application ratios have positive

effects on the AK of RCA. For instance, higher application ratios lead to higher concentrations of AK.

The effects of biochar types and application ratios on the plant weight and height were presented in Table 2. Plant weight was the highest in DMB10 (around  $395.3 \pm 91.0$  g), which was significantly ( $p < 0.05$ ) higher than that of RCA. However, the tallest height of the plant was found in WB5, which was 57% higher than that of RCA. It has been noted that a higher amount of biochar application ratio enhanced plant weight and height when DMB and PWB were applied. For example, plant performance in DMB10 and PWB10 were better than that in DMB5 and PWB5, respectively. However, the higher amount of WB application ratio inhibited plant growth. The weight and height of the plant were reduced by 26% and 14% in WB10 compared with that in WB5. Generally, the weight and height of the plant in biochar amended RCA was at least 227% and 38% higher than that in RCA.

#### 3.2 Effects of biochar on enzyme activities

The results of enzyme activities, including dehydrogenase, urease, and phosphatase, in different test conditions were presented in Fig. 2. It has been noted that the highest dehydrogenase activity was observed in DMB10, reached at 3.5 mg/(g·h) (Fig. 2(a)). Followed by DMB5, around 2.7 mg/(g·h). Only when DMB was applied in RCA, the dehydrogenase activity was higher than in raw soil (i.e., CDG). Higher amount of WB inhibited dehydrogenase activity. For example, this enzyme activity in WB5 was 16% higher than in WB10. However, the enzyme activity in PWB5 was 153% lower than in PWB10. The effects of different test treatments on urease activity were presented in Fig. 2(b). The highest urease activity was found in CDG, around 21.8 mg/(g·h). Biochar application reduced urease activity in RCA regardless

**Table 1** Effects of biochar types and application ratio on the RCA properties after 1 year study

Properties	pH	EC (μs/cm)	OM (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)
WB5	8.9 ± 0.1a	350.3 ± 84.9ab	52.4 ± 4.6c	20.2 ± 4.1c	28.7 ± 3.5bc	273.0 ± 26.8cde
WB10	8.8 ± 0.1ab	332.0 ± 54.8ab	87.3 ± 12.9b	19.0 ± 2.4c	27.0 ± 3.1bc	392.0 ± 55.7bcd
DMB5	8.7 ± 0.2abc	308.7 ± 29.5ab	57.7 ± 15.9c	21.3 ± 1.5c	63.1 ± 13.3b	493.7 ± 118.1bc
DMB10	8.8 ± 0.1ab	308.7 ± 55.3ab	127.0 ± 16.8a	26.0 ± 0.9bc	278.7 ± 55.0a	1196.7 ± 259.3a
PWB5	8.9 ± 0.1a	339.3 ± 88.5ab	86.1 ± 15.1b	19.7 ± 0.7c	59.5 ± 8.6b	482.2 ± 145.6bcd
PWB10	8.5 ± 0.2c	463.3 ± 296.7a	120.3 ± 12.0a	18.8 ± 2.6c	29.5 ± 1.0bc	545.9 ± 32.2b
CDG	8.2 ± 0.1d	160.1 ± 17.2b	19.4 ± 0.4d	77.8 ± 13.1a	6.0 ± 1.4c	53.8 ± 14.4e
RCA	8.6 ± 0.2bc	206.0 ± 26.1b	16.9 ± 1.4d	36.1 ± 11.2b	24.9 ± 5.4bc	246.5 ± 23.5de

Note: Value is indicated by mean ± standard error,  $n = 3$ . Unlike letters (i.e., a, b, c, d, e) indicate a significant difference between any two conditions ( $p < 0.05$ ).

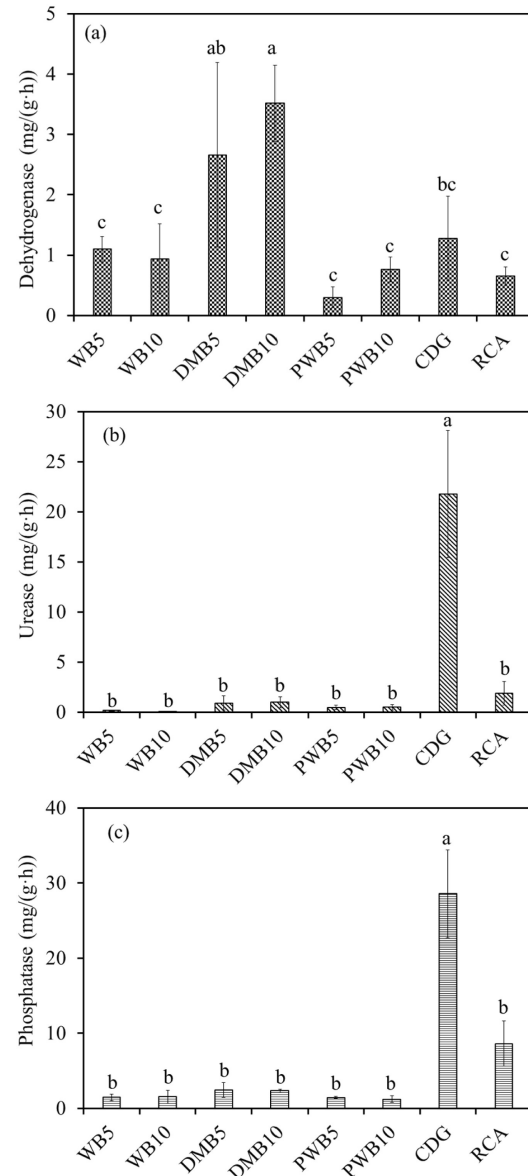
**Table 2** Effects of biochar types and application ratio on the plant performance after 1 year study

