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Soil Biocrusts May Exert a Legacy Impact on the Rhizosphere Microbial Community of Plant Crops

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Abstract: Biological soil crusts (biocrusts) play important ecological roles in many ecosystems, but their legacy effects in subtropical agricultural systems are poorly understood. This study investigated how biocrusts impact soil properties and subsequent crop rhizosphere microbiomes. Soil with (+BC) and without (−BC) biocrusts was cultivated and used to grow pepper plants in a greenhouse experiment. Soil physicochemical properties and microbial communities in the pre-planting soils, and microbial communities in crop rhizosphere were analyzed. The results showed that soils with biocrust had significantly higher organic matter, total nitrogen, alkaline hydrolyzable nitrogen, total phosphorus, and total potassium content. Microbial community structures differed significantly among treatments, with −BC soils exhibiting higher microbial diversity in pre-planting conditions, while +BC soils showed higher diversity in crop rhizosphere soils. Soil properties, especially extractable potassium, total nitrogen, and organic matter content, were significantly correlated with rhizosphere microbial community structure. Additionally, our results showed that the first principal coordinate (PCoA1) of soil microbial community structure was significantly correlated with rhizosphere microbiota. Multiple regression analysis revealed that pre-planting soil microbial diversity indices and certain soil physicochemical properties could predict crop rhizosphere soil microbial diversity. Our results demonstrate that biocrusts can enhance soil fertility and alter microbial communities in subtropical agricultural soils, with persistent effects on the crop rhizosphere microbiome. This study provides new insights into the ecological legacy of biocrusts in managed subtropical ecosystems and their potential agricultural implications.

Keywords: biological soil biocrust; rhizosphere microbiome; subtropical agroecosystem; soil fertility; microbial diversity

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1. Introduction

Biological soil crusts, or biocrusts, are complex communities of microorganisms and small plants—including bacteria, algae, fungi, lichens, and mosses—that form a living layer within the top centimeter of soil surfaces in various ecosystems [1,2]. Biocrusts are distributed worldwide and have been studied in many countries and dryland soil ecosystems [3–5]. Despite earlier research indicating neutral or even adverse impacts of

biological soil crusts on plant development, recent global studies have overwhelmingly demonstrated that these biocrusts play a beneficial role in promoting plant growth across various ecosystems worldwide [6–8], suggesting the existence of complex, potentially symbiotic relationships between biocrust communities and vegetation.

While the dynamics and ecology of biocrust in arid and semi-arid land have been well documented over the last decade [9,10], fewer investigations have been undertaken in humid and semi-humid regions, despite the fact that climatic and edaphic conditions of large areas seem to be well suited for the development of rich terrestrial cryptogrammic vegetation in these regions [11–13]. In subtropical and tropical agroecosystem, biological soil crust could also develop due to winter fallowing, short-term favorable weather conditions [14]. However, these biocrusts generally die out and decay at the soil surface or are buried into soils with tillage or plowing. There are no studies that have fully characterized the effects of these crusts on soils and crops.

Biocrusts may have the ability to fix CO_2, N_2, and solubilize phosphate, leading to an increase in organic matter and nutrient content, which are then released during the biocrusts' decaying and become available to crops. Biological soil crusts play a crucial role in enhancing carbon and nutrient biogeochemical cycles across diverse ecosystems, including arid, semi-arid, and cold environments, thereby significantly influencing ecosystem functioning and productivity [15,16]. For example, a global meta-analysis showed that the SOC content under biocrusts was 71% higher than that of soil without crust cover [17]. Biological N_2 fixation is one of the key biocrust processes in arid and semi-arid ecosystems [18,19], and humid and semi-humid regions [20,21]. Biocrust microorganisms, including cyanobacteria, algae, and fungi, enhance soil phosphorus (P) availability through multiple mechanisms. These include the production of phosphatases for enzymatic P release, the secretion of organic acids to liberate mineral-bound P, and the excretion of H^+ ions during respiration, which dissolves carbonate-bound P [22,23]. These processes collectively increase P accessibility for plants and soil organisms. Indeed, phosphatase activity has been shown to increase under developed biocrusts compared to areas with bare soil [24]. In addition, biocrust could also improve soil stability [25], enhance soil moisture [26], and interact with plant roots through either nutrient mass flow or fungal networks [27]. In well-established biocrusts, the abundance of carbohydrate-C contributes to the enhanced water solubility of organic C, facilitating its downward migration and consequently promoting the genesis of soil [28,29]. However, the potential impact of biocrusts on subsequent crop growth within agricultural ecosystems remains unclear.

