Thai Environmental Engineering Journal Vol. 39 No. 2 (2025) : 19-30
www.eeat.or.th

The Variability in Soil Community and Its Enzymatic Function to Plant Promotion after Biochar Addition

Mujalin Pholchan[1*], Jearanai Thassana[2] and Khajitmanee Muangprom[3]

[1,2,3]Environmental Technology, Faculty of Science,
Maejo University, Chiangmai 50290, Thailand
[*]E-mail : mujalin@mju.ac.th

Article History; *Received: 1 July 2025, Accepted: 19 August 2025, Published: 28 August 2025*

Abstract

The widespread use of chemical inputs in contemporary agriculture has raised concerns regarding long-term soil health and sustainability. Biochar (BC), a carbon-rich byproduct of biomass pyrolysis, presents a viable alternative, offering improvements in soil structure, nutrient retention, and microbial activity. This study investigates the effects of modified biochar on soil microbial diversity and associated metabolic pathways that contribute to soil fertility and plant productivity. Five soil samples (D1 control without BC, D2 paddy field 1, D3 paddy field 2, D4 eggplant field, and D5 long yard bean field) were collected from agricultural sites for microbial community profiling. Metagenomic DNA was extracted and analyzed using QIIME2. A total of 371 microbial species were identified across samples. D1 exhibited dominance of Proteobacteria, Acidobacteriota, and Halobacterota. In contrast, D2–D5 showed increased abundance of Firmicutes and Actinobacteriota, with the emergence of phyla such as WPS-2 and the enrichment of functional genera including the ammonia-oxidizing bacterium MND1. Bacilli were detected in all samples, with the highest relative abundance observed in D5 (8.34%). Notably, Bacillus sp.—a component of the applied BC—was more abundant in D4 (0.12%) and D5 (0.58%) than in D1 (0.02%), indicating BC's potential to retain and promote beneficial microbial taxa. Microbial richness was highest in D2 (4887 OTUs), exceeding that of the control (D1, 4517 OTUs), confirming the diversity-enhancing effect of BC. D4 exhibited the most distinct microbial community (3403 OTUs). Functional annotation revealed increased abundance of proteins such as COG1595 and key enzymes including EC:2.7.7.7 and EC:1.6.5.3, which are associated with nucleotide synthesis and redox metabolism. The metabolic pathway PWY-3781 was most prominent post-BC application, with pathways PWY-7111, PWY-6277, and PWY-6122 also enriched in D3 compared to D1.These findings demonstrate that biochar promotes microbial diversity, enhances beneficial microbial populations, and activates key metabolic pathways, thereby contributing to improved soil quality and supporting sustainable agricultural practices.

Keywords : biochar; microbial diversity; metabolic pathways; plant growth; soil fertility

Introduction

Contemporary agriculture relies on chemicals to achieve high-quality yields. However, the long-term and repeated use of chemicals in the same area not only decreases soil quality but also disrupts and damages the soil microbial system. Therefore, the shift towards using biological materials instead of chemicals is an alternative that should be given attention. Biochar is an agricultural waste from the pyrolysis process. It is an environmentally friendly biological material at a low cost that has been widely applied in environmental studies due to its various properties e.g. high carbon content, large surface area, high cation exchange capacity, and stable structure [1]. Due to its adsorption capacity, biochar is an effective biological material for eliminating contaminants [2], including heavy metal pollutants [3] and organic contaminants such as dyes [4]. Moreover, biochar is also used to improve soil quality and promote plant growth. Biochar has been used to examine soil properties and the growth of plants. It helps increase the pH of the soil and promotes higher final biomass, root biomass, plant height, and number of leaves [5].

In addition to its physical and chemical benefits, biochar also influences the soil microbiome. Previous studies have reported that biochar application can increase microbial diversity [6], including a substantial rise in beneficial genera such as Bacillus and Pseudomonas by up to 100% [7], both of which are known to promote plant growth and nutrient cycling. Some studies have also shown that biochar can increase soil enzyme activity, such as urease activity by 23.1%, alkaline phosphatase activity by 25.4%, and dehydrogenase activity by 19.8% [8]. Biochar has also been shown to enhance soil enzyme activities, with increases of 23.1% in urease, 25.4% in alkaline phosphatase, and 19.8% in dehydrogenase activity [8]. Despite these promising findings, most research has concentrated on short-term effects, leaving long-term impacts on microbial diversity, abundance, and functional dynamics less understood. Moreover, while biochar has been linked to enhanced microbial activity, its influence on specific microbial metabolic pathways, particularly those related to carbon and nitrogen cycling, requires further investigation. Given these knowledge gaps, this study presents a preliminary evaluation of the effects of modified biochar addition on soil microbial diversity and key metabolic pathways associated with plant growth and soil quality. While limited in duration and scope, the findings provide important baseline evidence that can guide future long-term investigations and support the development of sustainable agricultural practices and soil management strategies.

Methodology

Experimental design and biochar application

For the application of biochar in crop cultivation, the experiment was arranged in a randomized complete block design (RCBD) with plot sizes of 0.8×8.1 m. for eggplant 0.9×10.5 m for long yard bean and 10.5×9.5 m for rice. Two treatments of each crop were included: a control and a biochar-amended treatment. All treatments received fertilizer according to the recommended rates for each crop, with a 15-15-15 formula applied twice for all plants. Rice straw biochar was produced by pyrolysis at 500 °C for 1 hour, then sieved through a mesh smaller than 355 µm and subsequently encapsulated with microbial cells following a previously established method. The microbial culture consisted of a mixed culture of *Bacillus sp*. The modified biochar was applied to the experimental plots at the appropriate rate of 1% w/w prior to crop planting.