Test conditions	Weight (g)	Height (cm)
WB5	355.6 ± 150.7a	187.7 ± 10.2a
WB10	282.1 ± 151.3ab	164.7 ± 28.2ab
DMB5	279.6 ± 18.5ab	175.0 ± 22.0a
DMB10	395.3 ± 91.0a	186.3 ± 17.2a
PWB5	182.5 ± 54.9ab	167.3 ± 4.0ab
PWB10	259.2 ± 140.6ab	180.0 ± 21.2a
CDG	187.6 ± 37.2ab	146.0 ± 14.0ab
RCA	55.8 ± 11.6b	119.5 ± 34.5b

of biochar types and application ratios. The same trend has also been found in urease activities. For instance, WB5 has higher urease activity than WB10, while DMB5 and PWB5 had lower urease activity than DMB10 and PWB10, respectively. As for the phosphatase activity, the highest value was observed in CDG (28.6 mg/(g·h)) (Fig. 2(c)). There was a significant difference between CDG and RCA. Biochar application negatively affected the phosphatase activity, while there were no obvious variations between biochar application levels (i.e., 5% and 10%) and types (i.e., WB, DMB, and PWB).

### 3.3 Effects of biochar on richness and diversity of bacterial communities

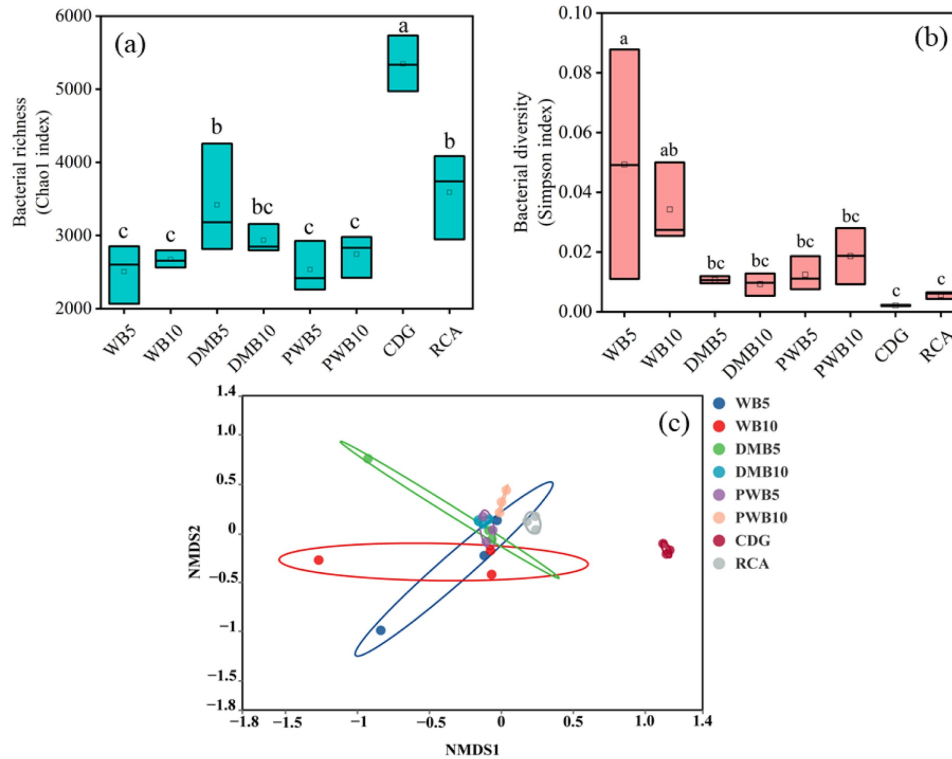
The bacterial richness in CDG was significantly ( $p < 0.05$ ) higher than that in RCA (Fig. 3(a)). However, no obvious difference was observed between DMB5 and DMB10 with RCA. A higher amount of DMB (i.e., 10% application ratios) would decrease bacterial richness compared with 5% application ratios. Nonetheless, WB and PWB application significantly reduced bacterial richness compared with RCA regardless of biochar application ratios. The Simpson index ( $\alpha$ -diversity) of bacterial communities was presented in Fig. 3(b). It has been noted that bacterial  $\alpha$ -diversity reduced by biochar application regardless of biochar types compared with RCA. Although the  $\alpha$ -diversity in RCA was slightly lower than that in CDG, this difference was not statistically significant ( $p > 0.05$ ). The WB and DMB application ratios enhanced bacterial  $\alpha$ -diversity, while 10% PWB application reduced it. Non-metric multidimensional scaling (NMDS) demonstrated that bacterial communities in the two groups could be distinguished along the primary coordinate axis (Fig. 3(c)). Biochar application changed the  $\beta$ -diversity of bacterial communities in RCA regardless of biochar types and application ratios.