There may be a close link between rhizopheric and biocrust microbiome. Early-succession biocrusts were formed when pioneer primary producers colonized bare ground through the production of exopolysaccharides that bind soil particles [30]. Microbial diversity detected in biocrusts is typically lower than that in soils [31,32]. However, bacterial phyla typically associated with plant root microbiomes, such as *Proteobacteria*, *Actinobacteriota* and *Bacteroidota* [33–35], have also been identified in biological soil crust communities [31,36], suggesting a potential overlap in microbial composition between these distinct soil habitats. The prevalence of these taxonomic groups in both the rhizosphere and cyanosphere can be attributed to their copiotrophic nature, allowing them to adapt to and thrive in the carbon-rich conditions characteristic of these environments [37,38]. Therefore, microbe living in biocrusts can be a source for new plant-growth-promoting microbes and metabolites [32,39]. As studies on the biocrust have only recently emerged, there are limited public datasets on the link between biocrust's and rhizospheric microbiomes available for our comparisons.

In tropical and subtropical agricultural ecosystems, under conditions such as the winter fallow period, etc., a layer of biocrust often grows on the surface. Generally, these biocrusts die on the surface and decay or are mixed into the soil as the land is tilled and the weather becomes less suitable. The legacy impact of biocrusts on soil physicochemistry and soil microbiome in the agroecosystem in these regions could be a combination of C and N input, and microbial migration. However, knowledge is limited about the legacy effect

of biocrust on the rhizosphereic microbiome in the agroecosystem. In this study, we aimed to reveal how these biocrusts affect soil physicochemical properties and the rhizosphere microbiome of subsequently planted crops. We hypothesize that (1) biocrusts could enhance soil CNP availability and alter the rhizosphere microbiome of subsequently planted crops; (2) biocrusts may influence rhizosphere microbial community structure by modifying soil physicochemical properties and serving as a source of crop rhizosphere microbiome.

2. Materials and Methods

2.1. Experimental Design

Soils were sampled from a corn–rice crop rotation field (110°16′42″ E, 21°21′17″ N) located at Zhanjiang in Guangdong province, China. The natural soil type in the area is red soil (Ultisol). The soil at the sampling site is paddy soil, specifically sandy loam. The region has a subtropical monsoon climate. The climate is characterized by warm and humid conditions, with distinct seasons. The average annual temperature ranges from 22 to 23 degrees Celsius, and the annual precipitation is around 1600 to 2000 mm. The climate of Zhanjiang is conducive to agricultural production and the development of various tropical and subtropical crops.

In November 2022, we collected approximately 1000 kg of soil from the sample site. The sampling was conducted within one week after the rice harvest, before the formation of biological soil crusts, which typically develop on these cultivated lands within several weeks post-harvest, depending on weather conditions. We collected soil from the surface to a depth of 0–20 cm, corresponding to the local plough layer, while avoiding ridge and waterlogged areas to ensure the representativeness of the cultivated soil. This soil was transported to a greenhouse (113°20′60″ E, 23°10′57″ N) in Guangzhou, where it was air-dried, sieved through a 1 cm nylon mesh to remove all visible plant residues, stones, and other impurities, and then thoroughly mixed. Afterward, this batch of soil was stored in an earthworm cultivation laboratory, where the temperature was maintained at approximately 26 °C by air conditioning throughout the year. The greenhouse experiment was initiated in November 2023 and consisted of two sequential phases. The first phase focused on soil preparation, establishing two distinct soil treatments in the greenhouse: one with a biocrust layer on the surface and a control without biocrust. The second phase involved planting pepper seedlings in both soil treatments. In one treatment, we simulated soil tillage by incorporating the biocrust into the top layer of soil. A total of 36 experimental pots were utilized, equally allocated between the two experimental phases. Detailed procedures are described in the following sections.

First, 36 truncated cone-shaped pots with a top diameter of 30 cm, a bottom diameter of 23.5 cm, and a height of 20 cm were prepared, and then were lined with non-woven fabric at the bottom to prevent soil leakage and then filled with 8 ± 0.2 kg of the soils. Subsequently, the 36 pots of soil were divided into two groups: one for biocrust growth (+BC) and one for non-biocrust growth (−BC). Both groups were placed in the greenhouse. To inhibit biocrust formation in the −BC treatment, a shading apparatus consisting of three layers of black shade cloth was installed 10 cm above the pots. Soil moisture was monitored gravimetrically every 3–5 days. Water was added to maintain the soil water content (mass of water/mass of dry soil) at 40%. To minimize disturbance to the soil surface, irrigation was carefully performed by adding water along the inner walls of the pots. During this period, biocrust was identified based on visual appearance. After about one month, the +BC group successfully developed biocrusts at soil surface, while in the −BC group, two samples showed signs of algae formation on the surface, but the rest did not develop biological crusts. In the later stage of this phase, we conducted destructive sampling on 9 +BC soil samples and 8 −BC soil samples, with the remaining samples reserved for the second phase of the experiment.