Soil sampling

Soil samples (D1: control without BC, D2: paddy field 1, D3: paddy field 2, D4: eggplant field, and D5: long yard bean field) were collected from approximately 15 locations following a zigzag random sampling pattern after planting at a depth of 0-15 cm. All samples originated from the same soil series, which had been used to grow rice and different types of vegetables, to ensure consistency in soil type. The samples were air-dried, ground, and sieved through an 80-mesh screen prior to chemical analysis. The analyzed parameters included soil pH, organic matter content, and cation exchange capacity (CEC) determined by the distillation method. Total nitrogen was measured using the Kjeldahl method (Multi EA 4000), available phosphorus was determined by the Bray II extraction method, and exchangeable potassium (K) was analyzed using atomic absorption

spectroscopy (AAS). All analyses were conducted at the Central Laboratory, Faculty of Agriculture, Chiang Mai University. Separate soil samples were collected for microbial diversity analysis. These were preserved in 50 mL centrifuge tubes with absolute ethanol at a 1:1 volume ratio and stored at −20 °C prior to DNA extraction.

DNA extraction and metagenomic analysis

Total DNA was extracted from the soil sample using the nucleospin® soil (Bio-rad laboratories, USA) following the manufacturer's instructions. The DNA samples were then sent for metagenomic sequencing analysis which was performed on Illumina HiSeq platform by Novogene Biological Information Technology (Tianjin, China). 16 S rRNA genes of distinct 16SV3–V4 regions were amplified used specific primers with the barcode. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs) and sequencing libraries were generated using NEBNext Ultra DNA Library Pre ® Kit for Illumina. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Sequences analysis were performed by Uparse software (Uparse v7.0.1001) (Edgar, 2013). Sequences with ≥97% similarity were assigned to the same OTUs. Representative sequence for each OTU was screened for further annotation. Alpha and Beta diversity analysis were calculated with QIIME2. and displayed with R software (Version 2.15.3). Venn and Flower diagram, Ternary plot as well as were relative abundances also generated [9].

Results and Discussions

Effect of Biochar on phylum-level shifts in soil microbial community structure

The microbial diversity analysis using the Metagenomic method in all soil samples revealed the presence of both Bacteria and Archaea. From Figure 1, which indicates the evolutionary relationships of microorganisms in each phylum and the abundance of microbial groups in the soil samples (D1 – D5) through a phylogenetic tree, various dominant microbial communities were identified (65 phyla, 154 classes, 331 orders, 434 families, 732 genera, and 371 species) such as *Bacillus* and *Alicyclobacillus* (Phylum Firmicutes), *Sphingomonas* (Phylum Proteobacteria), and *Nitrospira* (Phylum Nitrospirota).

Shifts in microbial diversity after BC addition

The relative abundance of soil samples in Figure 2 have 10 dominant microbial phyla. In control (D1) before BC were applied, Proteobacteria, Acidobacteriota, and Halobacterota were the most abundant microbial phyla. Proteobacteria are functions related to carbon, nitrogen, and sulfur cycling. Acidobacteriota survive in acidic environments and influencing nitrogen, phosphorus, and plant growth dynamics [10]. And Halobacterota, which is Archaea that survive in high-salinity environments and can promote plant growth [11]. According to the results, the soil from D1 is in extreme condition, which should improve for better microbial growth. The dominant genus after BC addition (D3) is Ammonia-oxidizing bacterium (MND1) (3.29%). Ammonia-oxidizing bacteria (AOB) plays a role in promoting root cytokinin synthesis that contributes to enhancing plant growth. Additionally, AOB is the primary bacteria in soil nitrification, leading to an increase of release nitrate (NO_3^-) in the soil [12]. The increased abundance of MND1 after BC addition indicates that biochar helps to enhance the population of beneficial soil microorganisms. This coincides with the extended and well-developed root systems observed in the samples where biochar was applied, indicating improved agricultural performance (data not shown).

In the areas where modified biochar (BC) were applied (D2-D5), relative abundance results showed an increase in microbial populations, along with new microbial phyla. For instance, *Candidatus* phylum Eremiobacterota (WPS-2), which is commonly found in bare soil environments [13], was detected after the addition of BC. It was found that the Firmicutes population increased in D2, D4 and D5, representing the phylum of BC-associated microorganisms in this study. Actinobacteriota populations significantly increased in D4 and D5. Similarly, a study on clay soil reported an increase in Actinobacteriota after applying biochar at 20 t/m³ [14].