**Fig. 2** Effects of different biochar types and application rates on enzyme activities. (a) Dehydrogenase activity, (b) urease activity, (c) phosphatase activity. Value is reported as mean ± standard error,  $n = 3$ ; Different letters represent a significant difference between any two conditions ( $p < 0.05$ ).

### 3.4 Identification of differential bacteria

The relative abundance of phylum belonging to Proteobacteria, Bacteroidota, Acidobacteriota, Chloroflexi, Actinobacteriota, Patescibacteria, and Verrucomicrobiota accounts for more than 80% of all bacteria in RCA and biochar amended RCA (Fig. 4(a)). However, these bacteria only accounts for 74% in CDG. It has been noted that Proteobacteria abundance



**Fig. 3** The effects of different biochar types and application rates on bacterial  $\alpha$ -diversity (a) and (b) and  $\beta$ -diversity (c) in substrates. Each test condition has three replicates and different letters represent a significant difference between any two conditions ( $p < 0.05$ ).

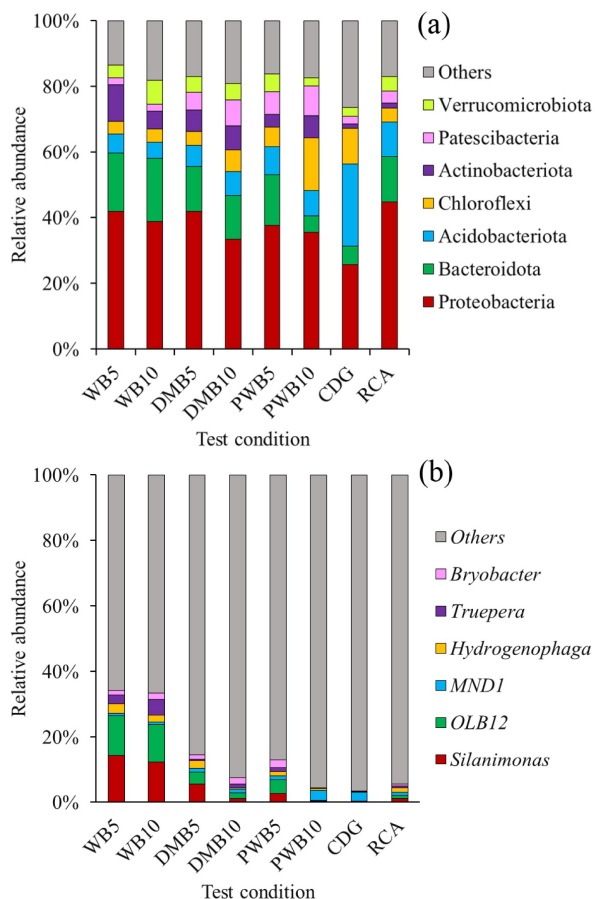
was the highest in RCA, while biochar application reduced this abundance regardless of biochar types. The Proteobacteria abundance in WB5, DMB5, and PWB5 was higher than in WB10, DMB10, and PWB10, respectively. The highest Acidobacteriota abundance was observed in CDG. Biochar addition reduced Acidobacteriota abundance regardless of biochar types and application ratios. Higher DMB and PWB application ratios (i.e., DMB10, PWB10) lead to higher Actinobacteriota abundance than that in DMB5 and PWB5, while 10% WB application ratios reduced the abundance by 120% compared with WB5. A higher biochar application ratio enhanced Patescibacteria abundance in RCA regardless of biochar types.

The variation of bacterial communities at the genus level that respond to different biochar types and application ratios was presented in Fig. 4(b). The highest *Silanimonas* and *OLB12* abundance were found in WB5 (around 4% and 12%, respectively), while 10% of WB addition decreased these two genus abundances. The same trend has been observed in DMB and PWB addition. The highest abundance of *Truepera* was in WB10, which was 74% higher than that in WB5. The higher amount of WB and DMB have positive effects on *Bryobacter*, while 10% PWB addition decreased

*Bryobacter* compared with PWB5.

### 3.5 The interaction of bacteria and biochemical properties

An investigation of canonical correlation analysis was conducted to determine soil chemical properties and enzyme activities influencing soil bacterial communities (Fig. 5). The first two axes of CCA explained 58.74% and 85.75% of the compositional variation for soil chemical properties and enzyme activities, respectively. The CCA results showed that soil chemical properties (i.e., pH, AP, AK, EC, and OM) significantly affected the bacterial communities in RCA and biochar amended RCA (Fig. 5(a)). However, soil AN has a dramatic influence on bacterial communities of CDG. It is widely accepted that when the correlation coefficient  $R^2 > 0.7$  and  $p < 0.05$ , there is significant collinearity between the variables. In this study, AN in soil chemical properties was considerable collinearity ( $R^2 = 0.902$ ,  $p = 0.000$ ) (Table S2). As for the enzyme activities, dehydrogenase activity was positively related to bacterial communities in biochar amended RCA regardless of biochar types and application ratios (Fig. 5(b)). However, urease and



**Fig. 4** Relative abundances of bacterial phylum (a) and genus (b) in substrates under different treatments.

phosphatase activities were positively related to bacterial communities in CDG. There was considerable collinearity, such as urease ( $R^2 = 0.918, p = 0.000$ ) and phosphatase ( $R^2 = 0.941, p = 0.000$ ) activities, in enzyme activities.