In the remaining 17 pots (9 +BC and 8 −BC), 5 g of soybean meal organic fertilizer was uniformly mixed into the soil of each pot. Meanwhile, we used small shovels to simulate plowing, thoroughly mixing the soil in the pots. By doing this, we incorporated

the biocrust into the 0–15 cm layer of the soil in the +BC treatment, which frequently occurred due to tillage and other farming practices in croplands. After watering each pot to field capacity, two pepper seedlings, approximately 15 cm tall, were transplanted into each. The 34 pepper seedlings were selected from 50 to ensure consistency in growth status. Two weeks after transplanting, one weaker seedling was removed from each pot, leaving only the stronger one to reduce experimental error due to seedling differences. Subsequently, routine management of the peppers was carried out, including regular weeding and watering based on weather conditions and actual soil moisture. During this process, one pepper plant in the +BC group died unexpectedly.

2.2. Soil Samplings

After successful biocrust growth in the +BC treatment, in mid-January 2023, to examine the impact of biological crusts on soil physicochemical properties and bacterial microbiome, we sampled the soil from the first phase. From each pot, we collected soil from 0–15 cm depth (including the biological crust), resulting in two soil types: pre-planting soil with biological crust growth ($+BC_{PPS}$) and pre-planting soil without biological crust growth ($-BC_{PPS}$). About 50 days after planting, we conducted destructive sampling, collecting rhizosphere soil from pepper plants for bacterial amplicon sequencing, yielding another two soil types: crop rhizosphere soil with biological crust growth ($+BC_{CRS}$), and crop rhizosphere soil without biological crust growth ($-BC_{CRS}$). The soil samples for physicochemical analysis (including $+BC_{PPS}$ and $-BC_{PPS}$) were immediately sieved through a 2 mm mesh, mixed thoroughly, and air-dried at room temperature after removing visible plant residues, stones, and other impurities. The samples for bacterial amplicon sequencing (including $+BC_{PPS}$, $-BC_{PPS}$, $+BC_{CRS}$ and $-BC_{CRS}$) were sieved immediately after collection and stored at −80 °C until analysis.

2.3. Soil Physicochemical Property Analyses

Soil samples from $+BC_{PPS}$ and $-BC_{PPS}$ were analyzed for physicochemical properties, including pH, organic matter (SOM), total N (TN), total P (TP), total K (TK), alkaline hydrolyzable N (N_{alk}), extractable P (P_{extrac}), and extractable K (K_{extrac}). All analyses were performed following the methods described by Liu et al. [40] with some modifications, except for the N_{alk} determination. Soil pH was determined in a 1:2.5 soil–water slurry using a glass pH electrode (FiveGO^{TM}, METTLER TOLEDO, Zurich, Switzerland). SOM was determined using the H_2SO_4-$K_2Cr_2O_7$ oxidation method. TN was quantified by the Kjeldahl acid digestion method. TP was quantified using the molybdate blue method after acid digestion. To measure TK, air-dried soil was acid-digested, and the resulting solution was analyzed. The soil N_{alk} content was determined following the method described by [41], where NH_3 produced from 2 g air-dried soil mixed with 7 mL 1 M NaOH solution for 24 h was measured. Soil P_{extrac} was extracted with 5 g air-dried soil via the Bray 1 method (0.03 M NH_4F and 0.025 M HCl, 50 mL) for 5 min. Soil K_{extrac} was extracted with 2.0 g air-dried soil in 100 mL 1 M CH_3COONH_4 solution for 5 min.

2.4. Soil Biocrust and Crop Rhizosphere Bacteria Analyses

Soil samples from all four types ($+BC_{PPS}$, $-BC_{PPS}$, $+BC_{CRS}$, and $-BC_{CRS}$) were analyzed for bacterial properties. For the amplification of the V3-V4 variable regions of the 16S rRNA gene, we utilized the Phusion Hot Start Flex 2X Master Mix [42]. The 16S rRNA gene amplification reaction was set up in a sterile microcentrifuge tube, containing 12.5 µL of master mix, 2.5 µL each of forward (341F) and reverse (805R) primers at 1 µM concentration, targeting the V3-V4 regions, 50 ng of template DNA, and nuclease-free water to reach a final volume of 25 µL. The PCR thermal cycling conditions were as follows: initial denaturation at 98 °C for 30 *s*, followed by 32 cycles of denaturation (98 °C, 10 s), annealing (54 °C, 30 s), and extension (72 °C, 45 s). The reaction concluded with a final extension at 72 °C for 10 min, followed by a hold at 4 °C.

Following the amplification and sequencing of 16S rDNA amplicons on the Illumina MiSeq/HiSeq platform, the raw data underwent several preprocessing steps. First, the data were demultiplexed based on barcode sequences, with both barcodes and PCR primer sequences removed. Paired-end reads were then merged using vsearch v2.14.2 to reconstruct the original tag sequences [43]. Quality filtering was performed using trimmomatic-0.36, retaining only tags with at least 75% of bases at Q20 or higher quality and removing any tags containing ambiguous nucleotides (N).

Chimeric sequences were then eliminated using vsearch to ensure data integrity. For species composition and diversity analysis, the cleaned tags were clustered into operational taxonomic units (OTUs) at 97% similarity using vsearch. Representative sequences from each OTU were taxonomically annotated using the RDP Classifier (Version 2.2) against the GreenGene database [44]. This process enabled a comprehensive analysis of species composition and abundance across various taxonomic levels (Kingdom, Phylum, Class, Order, Family, Genus, and Species), providing detailed insights into the microbial community structure and diversity of the samples.