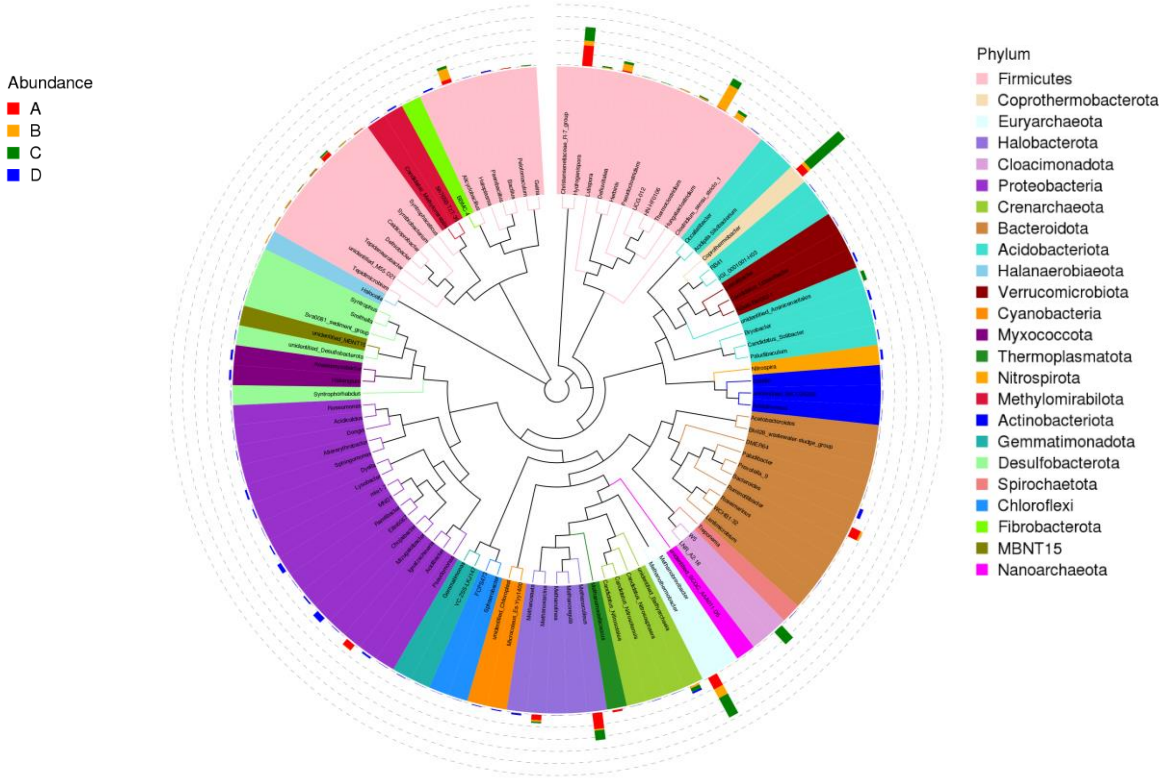

Figure 1 Phylogenetic tree and the abundance of the soil samples (D1 – D5)

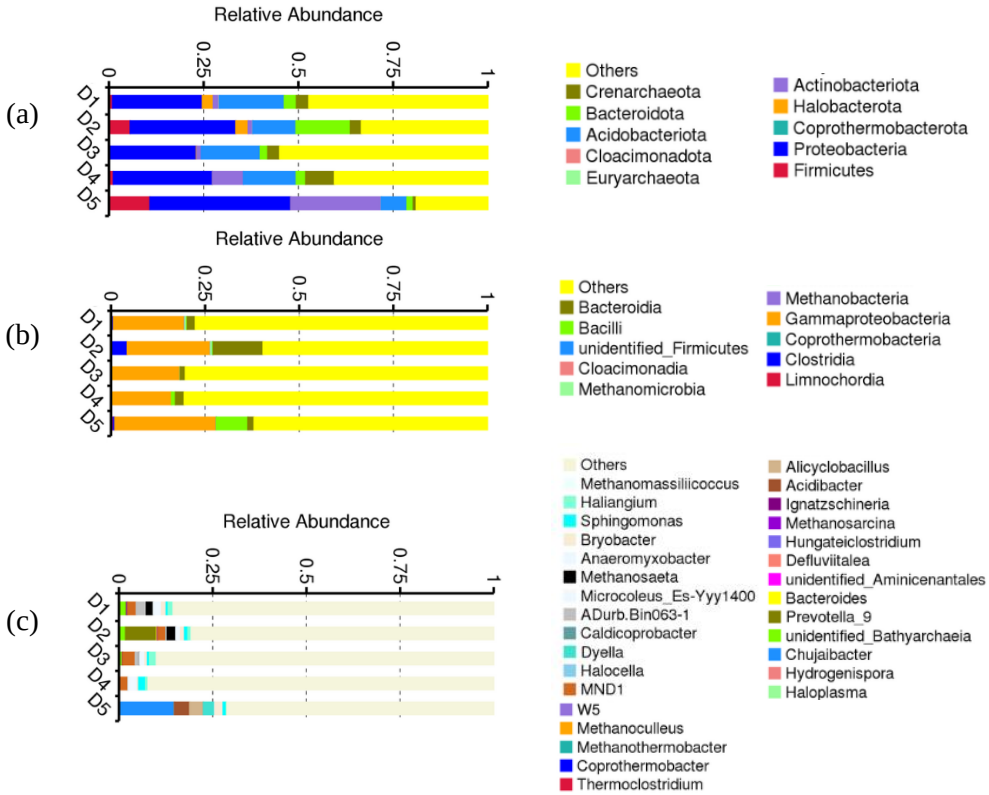

Figure 2 Relative abundances of major taxonomic groups from soil samples
(a) phylum level (b) class level (c) genus level

In Figure 3 shown that Bacilli (Phylum Firmicutes) is mostly found in D5 (8.34%), D4 (1.06%), D2 (0.18%), D1 (0.18%), and D3 (0.04%) respectively. This can improve the soil microenvironment by modulating the microbial community and soil quality through water and nutrient retention within the soil, as well as reducing soil salinity. Resulting in the enhancement of plant growth and an increase in yield [15]. According to the results, BC used in this study are a mixed culture (*Bacillus* sp.) that found in mostly test areas, it can imply that biochar can retain and maintain the soil-improving microorganisms in varying environmental conditions. Meanwhile, the microbial populations of D3 is decrease due to unsuitable soil conditions for microbial growth.