The spearman correlation between dominant bacterial phylum and soil chemical properties and enzyme activities was presented in the current study. The parameters for spearman correlation was presented in Table S3. It has been noted that pH has a significant ( $p < 0.001$ ) positive relationship with Bacteroidota, while EC has a significant ( $p < 0.001$ ) negative relationship with Acidobacteriota (Fig. 5(c)). The AN was positively correlated with Acidobacteriota, while it was negatively correlated with Actinobacteriota. The soil enzyme activity associated with bacterial phylum was presented in Fig. 5(d). Acidobacteriota has a significant ( $p < 0.01$ ) positive relationship with urease and phosphatase activities. A significant ( $p < 0.01$ ) negative relationship was observed between urease activity and Bacteroidota.

### 3.6 Co-occurrence networks of bacterial communities and metabolites

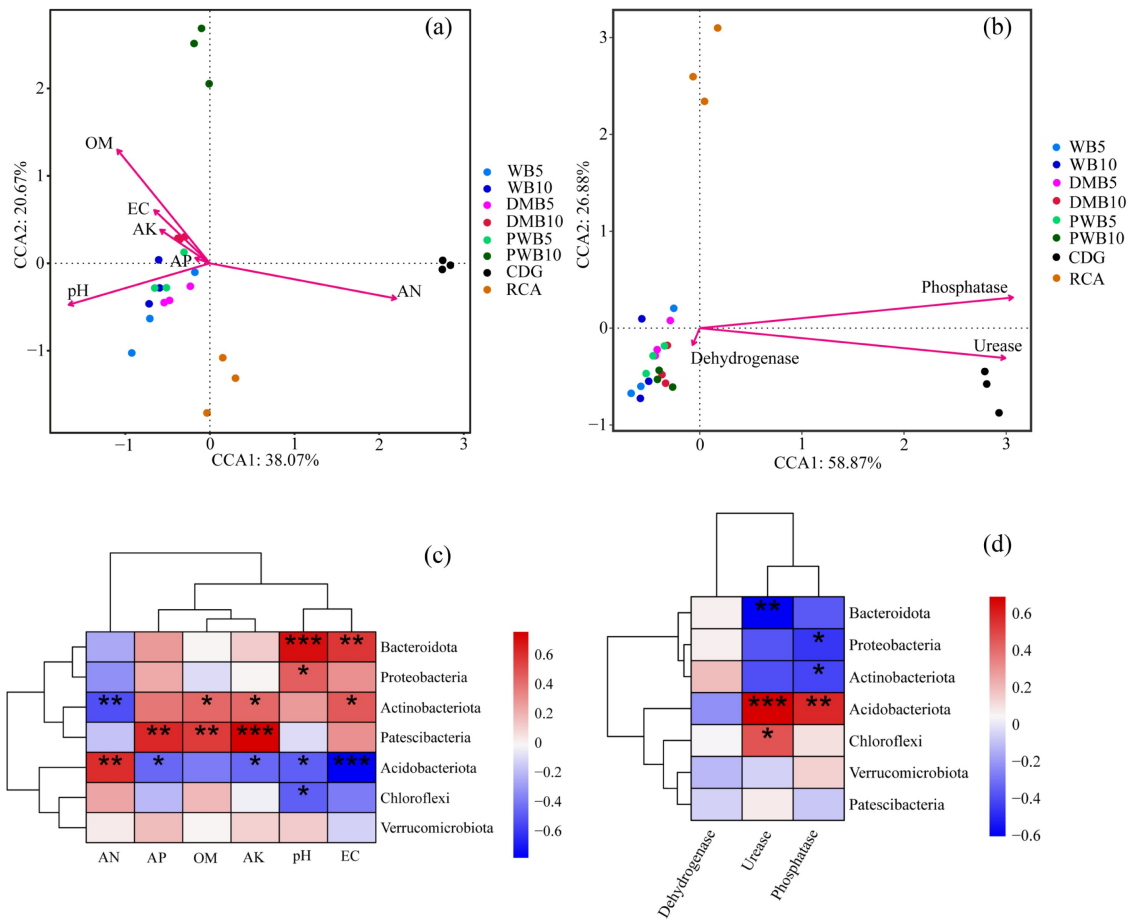
The co-occurrence networks were generated for bacterial communities at the phylum level and associated topological properties were calculated (Fig. 6 and Table 3). The network of the bacterial communities consisted of 49 nodes and 392 edges in RCA. The nodes and edges in WB10 and PWB10 were lower than that in WB5 and PWB5, respectively. It has been noted that the highest positive/negative edge ratio was observed in WB5 ( $P/N = 3.838$ ). Normally, the positive edges were greater than negative edges, except for DMB10 and RCA. Based on the topological properties of the networks, the keystone species were changed with different test treatments. The bacterial networks hosted some keystone species belonging to the phyla Proteobacteria (ranged from 30.61 to 59.18%), Bacteroidota (ranged from 2.17 to 20.41%), and Acidobacteriota (ranged from 6.12 to 36.73%) were the most abundant phylum (Table S4).