2.5. Data Analyses

All data are presented as mean ± standard error (SE) unless otherwise stated. All statistical analyses were performed using *R* (version 4.3.2) [45]. To examine the effect of soil biocrust growth on soil physicochemistry, a *t* test was conducted. To assess the similarity of microbial communities across sample groups, we calculated the Jaccard index (*J*) for each pair of groups. The Jaccard index was computed as the ratio of the number of shared OTUs to the total number of unique OTUs in both groups combined. The statistical significance of the observed similarities was evaluated using permutation tests with 999 randomizations. To assess the significance of compositional differences between groups, we conducted a pairwise permutational multivariate analysis of variance (perMANOVA) using adonis function [46]. Pairwise comparisons were performed for each combination of groups, using Bray–Curtis distance matrices to quantify dissimilarities. The analysis involved 999 permutations to ensure robust statistical inference. Principal Coordinate Analysis (PCoA) was employed to visualize the compositional dissimilarities among samples based on Bray–Curtis distances using the cmdscale function, calculated from the OTU table. We employed the envfit function to examine the relationships between soil physicochemical properties/pre-planting soil microbiome and the crop rhizosphere microbial community composition as determined by PCoA [47]. In the analysis, the pre-planting soil microbiome community structure was represented by the first and second principal components from the PCoA [48]. Linear regression analyses were used to explore response of rhizosphere biodiversity indices to soil property changes. The best models including different explanatory variables number were obtained using the regsubsets function based on higher adjusted R^2 in 'leap' package [49]. To avoid overfitting, models with more than 2 independent variables were not considered.

3. Results

3.1. Soil Physicochemical Property

The presence of biocrusts significantly influenced several soil physicochemical properties in the pre-planting soil (Table 1). Soil organic matter (SOM) content was significantly higher in the with-biocrust (+BC) treatment compared to the without-biocrust (−BC) treatment (33.5 ± 2.0 g/kg vs. 22.7 ± 0.4 g/kg, $p < 0.001$). Similarly, total nitrogen (TN) content was significantly elevated in the +BC treatment (0.147 ± 0.007% vs. 0.100 ± 0.002%, $p < 0.001$). Alkaline hydrolyzable nitrogen (N_{alk}) also showed a significant increase in the +BC treatment (121.4 ± 3.6 mg/kg vs. 98.0 ± 2.7 mg/kg, $p < 0.001$). Total phosphorus (TP) content was significantly higher in the +BC treatment (0.166 ± 0.009% vs. 0.131 ± 0.002%, $p = 0.005$), while extractable phosphorus (P_{extrac}) showed no significant difference between treatments ($p = 0.803$). Both total potassium (TK) and extractable potassium (K_{extrac}) were significantly higher in the +BC treatment (TK: 0.360 ± 0.028% vs. 0.236 ± 0.003%, $p = 0.002$;

K_{extrac}: 138.1 ± 9.6 mg/kg vs. 60.8 ± 1.1 mg/kg, $p < 0.001$). Soil pH did not differ significantly between the +BC and −BC treatments (5.49 ± 0.13 vs. 5.29 ± 0.07, $p = 0.197$).

Table 1. Comparisons of soil physicochemical property between with-biocrust (+BC) and without-biocrust (−BC) treatments of pre-planting soil.

	+BC	−BC	*p*-Value
pH	5.49 (0.13)	5.29 (0.07)	0.197
SOM (g/kg)	33.5 (2.0)	22.7 (0.4)	<0.001
TN (%)	0.147 (0.007)	0.100 (0.002)	<0.001
N_{alk} (mg/kg)	121.4 (3.6)	98.0 (2.7)	<0.001
TP (%)	0.166 (0.009)	0.131 (0.002)	0.005
P_{extrac} (mg/kg)	175.0 (10.3)	177.8 (3.5)	0.803
TK (%)	0.360 (0.028)	0.236 (0.003)	0.002
K_{extrac} (mg/kg)	138.1 (9.6)	60.8 (1.1)	<0.001

Notes: Data are presented as mean (standard error). pH: soil pH; SOM: soil organic matter; TN: total soil N; N_{alk}: alkaline hydrolyzable N; TP: total soil P; P_{extrac}: extractable P; TK: total K; K_{extrac}: extractable K. *p*-values were obtained using paired *t* tests.