These results align with research on biochar and bioorganic fertilizer (BOF) containing *Bacillus* sp., which similarly enhanced microbial diversity in saline-alkaline soil, comparable to the BC used in this study. The study found Actinobacteriota present in soils both with and without biochar and BOF. Moreover, Firmicutes population also increased after biochar or BOF application [16], consistent with the above experimental results. The research demonstrated that biochar and BOF alter microbial diversity and community structure, with the application of both biochar with BOF resulting in more effective improvements than either biochar or BOF alone [16]. Indicating that the BC addition in this study supports the efficient application of biochar.

Effects of biochar on soil microbial diversity and abundance

The highest microbial diversity was observed in D2 (n = 4,887), whereas the lowest was in D5 (n = 1,436). In comparison, D2 also had higher microbial diversity than D1 (n = 4,517), as shown in Figure 4. Differences in fertilizer use and field management between sites may have contributed to variations in microbial communities, which should be considered when interpreting the results. Table 1 presents the baseline soil physicochemical properties (pH, organic matter, CEC, texture, moisture content) for all sampling sites. The lowest fertility indicators (OM, nutrients, CEC) found from D1 (control), representing poor baseline soil without amendments. However, moderate OM and nutrient levels in the paddy fields (D2 and D3) appeared after biochar addition, while D3 had the highest TN, indicating that rice–soil systems can retain nitrogen more effectively. In addition, very high levels of P, K, and NO_3^- were found in D4 and D5, reflecting either intensive fertilization or the effect of biochar addition on enhancing soil K and P availability. Although the variation in microbial communities may be partially explained by differences in baseline soil physicochemical properties, previous research has shown that biochar can significantly enhance soil microbial diversity [6]. Therefore, it can be concluded that biochar contributes to increasing microbial diversity. Although this study was based on a one-time application of biochar and its short-term effects may not have a significant impact on soil bacterial communities, it did enhance certain soil conditions. These improvements included an increase in soil pH, enhanced soil moisture retention, higher concentrations of potassium and phosphorus, and an increase in cation exchange capacity (CEC). In terms of long-term effects, Zhang et al. (2022) found that biochar significantly improved soil fertility and increased the relative abundance of Proteobacteria, while the abundance of Acidobacteria decreased.

However, there is specificity of microorganisms that depends on the environmental conditions. According to the quantity of microorganisms, as shown in Figure 5, the highest and lowest specifics and differences of microorganisms are D4 (3403 OTUs) and D5 (1280 OTUs) respectively. According to the experimental results, 3 microbial species were found in all soil samples: *Bacillus* sp., *Clostridium* sp. (*Clostridium sensu stricto 1*), and *Arenimonas* sp. In addition, unidentified_Chloroplast was also found in all soil samples, which due to residual genetic material from Cyanobacteriia.

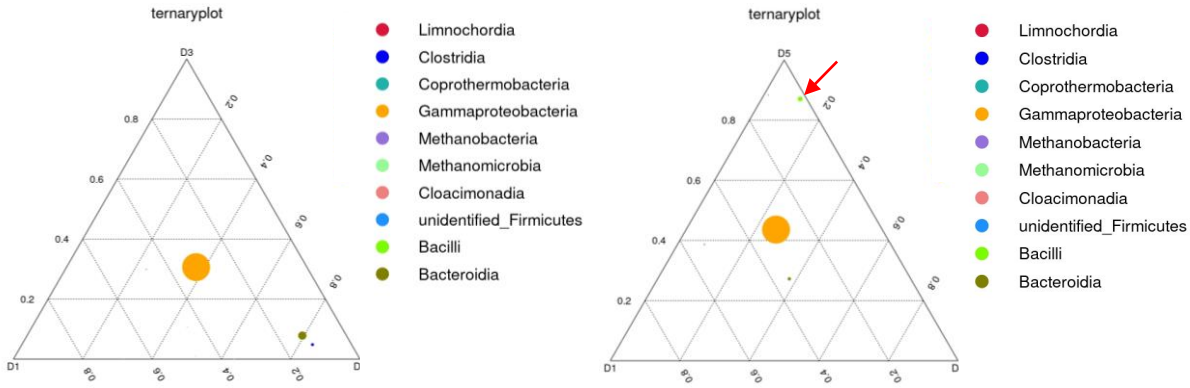

Figure 3 Ternary plot of soil samples (a) D1, D2, D3 (b) D1, D4, D5

Table 1 Soil properties for D1 (control without BC), D2 (paddy field 1), D3 (paddy field 2), D4 (eggplant field), and D5 (long yard bean field)

Soil samples	Organic matter (%)	Total Nitrogen (%)	Available Phosphorus (mg/kg)	Exchangeable Potassium (mg/kg)	Nitrate (NO_3) (mg/kg)	CEC (meq/100g)
Control without BC (D1)	0.67	0.08	14.99	0.75	28.45	2.92
Paddy field 1 (D2)	0.81	0.03	51.17	112.25	21.46	5.34
Paddy field 2 (D3)	1.64	0.15	46.78	91.00	35.79	3.63
Eggplant field (D4)	1.85	0.02	147.92	517.00	82.04	5.34
Long yard bean field (D5)	1.20	0.08	81.96	365.50	179.68	6.66