The application of biochar affected both the diversity of bacterial communities and their metabolic processes, as well as the variety of metabolites produced (Fig. 7). The majority category of the identified metabolites, accounting for more than 10% of the total amount identified, were Amimo acid metabolism, Carbohydrate metabolism, Metabolism of cofactors and vitamins, Metabolism of terpenoids and polyketides. The pathway sequences distribution of these four different metabolisms was the highest in DMB10. It has been noted that Amino acid metabolism, Carbohydrate metabolism, and Metabolism of cofactors and vitamins in RCA and biochar-amended RCA were higher than that in raw soil (i.e., CDG).

## 4 Discussion

### 4.1 Biochar improves the fertility of RCA and plant performance

The implementation of RCA is now a global issue that requires a solution. In the current research, it was utilized as a substrate for green roofs. However, higher pH and lower fertility of RCA inhibited plant growth (Guo et al., 2023), thus, biochar was added into RCA. Biochar application improved the EC, OM, AP, and AK of RCA regardless of biochar types and application ratios (Table 1). Upon application, biochar improved nutrient retention by promoting the development of an organic layer which was enhanced by hydrophilicity and redox activity (Hagemann et al., 2017). The

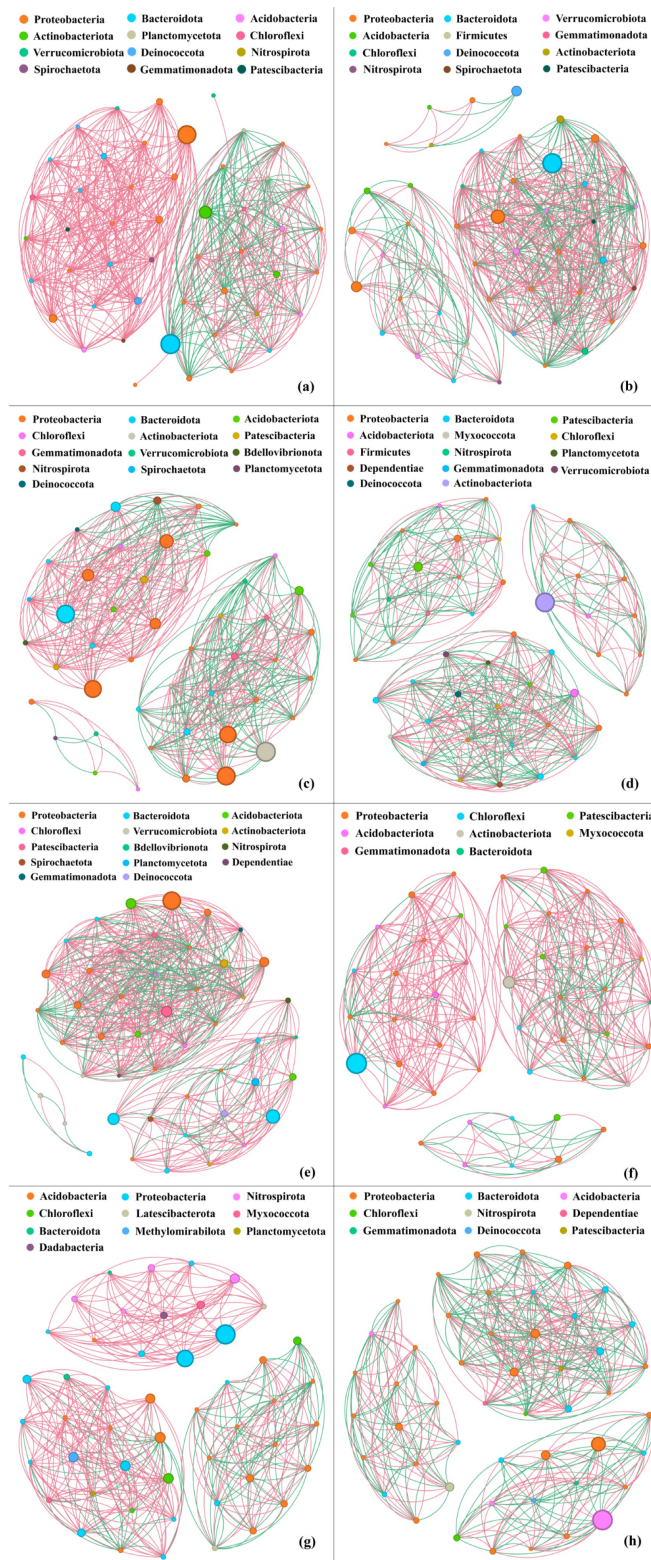


**Fig. 5** Canonical correlation analysis of (a) soil chemical properties with bacterial community structure, (b) soil enzyme activity with bacterial community structure at phyla level. Heatmap of Spearman correlation between dominant bacterial phylum and soil chemical properties (c) and soil enzyme activity (d)

abundant carbon content in biochar was the reason for the significant increase in soil organic carbon (Gautam et al., 2021). Therefore, higher biochar application rates lead to higher OM. The development of OM on biochar surfaces was attributed to the adsorbing of organic molecules from the substances consequently increasing the fertility of substrates (Yuan et al., 2019). Biochar can enhance substrates structure and availability by serving as a source of  $Ca^{2+}$  and  $Mg^{2+}$ . Moreover, it may be feasible to enhance aggregate stability and hydraulic conductivity, both crucial for improving substrate water movement and drainage (Fouladidorhani et al., 2020). The fertility of RCA can be enhanced after biochar application, which promotes a more favorable environment for plant growth. Higher EC of RCA after biochar application might be related to the certain cations on the surface of biochar may not attach strongly or bond via electrostatic forces (Chintala et al., 2014), resulting in their dissolution as soluble salts in

the substrates. Liao et al. (2024) observed that biochar increased soil salinity, impeding plant growth and leading to plant mortality. However, higher salt in the substrates did not inhibit plant growth in the current study. This might because the plant species used in current study was tolerant to salinity.