3.2. Microbial Community in Pre-Planting and Crop Rhizosphere Soils

Analysis of the bacterial community composition across four sample groups revealed distinct phylum-level profiles (Figure 1). The $+BC_{PPS}$ group was characterized by a high abundance of Cyanobacteria (49.5%), followed by Proteobacteria (29.8%) and Chloroflexi (15.7%). This composition differed markedly from the other groups, particularly in the dominance of Cyanobacteria. In contrast, the $-BC_{PPS}$ group showed a more balanced distribution among the top phyla, with Proteobacteria (29.1%), Actinobacteria (23.6%), and Chloroflexi (12.6%) being the most prevalent. The $+BC_{CRS}$ group exhibited a similar pattern to $-BC_{PPS}$, with Proteobacteria (29.4%), Actinobacteria (22.6%), and Bacteroidetes (19.2%) as the dominant phyla. The $-BC_{CRS}$ group demonstrated a unique profile, with Proteobacteria (23.5%) and Actinobacteria (18.6%) remaining prominent but showing a notable increase in Acidobacteria (7.7%) compared to other groups. Interestingly, Cyanobacteria, which was dominant in $+BC_{PPS}$, showed minimal presence in $-BC_{CRS}$ (0.3%).

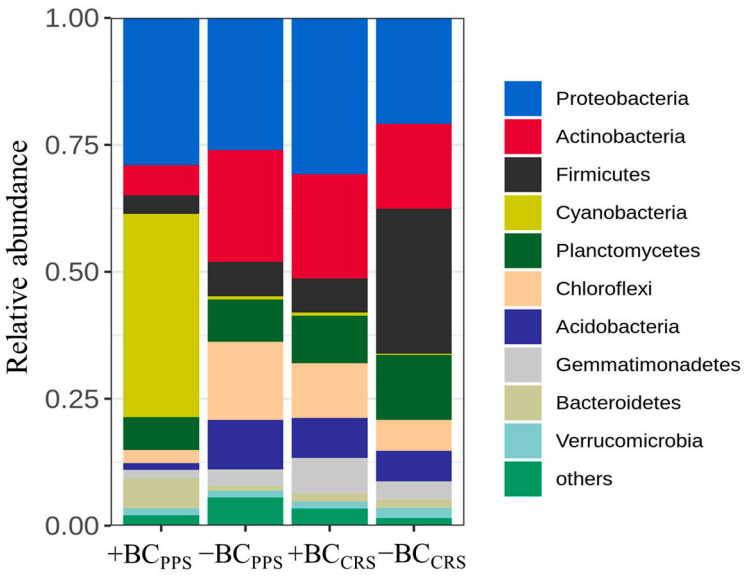

Figure 1. Stacked bar chart of bacterial abundance at phylum level. $+BC_{PPS}$: pre-planting soil with biocrust; $-BC_{PPS}$: pre-planting soil without biocrust; $+BC_{CRS}$: crop rhizosphere soil with biocrust; $-BC_{CRS}$: crop rhizosphere soil without biocrust.

Pairwise comparisons of microbial communities across different soil types revealed varying degrees of similarity and shared operational taxonomic units (OTUs) (Table 2). The

highest similarity was observed between $+BC_{CRS}$ and $-BC_{CRS}$ ($J = 0.772$, $p < 0.001$), with 4854 shared OTUs. This was followed by the comparison between $-BC_{PPS}$ and $+BC_{CRS}$ ($J = 0.718$, $p < 0.001$), which shared 4685 OTUs. The lowest similarity was found between $+BC_{PPS}$ and $-BC_{CRS}$ ($J = 0.557$, $p < 0.001$), with 3486 shared OTUs. PerMANOVA analysis indicated significant differences in microbial community composition across all soil type comparisons ($p < 0.001$). The highest proportion of variance explained by group differences was observed between $-BC_{PPS}$ and $+BC_{CRS}$ ($R^2 = 0.709$, $p < 0.001$), while the lowest was between $+BC_{CRS}$ and $-BC_{CRS}$ ($R^2 = 0.372$, $p < 0.001$). Interestingly, the presence or absence of biocrust appeared to influence microbial community composition. For instance, pre-planting soils with and without biocrust ($+BC_{PPS}$ vs. $-BC_{PPS}$) showed a moderate level of similarity ($J = 0.643$, $p < 0.001$) and a significant proportion of variance explained by group differences ($R^2 = 0.629$, $p < 0.010$).

Table 2. Pairwise comparison of microbial community difference across soil types.

	$-BC_{PPS}$	**$+BC_{CRS}$**	**$-BC_{CRS}$**
$+BC_{PPS}$	Shared OTUs: 3866 Similarity: $J = 0.643$ *** $R^2 = 0.629$ **	Shared OTUs: 3783 Similarity: $J = 0.584$ *** $R^2 = 0.614$ ***	Shared OTUs: 3486 Similarity: $J = 0.557$ *** $R^2 = 0.477$ ***
$-BC_{PPS}$	-	Shared OTUs: 4685 Similarity: $J = 0.718$ *** $R^2 = 0.709$ ***	Shared OTUs: 4229 Similarity: $J = 0.646$ *** $R^2 = 0.524$ ***
$+BC_{CRS}$			Shared OTUs: 4854 Similarity: $J = 0.772$ *** $R^2 = 0.372$ ***

Notes: For each pairwise comparison, the table shows the number of shared OTUs, Jaccard similarity index (J), and PerMANOVA results (R^2 values). The Jaccard similarity index ranges from 0 to 1, with higher values indicating greater similarity. PerMANOVA R^2 values represent the proportion of variance explained by group differences. Asterisks indicate statistical significance levels: ** $p < 0.010$, *** $p < 0.001$. $+BC_{PPS}$: pre-planting soil with biocrust; $-BC_{PPS}$: pre-planting soil without biocrust; $+BC_{CRS}$: crop rhizosphere soil with biocrust; $-BC_{CRS}$: crop rhizosphere soil without biocrust.