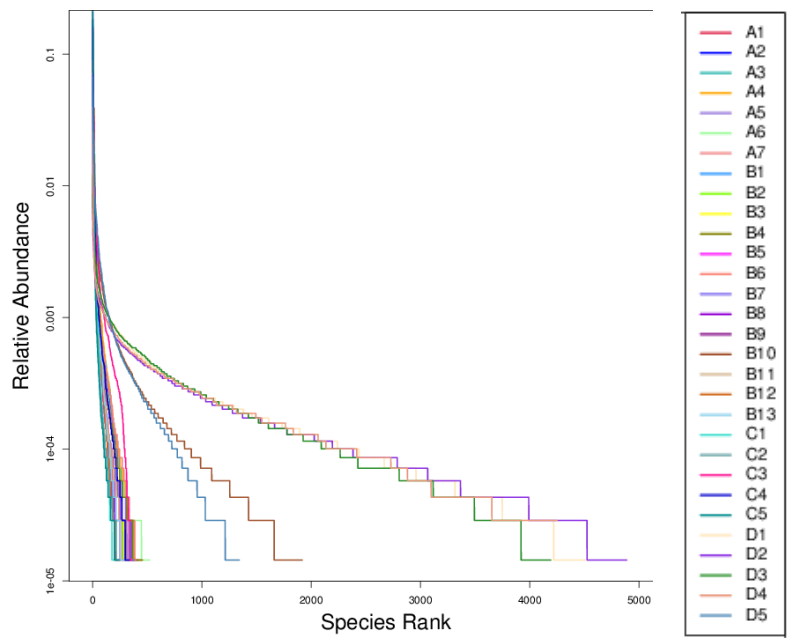

Figure 4 Species Rank showing Relative abundance of soil samples (D1 – D5)

Upon the addition of BC, a significant increase in *Bacillus* sp. was observed in D4 (0.0012054) and D5 (0.0057687) compared to D1 (0.0001722). As D1, D2, and D3 samples are collected from paddy fields, there is a higher correlation in soil microbiomes than D4 and D5 as shown in Figure 5. Conversely, following the addition of BC, changes in microbial diversity were observed, there is no presence identical microorganisms in D2–D3–D4–D5 (0), despite these soil samples are of the same type but cultivated with different plants. This clearly indicates that BC addition contributes to enhancing microbial diversity.

Biochar-Induced Enhancement of Soil Enzyme Activity and Metabolic Pathways

The analysis of enzymes or metabolic pathways can predict soil health and fertility. The functions of various enzymes can refer to a significant role in energy transfer, organic matter breakdown, and nutrient cycling from soil microorganisms. The enhancement of enzyme activity would affect the metabolic pathways of bacteria, leading to several reaction end-products based on their different functional contributions. The results of enzyme analysis from 5 soil samples revealed that D1 contained 10 distinct enzymes and Clusters of Orthologous Groups (COGs). The most abundant enzyme and COG found in D1 were EC:1.6.5.3 and COG0438, respectively. As shown in Table 2, the enzymes that increased after BC addition were EC:2.7.7.7, EC:3.6.4.12, EC:1.6.5.3, EC:2.1.1.72, and EC:1.2.7.11.

EC:2.7.7.7 is directly responsible for DNA synthesis and involved in transferring phosphorus-containing groups, specifically nucleotidyl transferases. EC:3.6.4.12 are also called molecular motors. This enzyme involved in unwinding DNA during DNA replication, DNA repair and recombination, chromosome segregation, and transcription initiation. Recently discovered that DNA helicases may involve in plant DNA recombination, as their abundance becomes prominent during the meiotic prophase of plants [17]. Additionally, DNA helicases is associated with the hydrolysis of phosphoric ester bonds in polynucleotides. EC:1.6.5.3 is an energy-conserving enzyme complex (Complexes I, 1, 2, 3), which is the first phosphorylation site of mitochondria and the respiratory complexes. This enzyme belongs to the hydrolase and amidase groups that are responsible for catalyzing the hydrolysis of the amide bond between the carbonyl group and the nitrogen atom. EC:1.6.5.3 was the most abundant enzyme in samples D1–D4, except for D5. EC:2.1.1.72 is responsible for producing a species-characteristic methylation pattern on adenine residues in a specific short base sequence in the host cell DNA, and is associated with S-adenosylmethionine–protein-arginine N-methyltransferases. And EC:1.2.7.11 is an essential enzyme in microbial one-carbon metabolism, belonging to the 2-oxoacid oxidoreductase group. This enzyme oxidatively decarboxylates different 2-oxoacids to form their CoA derivatives.