It has been noted that biochar application either promotes or inhibits plant growth, which depends on the soil properties, biochar types, and application ratios (Murtaza et al., 2023). For instance, the utilization of poultry manure-derived biochar in loam soil at a rate of 400 kg/ha improved the dry and fresh weights of pepper and tomato plants (Vaccari et al., 2015). Biochar enhanced substrate properties, with a 17% increase in available nitrogen and a 13% increase in total carbon, leading to improved plant root, shoot, and leaf morphology. The plants exhibited a higher leaf number in biochar-treated substrates than the untreated (Yang et al., 2022). This might be because that biochar



**Fig. 6** The molecular ecological networks of bacterial communities under different test conditions. (a) WB5, (b) WB10, (c) DMB5, (d) DMB10, (e) PWB5, (f) PWB10, (g) CDG, (h) RCA. The edge with red color represents a positive relationship, while the green color represents a negative relationship.

**Table 3** The number of nodes and edges in the networks of bacterial communities under different test conditions

Topological properties	Nodes	Edges	Positive edges	Negative edges	Positive/negative edge ratio (P/N)
WB5	48	508	403	105	3.838
WB10	47	479	303	176	1.722
DMB5	47	430	284	146	1.945
DMB10	48	381	188	193	0.974
PWB5	48	504	340	164	2.073
PWB10	46	371	287	84	3.417
CDG	49	382	288	94	3.064
RCA	49	392	194	198	0.980

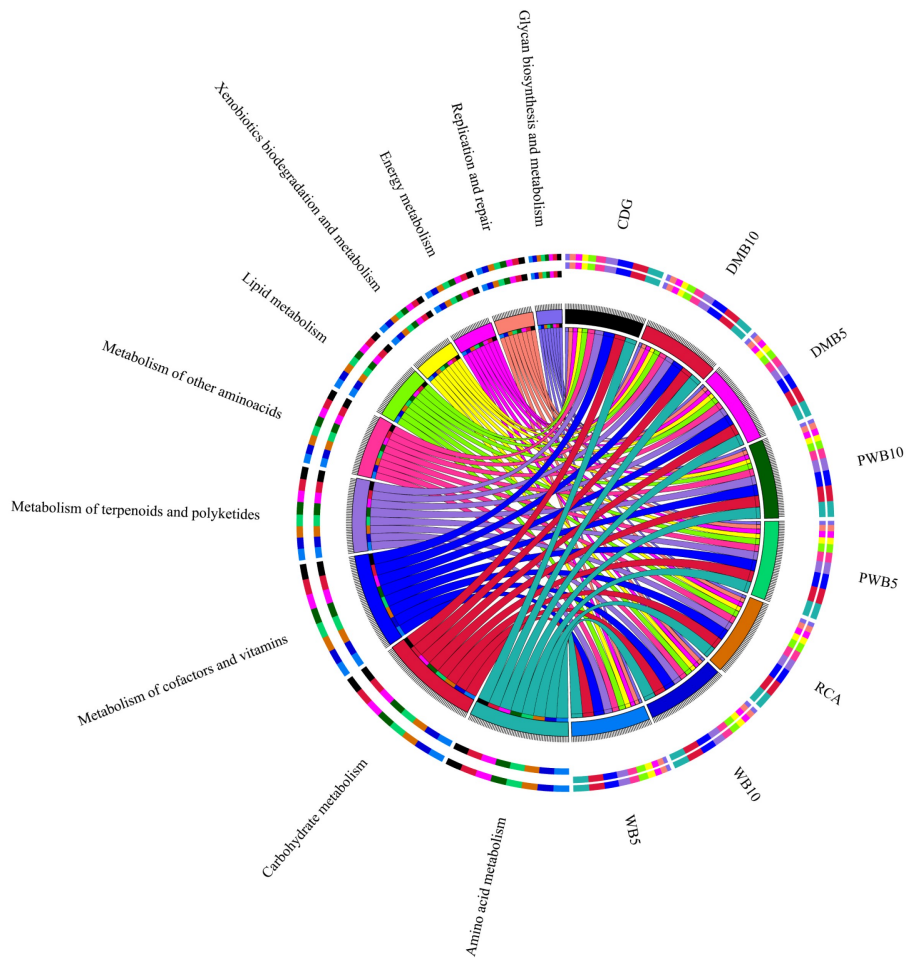
application enhanced the water retention by changing soil pore structure (Li et al., 2018b; 2025). The application rates and feedstock types of biochar significantly influenced the development of clover, mung beans, and wheat in the laboratory test (Solaiman et al., 2012). Nevertheless, the results in the current study shows that biochar application enhanced plant growth regardless of biochar types and application ratios. This might be due to the fertility of RCA was relatively lower compared to raw soil (CDG). Higher amount of WB application causes a decrease both in plant weight and height in this study. Artiola et al. (2012) indicated that the use of pine-derived biochar at 2% with sandy-loam and alkaline soil adversely impacted lettuce growth in the biochar-amended soil. It has been reported that free radicals in biochars generated substantial OH radicals in the aqueous phase, which markedly impeded plant growth by damaging their plasma membranes (Liao et al., 2014). Biochar application in RCA enhanced plant growth, which lead to the decrease of greenhouse gases emissions and increase of CO<sub>2</sub> uptake (Kravchenko et al., 2024b). Moreover, the utilization of RCA for green roofs aligns with carbon neutrality strategies (Kravchenko and Besklubova, 2024a). This suggested that biochar amended RCA is a suitable substrate for green roof systems. Overall, WB at 5% and DMB at 10% were the best treatments for plant performance in terms of plant weight and height.