Furthermore, Principal Coordinate Analysis (PCoA) was conducted to visualize the differences in microbial community composition among the four soil types (Figure 2). The relative positions of the groups in the PCoA plot generally corresponded to the PerMANOVA R^2 values. The $+BC_{CRS}$ and $-BC_{CRS}$ exhibited the least separation. In addition, the PCoA showed that soil samples without biocrust showed greater internal variability, with $-BC_{CRS}$ exhibiting the highest degree of within-group variation among all four soil types.

The diversity indices of microbial communities were analyzed and compared between +BC and −BC treatments for both pre-planting soil and crop rhizosphere soil (Table 3). The results revealed significant differences in microbial diversity across all measured indices. In the comparison between $+BC_{PPS}$ and $-BC_{PPS}$ groups, all three diversity indices showed statistically significant differences ($p < 0.001$ for all indices). The $-BC_{PPS}$ group exhibited consistently higher diversity across all measures. Specifically, the observed species count (OS) in the $-BC_{PPS}$ group (3548.8 ± 24.1) was substantially higher than in the $+BC_{PPS}$ group (2228.8 ± 92.5). This trend was mirrored in the Shannon diversity index (H), with $-BC_{PPS}$ showing a higher value (9.80 ± 0.03) compared to $+BC_{PPS}$ (6.84 ± 0.38). The Chao1 richness (Chao1) further confirmed this pattern, with $-BC_{PPS}$ (4216.3 ± 39.9) demonstrating greater species richness than $+BC_{PPS}$ (2968.8 ± 89.8). A similar pattern of significant differences was observed between the $+BC_{CRS}$ and $-BC_{CRS}$, albeit with a reversal in the direction of the relationship. The $+BC_{CRS}$ consistently showed higher diversity indices compared to the $-BC_{CRS}$. The OS was significantly higher ($p = 0.007$) in $+BC_{CRS}$ (3822.5 ± 42.1) than in $-BC_{CRS}$ (2613.8 ± 323.7). The H also indicated significantly higher diversity ($p = 0.001$) in $+BC_{CRS}$ (9.84 ± 0.05) compared to $-BC_{CRS}$ (8.24 ± 0.30). Correspondingly, the Chao1 was significantly higher ($p = 0.003$) in $+BC_{CRS}$ (4570.0 ± 51.4) than in $-BC_{CRS}$ (3175.0 ± 328.3).

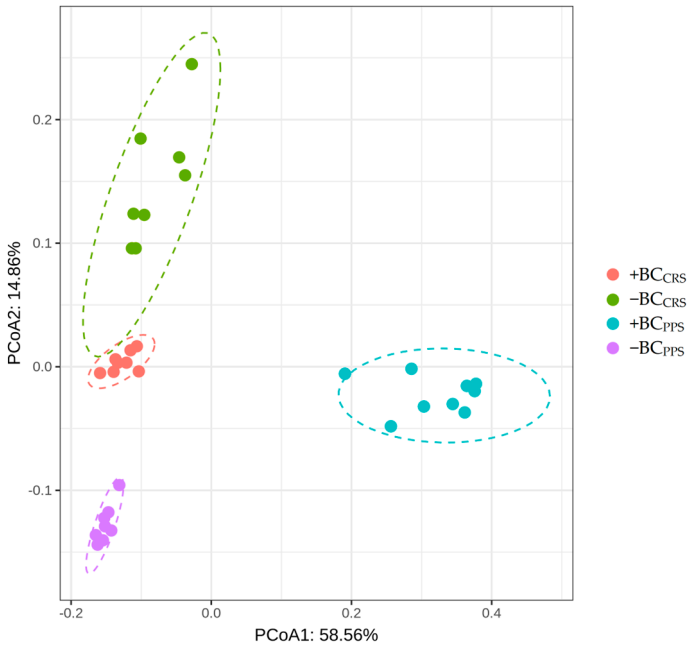

Figure 2. Principal Coordinate Analysis (PCoA) of microbial community composition in different soil samples. $+BC_{PPS}$: pre-planting soil with biocrust; $-BC_{PPS}$: pre-planting soil without biocrust; $+BC_{CRS}$: crop rhizosphere soil with biocrust; $-BC_{CRS}$: crop rhizosphere soil without biocrust.

Table 3. Comparisons of alpha diversity between with-biocrust (+BC) and without-biocrust (−BC) treatments in pre-planting and crop rhizosphere soils.