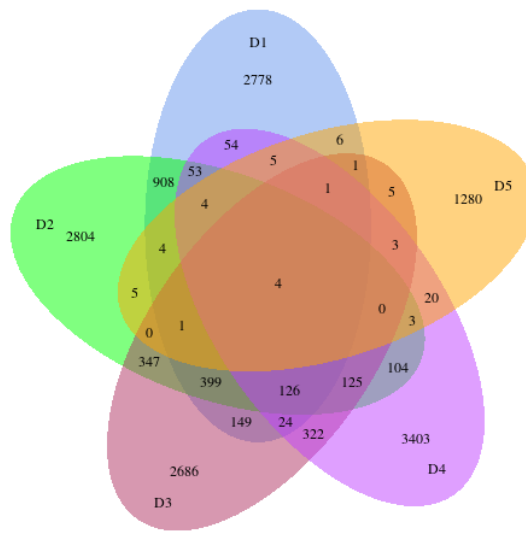

Figure 5 Venn diagram of soil samples

Table 2 Comparison of prominent enzymes from soil samples

Enzyme Commission (EC) number	Accepted Name	Soil samples				
		D1	D2	D3	D4	D5
EC:2.7.7.7*	DNA-directed DNA polymerase	0.013315	0.013435	0.013768	0.012851	0.012729
EC:3.6.4.12*	DNA helicase	0.011838	0.012217	0.012156	0.011122	0.011131
EC:1.6.5.3*	NADH:ubiquinone reductase	0.014261	0.013716	0.014998	0.014136	0.012556
EC:1.2.7.3	2-oxoglutarate synthase	0.003573	0.003992	0.003460	0.002429	0.001763
EC:2.7.13.3	histidine kinase	0.009984	0.009620	0.010220	0.009129	0.010377
EC:2.7.7.6	DNA-directed RNA polymerase	0.005981	0.005886	0.005858	0.006145	0.005445
EC:3.5.1.28	N-acetylmuramoyl-L-alanine amidase	0.001646	0.001979	0.001638	0.001739	0.002381
EC:5.2.1.8	peptidylprolyl isomerase	0.008104	0.008900	0.007880	0.007046	0.006900
EC:2.1.1.72*	site-specific DNA-methyltransferase (adenine-specific)	0.003819	0.003882	0.004042	0.003782	0.002511
EC:1.2.7.11*	2-oxoacid oxidoreductase (ferredoxin, OFOR)	0.002606	0.002802	0.002557	0.001996	0.001566

Note: (*) The increased enzyme after BC addition.

The results indicate that the increase in EC:2.7.7.7 reflects an enhancement in DNA synthesis, suggesting that BC contributes to the growth of microbial populations in the soil. Additionally, the higher levels of EC:1.6.5.3 compared to other enzymes demonstrate that the main activity in all soil samples is electron exchange that occurs in the electron transport chain of oxidative phosphorylation.

Interestingly, the analysis of COGs (Table 3) shown that COGs highly increased after BC addition were COG0745, COG1595, and COG1132. COG0745 belongs to the OmpR family and is involved in transcription as a DNA-binding response regulator with REC and winged-helix (wHTH) domains. The abundance of COG0745 increased Obviously in D3 – D5. COG1595 belongs to the sigma24 family and is involved in transcription as a DNA-directed RNA polymerase specialized sigma subunit. The abundance of COG1595 increased in all soil samples after the addition of BC. And COG1132 is a transport protein associated with the ABC-type multidrug and LPS transport system, specifically the ATPase and permease component MsbA. Its abundance increased Obviously in D3 – D5. From the experimental results, the analysis of COGs revealed the metabolic activity and corresponding essential proteins of the bacterial community, with COG1595 identified as a key protein in the soil with BC addition.

The prediction analysis of metabolic pathways is the analysis within soil samples using PICRUSt2. The most 10 abundant metabolic pathways are shown in Figure 6. According to the metabolic pathway analysis of the samples after BC addition (D3), there was a noticeable increase in the proportion of metabolic pathways compared to the control without BC (D1). The most abundant metabolic pathway found in all samples was PWY-3781, or Aerobic Respiration I (cytochrome c). PWY-3781 is an aerobic respiration pathway that parallels the transport of electrons from the oxidation of NADH, leading to ATP synthesis. This metabolic pathway is performed in plants, animals, and some bacteria via complexes I-IV. Complex I accepts NADH, while complex II accepts the TCA cycle intermediate succinate. The flow of electrons causes protons to be pumped from the matrix side to the intermembrane space, creating a proton gradient. These protons then flow back through the ATP-synthesizing complex in the mitochondrial or bacterial inner membrane, resulting in ATP synthesis. In microorganisms, NADH:ubiquinone oxidoreductases can also catalyze the reoxidation of cytosolic NADH to regenerate NAD^+ such as *Saccharomyces cerevisiae* [18-21]. This is consistent with the above experimental results that showed the presence of EC:1.6.5.3 (Table 1).

Table 3 Comparison of prominent COGs from soil samples

COGs number	Classification	Accepted Name	Soil samples				
			D1	D2	D3	D4	D5
COG0438	TRANSFERASE	Lipopolysaccharide 1,6-galactosyltransferase	0.006011	0.005790	0.006285	0.005686	0.005220
COG1131	TRANSPORT PROTEIN	Ribosome-associated ATPase	0.003302	0.003157	0.003546	0.003429	0.003814
COG0745*	TRANSCRIPTION	DNA-binding response regulator	0.003535	0.003512	0.003603	0.003609	0.004415
COG0642	TRANSFERASE	Signal transduction histidine kinase	0.005481	0.005205	0.005756	0.005157	0.004529
COG1595*	TRANSCRIPTION	DNA-directed RNA polymerase specialized sigma subunit	0.003755	0.003787	0.004022	0.003930	0.004142
COG1132*	TRANSPORT PROTEIN	-	0.002134	0.002103	0.002310	0.002238	0.002301
COG1028	OXIDOREDUCTASE	NAD(P)-dependent dehydrogenase	0.004781	0.004389	0.005080	0.005100	0.005066
COG0451	ISOMERASE	Nucleoside-diphosphate-sugar epimerase	0.004911	0.004375	0.005100	0.004508	0.003938
COG0840	SIGNALING PROTEIN	Methyl-accepting chemotaxis protein (MCP)	0.001795	0.002027	0.001589	0.001252	0.001522
COG1136	MEMBRANE PROTEIN	-	0.002867	0.002682	0.002856	0.002441	0.002435