#### 4.2 Effects of biochar on enzyme activities and bacterial diversity

Enzyme activities indicate substrate quality and are involved in biochemical cycles, with their activities affected by substrate type and the biochar application rate. Dehydrogenase, urease, and phosphatase activities participate in nutrient recycling such as C, N, and P (Yang et al., 2016). The enzyme activities in the current study showed that biochar application reduced urease

and phosphatase activities in RCA regardless of biochar types and application rates. This might be related to higher EC induced by biochar application. According to Heng et al. (2022), higher salt stress reduces microbial community richness, which is bound to decrease the major amount of enzymes secreted by microorganisms into the substrates. Moreover, salt alters the ecological environment of substrates, and osmotic stress and ion toxicity induced by the salt can inhibit the activities of the soil enzymes (Tang et al., 2020). Besides, the incorporation of biochar adversely impacts bacteria, hence influencing enzyme production (Huang et al., 2017). Biochar can adsorb various organic and inorganic compounds and inhibit certain enzymes or their substrates through adsorption or by impeding the active sites of reactions (Elzobair et al., 2016). Furthermore, materials with a high specific surface area and porosity may render substrates ineffective in mitigating degradation (Chen et al., 2013). However, WB and DMB applied in the RCA enhanced dehydrogenase activity regardless of application ratio (Fig. 2(a)). This may pertain to the enhanced substrate characteristics and the incorporation of various labile carbon molecules in the biochar for enzymatic processes (Khadem and Raiesi, 2017). The variability in enzyme activity performance across substrates may be attributed to factors such as material sources, production methods, substrate characteristics, amendment content, and the timing of enzyme activity assessments, all of which can significantly influence soil enzyme responses to additives (Tang et al., 2020). Overall, WB and DMB have positive effects on dehydrogenase activity, while biochar application reduced urease and phosphatase activities in RCA.

It has been noted that bacterial richness and  $\alpha$ -diversity in CDG were significantly higher than that in RCA and biochar treatments (Figs. 3(a) and 3(b)). The cement paste portion of the RCA has a high content of calcium hydroxide (Ca(OH)<sub>2</sub>), which has a negative impact on the bacterial communities. The interesting



**Fig. 7** The circus of KEGG-predicted metabolic pathways under different test conditions.

result of the current study is that biochar application reduced bacterial richness and diversity regardless of its type and application ratio. According to [Ng et al. \(2023\)](#), biochar application help plants to specialize soil microbes that favor plant growth. Biochar directly improved substrate microclimate conditions that promote plant growth and productivity ([Xiang et al., 2023](#)). The  $\beta$ -diversity showed that biochar application was different with RCA, suggested that biochar has shaped distribution of bacterial communities ([Fig. 3\(c\)](#)). It has been reported that the application of biochar potentially reduced fungal and bacterial diversity in alkaline, fine-textured soils ([Li et al., 2020](#)). Biochar accelerates the proliferation of specific microbes and alters the composition of the soil microbial community, leading to enhanced microbial biomass but diminished variety ([Khodadad et al., 2011](#)). Thus, biochar application in RCA might increase certain bacterial species and reduce their richness and diversity in current study.

### 4.3 Biochar alters the bacterial composition and metabolism

Biochar application changes the bacterial community composition in RCA at phylum and genus level ([Fig. 4](#)). It has been reported that Bacteria from Bacteroidetes, Firmicutes, and Actinobacteria were predominated in biochar-amended soils ([Abujabhah et al., 2018](#)), whereas bacteria from Acidobacteria, Proteobacteria, and Planctomycetes were typically reduced following biochar application ([Xu et al., 2014](#)). A similar result has been found in the current study. For instance, the Proteobacteria abundance decreased after biochar application in RCA, and a higher biochar application ratio has negative effects on their abundance. It was the dominant phylum in the substrates. Proteobacteria are copiotrophic organisms responsible for methanotrophic activity and the reduction of greenhouse gas emissions ([Kersters et al., 2006](#)). This suggested that biochar application inhibited