Soil Type		OS	H	Chao1
$+BC_{PPS}$		2228.8 (92.5)	6.84 (0.38)	2968.8 (89.8)
$-BC_{PPS}$		3548.8 (24.1)	9.80 (0.03)	4216.3 (39.9)
	p-value	<0.001	<0.001	<0.001
$+BC_{CRS}$		3822.5 (42.1)	9.84 (0.05)	4570.0 (51.4)
$-BC_{CRS}$		2613.8 (323.7)	8.24 (0.30)	3175.0 (328.3)
	p-value	0.007	0.001	0.003

Notes: Data are presented as mean (standard error). OS: number of observed species; H: Shannon's diversity index; Chao1: Chao1 diversity index. $+BC_{PPS}$: pre-planting soil with biocrust; $-BC_{PPS}$: pre-planting soil without biocrust; $+BC_{CRS}$: crop rhizosphere soil with biocrust; $-BC_{CRS}$: crop rhizosphere soil without biocrust. *p*-values were obtained using paired *t* tests.

3.3. Relationships Between Rhizosphere Microbiome and Soil Properties

The analysis reveals that several soil properties have significant correlations with the rhizosphere microbial community structure (Table 4). The strongest correlation is observed with PCoA1 (r^2 = 0.807, p = 0.001), which explains 58.56% of the total variation in the microbial community structure. Among the soil physicochemical properties, K_{extrac} shows the highest correlation (r^2 = 0.687, p = 0.001), followed by TN (r^2 = 0.568, p = 0.004) and SOM (r^2 = 0.564, p = 0.005). Other soil properties that demonstrate significant correlations include N_{alk} (r^2 = 0.520, p = 0.010), TK (r^2 = 0.453, p = 0.019), and TP (r^2 = 0.422, p = 0.032). In contrast, soil pH (r^2 = 0.156, p = 0.316) and P_{extrac} (r^2 = 0.002, p = 0.992) show weak and non-significant correlations with the microbial community structure. Similarly, PCoA2 and PCoA3, which explain 14.86% and 10.93% of the total variation, respectively, do not exhibit significant correlations ($p > 0.05$) with the rhizosphere microbial community.

Table 4. Results of environmental vectors fitting analysis showing the relationships between soil properties (including soil physicochemistry and microbial community) and crop rhizosphere microbial community.

	r^2	*p*-Value
pH	0.156	0.316
SOM	0.564	0.005
TN	0.568	0.004
N_{alk}	0.520	0.010
TP	0.422	0.032
P_{extrac}	0.002	0.992
TK	0.453	0.019
K_{extrac}	0.687	0.001
PCoA1	0.807	0.001
PCoA2	0.012	0.923
PCoA3	0.005	0.965

Notes: r^2 was calculated using the envfit function in R to assess the correlation strength.; *p*-values correspond to the significance of the correlations. K_{extrac}: extractable K; TN: total soil N; N_{alk}: alkaline hydrolyzable N; SOM: soil organic matter; TK: total K; P_{extrac}: extractable P; pH: soil pH; TP: total soil P. PCoA 1, 2, and 3 refer to the sample scores on the first three principal coordinate axes obtained from our Principal Coordinate Analysis (PCoA) of the microbial community data in pre-planting soils. PCoA1, PCoA2, and PCoA3 explain 58.56%, 14.86%, and 10.93% of the total variation, respectively.

The relationship between crop rhizosphere microbial diversity indices and soil physicochemical factors, as well as pre-planting soil microbial diversity indices, was analyzed using multiple regression models (Table 5). For the Chao1 diversity index of crop rhizosphere soil ($Chao1_{CRS}$), the optimal model included the Chao1 diversity index of pre-planting soil ($Chao1_{PPS}$) as an independent variable, yielding an adjusted R^2 of 0.474 with a *p*-value of 0.002. Additionally, when N_{alk} and K_{extrac} were included as predictors, the adjusted R^2 improved to 0.573, with a *p*-value of 0.002. In the case of the number of observed species of crop rhizosphere soil (OS_{CRS}), the model incorporating observed species number of pre-planting soil (OS_{PPS}) as a predictor achieved an adjusted R^2 of 0.403 and a *p*-value of 0.005. The inclusion of N_{alk} and K_{extrac} further increased the adjusted R^2 to 0.592, with a *p*-value of 0.001. For Shannon's diversity index of crop rhizosphere soil (H_{CRS}), the model with Shannon's diversity index in pre-planting soil (H_{PPS}) as a predictor resulted in an adjusted R^2 of 0.514 and a *p*-value of 0.001. The inclusion of SOM and P_{extrac} significantly enhanced the model's performance, achieving an adjusted R^2 of 0.764 with a *p*-value of less than 0.001.

Table 5. Results of all-subset linear regression analysis for crop rhizosphere microbial diversity indices with soil and microbial factors.