Note: (*) The increased COGs after BC addition

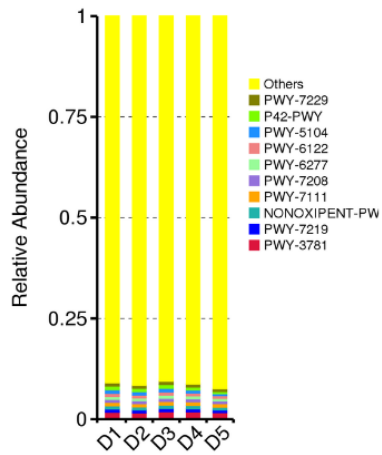

Figure 6 Prediction analysis of metabolic pathways in soil samples

Moreover, the metabolic pathways that increased after BC addition were PWY-7111, PWY-6277, and PWY-6122. PWY-7111 is the Pyruvate Fermentation to Isobutanol (engineered) pathway, where isobutanol is a higher alcohol and a by-product of yeast fermentation. This metabolic pathway is commonly engineered in microbial hosts such as *Saccharomyces cerevisiae*, *Escherichia coli*, and *Bacillus subtilis*. In this pathway, the last two reactions of the Ehrlich pathway are combined with the pathway for valine biosynthesis from pyruvate through the mutual intermediate 3-methyl-2-oxobutanoate to produce isobutanol [22, 23]. PWY-6277 is the superpathway of 5-aminoimidazole ribonucleotide (AIR) biosynthesis. This metabolic pathway's end product is 5-amino-1-(5-phospho-β-D-ribosyl)imidazole (AIR), which is a key intermediate in the biosynthesis of purine nucleotides and thiamine. AIR is synthesized from 5-phospho-α-D-ribose 1-diphosphate (PRPP) through 5 steps. PWY-6122 is the 5-aminoimidazole ribonucleotide biosynthesis II pathway, which parallels the PWY-6277 as it also produces AIR as the end product. However, this metabolic pathway is found in *E. coli*, as the third step is catalyzed by a different enzyme compared to PWY-6277. The metabolic

pathways that increased after BC addition are associated with the production of various compounds, such as isobutanol and AIR.

According to the results, both the abundance of soil microbiome and microbial diversity were observed, along with the effectiveness of BC on soil microorganisms. Additionally, enzymes and metabolic pathways were observed as well. By examining the changes in metabolic pathways, several observations can be used for further research, such as soil quality, microbial activity and diversity, as well as soil enzyme activity. The results indicate that BC contributed to increasing microbial diversity and populations in soil, leading to enhanced microbial activity and an increase in enzyme quantities, which are linked to important metabolic pathways that promote plant growth and soil quality. However, the scope of the research is limited in terms of the range of biochar addition and the investigation of long-term effects and beneficial microorganisms, so further studies are required within this context.

Conclusions

The microbial diversity analysis results of soil samples (D1-D5) revealed 65 phyla, 154 classes, 331 orders, 434 families, 732 genera, and 371 species. Before the addition of BC (D1), Proteobacteria, Acidobacteriota, and Halobacterota were the most abundant microbial phyla, indicating that the soil was in an extreme condition. Regarding the soil after the addition of BC (D2-D5), there was an increase in the populations of Firmicutes and Actinobacteriota, along with new microbial phyla, such as WPS-2, and dominant genera, such as ammonia-oxidizing bacterium (MND1) (3.29%), which is a primary bacterium in soil nitrification.

Additionally, Bacilli have been found as well (D5 (8.34%), D4 (1.06%), D2 (0.18%), D1 (0.18%), and D3 (0.04%)), which are microorganisms that can improve the soil microenvironment. *Bacillus* sp. was observed in D4 (0.12%) and D5 (0.58%) compared to D1 (0.02%). Since BC used in this study is a mixed culture of *Bacillus* sp., it can be implied that biochar can retain and maintain soil-improving microorganisms under varying environmental conditions and also indicates that biochar helps to enhance the population of beneficial soil microorganisms. The results showed that the highest microbial diversity is in D2 (n=4887), which is higher than in D1 (n=4517). Therefore, it can be concluded that BC contributes to increasing microbial diversity. And also found that D4 (3403 OTUs) has the highest specifics and differences in microbiome.

Moreover, COG1595 is a key protein in the soil after BC addition, and it was also found that enzymes, including EC:2.7.7.7 and EC:1.6.5.3, increased after BC addition. This increase suggests that BC contributes to the enhancement of DNA synthesis, leading to an increase in microbial populations in the soil. Furthermore, the main activity of the soil microbiome involves electron exchange, which occurs in the electron transport chain of oxidative phosphorylation.

The most prominent metabolic pathway is PWY-3781 (Aerobic Respiration I (cytochrome c)), which aligns with the presence of EC:1.6.5.3. After the addition of BC (D3), several metabolic pathways increased compared to the control without BC (D1). The metabolic pathways that increased include PWY-7111, PWY-6277, and PWY-6122. In conclusion, biochar can support soil-improving microorganisms and increase microbial populations that enhance microbial activity, resulting in greater microbial diversity and improved soil fertility, which in turn supports better plant health and growth.

Acknowledgement

This research was financially supported by the Fundamental Fund in the year 2024 no. MJU 1-67-08-002.

References

[1] Rizwan, M., Ali, S., Qayyum, M.F., Ibrahim, M., Zia-ur-Rehman, M., Abbas, T. and Ok, Y.S. 2016. Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: a critical review. Environmental science and pollution research international. 23(3): 2230-48.

[2] Kaetzl, K., Lübken, M., Nettmann, E., Krimmler, S. and Wichern, M. 2020. Slow sand filtration of raw wastewater using biochar as an alternative filtration media. Scientific reports, 10(1): 1229.

[3] Yang, W., Wang, Z., Song, S., Han, J., Chen, H., Wang, X., Sun, R., and Cheng, J. 2019. Adsorption of copper (II) and lead(II) from seawater using hydrothermal biochar derived from Enteromorpha. Marine pollution bulletin, 149: 110586.