methanotrophic activity. The Acidobacteria abundance was also reduced by biochar application. Acidobacteria is oligotrophic and exhibits limited adaptability to nutrient-dense soil (Dai et al., 2018). This might be because that biochar application enhanced the nutrients of RCA (Table 1). Furthermore, using biochar enhanced Nitrospirae and Verrucomicrobia populations in black clay loam and brown sandy loam soils, while their presence diminished in loamy red soils (Abujabhah et al., 2018). The relative abundance of Chloroflexi grew in a field but was reduced in laboratory incubation research following the application of rice straw biochar (Xu et al., 2014). In contrast, bamboo biochar treatment in the field resulted in a reduction of Chloroflexi abundance (Chen et al., 2015). However, those bacterial phylum in the current study were performed differently after biochar application. This might be related to the type of substrates and biochar. The *Silanimonas* abundance was enhanced after WB application in the current study (Fig. 4(b)). *Silanimonas* is an autotrophic denitrifying bacteria (Liu et al., 2021). Consequently, they significantly contribute to the elimination of TN in RCA. Thus, *Silanimonas* was able to remove nitrogen through autotrophic denitrification (Xu et al., 2024).

The co-occurrence of bacterial networks in the current study presented that the network with different biochar applications had their unique characteristics (Fig. 6). The greater the complex signifies a steadier ecological function (Jing et al., 2023). For instance, different microorganisms interact to form complex ecological networks that affect community dynamics and ecosystem stability through relationships, such as predation, competition, and symbiosis. Complex network structures often help to enhance the resilience and recovery of ecosystems. Therefore, the steady ecological function can be found in WB at both application ratios. As for DMB and PWB, only 5% of application rates have contribution to the steady ecological function in RCA. The edges delineate the distance between two nodes, facilitating the exchange of substances, information, and energy among microorganisms (Li et al., 2020). Thus, the higher exchange of substances can be found in WB5. Jing et al. (2023) assert that negative edges (relationships) frequently encompass competition. Thus, the competition in DMB10 has become serious. The keystone species in different treatments performed varied (Table S5). The dominated species in RCA and biochar amended RCA were Proteobacteria and Bacteroidota. However, Proteobacteria and Acidobacteriota were the dominant species for CDG. It has been observed that biochar application enhanced

the percentage of Acidobacteriota in RCA, which indicates that biochar application shaped the bacterial communities into raw soil composition. The result of predicted metabolic pathways showed that the major metabolism pathways were highest in DMB10 (Fig. 7), indicating this treatment stimulates the metabolism activities in the RCA.

#### 4.4 The interaction of RCA properties with bacterial communities

In this study, the pH shaped bacterial communities in RCA with positive effect (Fig. 5(a)), while Acidobacteriota and Chloroflexi have a significant negative relationship with pH (Fig. 5(c)). It has been observed that AN was the dominant factors that influencing bacterial communities in CDG (Fig. 5(a)). The positive relationship was found between Acidobacteria and AN. Acidobacteriota governs biogeochemical cycles, decomposes biopolymers, and enhances plant growth (Kalam et al., 2020). Chloroflexi are generalists regarding habitat and substrate, and they play crucial roles in the carbon cycle and humified stabilization of soil organic matter (Zhang et al., 2022). Ren et al. (2020) assert that the growth and reproduction of Chloroflexi are not contingent upon soil nutrient availability, as these gram-negative bacteria possess the capability for autotrophic metabolism through photosynthesis. This indicates that higher pH will inhibit nutrient cycling and plant growth. A significant positive relationship can be found between Patescibacteria with AP, OM, and AK (Fig. 5(c)). Patescibacteria has optimized numerous functions while gaining advantages such as evading phage infection to adapt to the extreme environment (Tian et al., 2020). Thus, improving RCA properties by biochar application might enhance Patescibacteria abundance. The enzyme activities, such as urease and phosphatase, were positively related to CDG (Fig. 5(b)). The significant positive relationship between Acidobacteriota and these two enzyme activities (Fig. 5(d)). Dehydrogenase activity was positively correlated with RCA, while no significant relationship was observed between bacterial communities and dehydrogenase activity. This suggested that biochar application was more likely to impact dehydrogenase activity.

## 5 Conclusions

This column test investigates different biochar types and application ratios on biochemical properties and

bacterial communities of RCA, the following conclusions can be drawn:

(1) Biochar addition improved the OM, AP, and AK of RCA regardless of biochar types and ratios, which might be the higher plant growth in biochar amended RCA. Nevertheless, biochar application enhanced EC at least by 33% of RCA, suggesting that the salinity of biochar amended RCA can be accepted for the selected plant in current study. However, the urease and phosphatase activities in biochar amended RCA was lower compared with RCA. This suggested that higher salinity caused by biochar inhibited the urease and phosphatase activities in RCA.

(2) The bacterial richness and  $\alpha$ -diversity in RCA and biochar amended RCA were lower than that in CDG, indicating that high content of calcium hydroxide in RCA has a negative effect on the bacterial communities. Biochar application reduced bacterial richness and  $\alpha$ -diversity, while enhanced plant growth. This suggested that biochar application help plants to specialize soil microbes that favor plant growth, and directly improves substrate microclimate conditions that promote plant growth and productivity.

(3) The dominated bacterial phylum in RCA were Proteobacteria and Bacteroidota, while Proteobacteria and Acidobacteriota were the dominant phylum for CDG. Biochar application enhanced the percentage of Acidobacteriota in RCA, indicating that biochar application shaped the bacterial communities into raw soil composition.

**Conflict of Interests** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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