[4] Park, J.-H., Wang, J., Meng, Y., Delaune, R. and Seo, D.-C. 2019. Adsorption/desorption behavior of cationic and anionic dyes by biochars prepared at normal and high pyrolysis temperatures. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 572: 274-282.

[5] Carter, S., Shackley, S., Soh.i, S., Tan, B., and Haefele, S. 2013. The Impact of Biochar Application on Soil Properties and Plant Growth of Pot Grown Lettuce (Lactuca sativa) and Cabbage (Brassica chinensis). Agronomy. 3: 404-418.

[6] Wenhuan X., Hanmei X., Manuel D.-B., Michael J. G., Xiaoming Z. and Honghua R. 2023. Global meta-analysis reveals positive effects of biochar on soil microbial diversity, Geoderma, 436: 116528.

[7] Gebisa, L. 2018. Effects of Biochar Application on Beneficial Soil Organism Review. Interantional Jounal of research in agriculture and forestry. 7: 6-12.

[8] Pokharel, P., Ma, Z. and Chang, S. 2020. Biochar increases soil microbial biomass with changes in extra- and intracellular enzyme activities: a global meta-analysis. Biochar, 2.

[9] Edgar, R.C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods, 10(10): 996-998.

[10] Gonçalves, O., Fernandes, A., Tupy, S., Ferreira, T., Almeida, L., Creevey, C. and Santana, M. 2023. Insights into Plant Interactions and the Biogeochemical Role of the Globally Widespread Acidobacteriota Phylum. Soil Biology and Biochemistry. 92: 109369.

[11] Hernández-Canseco, J., Bautista-Cruz, A., Sánchez-Mendoza, S., Aquino-Bolaños, T. and Sánchez-Medina, P. 2022. Plant Growth-Promoting Halobacteria and Their Ability to Protect Crops from Abiotic Stress: An Eco-Friendly Alternative for Saline Soils. Agronomy, 12(4): 804.

[12] Wang, X.-L., Zhen-Qiang S., Hao Y., Lin Q., Wei L., Jiang S. and Peng S. 2023. Unveiling the dual role of heterotrophic ammonia-oxidizing bacteria: enhancing plant regrowth through modulating cytokinin delivery. Frontiers in Microbiology. 14 : 1268442.

[13] Sheremet, A., Jones, G. M., Jarett, J., Bowers, R. M., Bedard, I., Culham, C., Eloe-Fadrosh, E. A., Ivanova, N., Malmstrom, R. R., Grasby, S. E., Woyke, T. and Dunfield, P. F. 2020. Ecological and genomic analyses of candidate phylum WPS-2 bacteria in an unvegetated soil. Environmental microbiology, 22(8): 3143-3157.

[14] Zhang, J. and Shen, J.-L. 2022. Effects of biochar on soil microbial diversity and community structure in clay soil. Annals of Microbiology. 72(1): 35.

[15] Xia, H., Hongguang, L., Ping, G., Pengfei, L., Qiang, X., Qian, Z., Mingyue, S., Qiang, M., Fuhai, Ye. and Weizhen Y. 2025. Study of the mechanism by which Bacillus subtilis improves the soil bacterial community environment in severely saline-alkali cotton fields. Science of The Total Environment. 958: 178000.

[16] Gu, Y.-y., Liang, X.-y., Zhang, H.-y., Fu, R., Li, M. and Chen, C.-j. 2023. Effect of biochar and bioorganic fertilizer on the microbial diversity in the rhizosphere soil of Sesbania cannabina in saline-alkaline soil. Frontiers in Microbiology. 14: 1-14.

[17] Sarwat, M. and Tuteja, N. 2018. Chapter 7 - DNA Helicase-Mediated Abiotic Stress Tolerance in Plants, in Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress Tolerance in Plants, S.H. Wani, Editor., Academic Press. 103-115.

[18] Feng, Y., Li, W., Li, J., Wang, J., Ge, J., Xu, D., Liu, Y., Wu, K., Zeng, Q., Wu,

J.-W., Tian, C., Zhou, B. and Yang, M. 2012. Structural insight into the type-II mitochondrial NADH dehydrogenases. Nature. 491: 478-82.

[19] Marres, C.A., S. de Vries, and Grivell, L.A. 1991. Isolation and inactivation of the nuclear gene encoding the rotenone-insensitive internal NADH: ubiquinone oxidoreductase of mitochondria from Saccharomyces cerevisiae. European Journal of Biochemistry. 195(3): 857-62.

[20] Luttik, M. A., Overkamp, K. M., Kötter, P., de Vries, S., van Dijken, J. P. and Pronk, J. T. 1998. The Saccharomyces cerevisiae NDE1 and NDE2 genes encode separate mitochondrial NADH dehydrogenases catalyzing the oxidation of cytosolic NADH. The Journal of biological chemistry. 273(38): 24529-24534.

[21] Small, W.C. and McAlister-Henn, L. 1998. Identification of a cytosolically directed NADH dehydrogenase in mitochondria of Saccharomyces cerevisiae. Journal of Bacteriology. 180(16): 4051-5.

[22] Savrasova, E. A., Kivero, A. D., Shakulov, R. S., and Stoynova, N. V. 2011. Use of the valine biosynthetic pathway to convert glucose into isobutanol. Journal of industrial microbiology and biotechnology. 38(9): 1287-1294.

[23] Blombach, B. and Eikmanns, B.J. 2011. Current knowledge on isobutanol production with Escherichia coli, Bacillus subtilis and Corynebacterium glutamicum. Bioengineered bugs. 2(6): 346-350.