Dependent Variables	Independent Variables	Adjusted R^2	*p* Value
$Chao1_{CRS}$	$Chao1_{PPS}$ **	0.474	0.002
	N_{alk} *, K_{extrac} **	0.573	0.002
OS_{CRS}	OS_{PPS} **	0.403	0.005
	N_{alk} *, K_{extrac} ***	0.592	0.001
H_{CRS}	H_{PPS} **	0.514	0.001
	SOM ***, P_{extrac} ***	0.764	<0.001

Notes: Initial models included all soil physicochemical variables and biodiversity indices measured in this study (refer to Tables 1 and 3 for details). The optimal models were obtained using the regsubsets function from the 'leap' package based on higher adjusted R^2. To avoid overfitness, models with more than two independent variables were excluded. Independent variables with negative effect are in italic font. PPS: pre-planting soil; CRS: crop rhizosphere soil. OS: number of observed species; H: Shannon's diversity index; Chao1: Chao1 diversity index. pH: soil pH; SOM: soil organic matter; TN: total soil N; N_{alk}: alkaline hydrolyzable N; TP: total soil P; P_{extrac}: extractable P; TK: total K; K_{extrac}: extractable K. * indicated $p < 0.050$, ** $p < 0.010$; and *** $p < 0.001$.

4. Discussion

4.1. The Effects of Biocrust Growth on Soil Physicochemistry

Our results demonstrate that biocrust-covered soils generally exhibited higher nutrient content and organic matter compared to soils without biocrusts (−BC), which aligns with the findings from previous studies [14,50]. Soil organic matter content was significantly higher in +BC soils. This increase in SOM can be attributed to the photosynthetic activity of cyanobacteria and algae within the biocrusts, which contribute to carbon fixation and organic matter accumulation [51]. The enhanced SOM in biocrust-covered soils may lead to improved soil structure and water-holding capacity, which are crucial for plant growth in arid and semi-arid environments [8,52]. N availability was significantly higher in +BC soils, consistent with the findings of Belnap [53] and Fick, Day, Duniway, Hoy-Skubik and Barger [25]. This increase can be explained by the presence of N-fixing cyanobacteria in biocrusts, which contribute to N input in nutrient-poor soils [51,54]. The higher N content in biocrust-covered soils may have important implications for plant nutrition and ecosystem productivity in nutrient-limited environments. Total soil P and K were also significantly higher in +BC soils. This enrichment of macronutrients in biocrust-covered soils has been reported in other studies [23,55] and may be due to the ability of biocrusts to trap and retain dust particles rich in these elements [56,57]. The higher nutrient content in biocrust-covered soils could potentially enhance soil fertility and support greater plant growth. Extractable K showed a marked increase in +BC soils, which could be attributed to the weathering of minerals by organic acids produced by biocrust organisms [15]. This increased availability of K may have important implications for plant nutrition and growth in these ecosystems. The lack of significant differences in pH and extractable P between +BC and −BC soils suggests that biocrusts may have limited influence on these parameters. This finding contrasts with some previous studies that reported changes in soil pH due to biocrust presence [58], highlighting the complex and potentially site-specific nature of biocrust effects on soil properties.

In conclusion, our findings demonstrate that biocrusts significantly alter soil physicochemical properties, leading to increased organic matter content and higher levels of essential nutrients. These changes in soil properties due to biocrust presence may have important implications for plant growth, nutrient cycling, and overall ecosystem functioning. Future research should focus on the long-term effects of biocrusts on soil development and their potential role in ecosystem restoration and management strategies.

4.2. The Effects of Biocrust Growth on Soil Microbial Community

Our study reveals significant impacts of biological soil crusts (biocrusts) on soil microbial community structure in both pre-planting and crop rhizosphere soils. Notably, soil biocrust growth at the soil surface prior to crop planting markedly altered the phylum-level composition, diversity, and community structure of soil microbiota.

In pre-planting soils, the presence of biocrusts led to a dramatic shift in microbial community composition, characterized by a high abundance of Cyanobacteria. This dominance of Cyanobacteria is consistent with previous studies highlighting their crucial role in biocrust formation and function [9]. The prevalence of Cyanobacteria likely contributes to increased soil stability and nutrient cycling, particularly N fixation, which are key ecological functions of biocrusts [51]. Interestingly, the influence of biocrusts on microbial diversity in pre-planting soils was contrary to our initial expectations. Soils without biocrusts exhibited significantly higher diversity across all measured indices compared to soils with biocrusts. This unexpected result suggests that while biocrusts increase the abundance of certain functional groups, they may simultaneously reduce overall microbial diversity in pre-planting soils. This could be due to the dominance of biocrust-forming microorganisms outcompeting other soil microbes [59], or alterations in soil physicochemical properties induced by biocrusts that favor specific microbial groups [60]. In contrast, the effect of biocrusts on crop rhizosphere soils showed a different pattern. Here, the presence of biocrusts was associated with significantly higher microbial diversity. This reversal in the diversity trend between

pre-planting and rhizosphere soils suggests a complex interaction between biocrusts, plant roots, and soil microbiota. The higher diversity in biocrust-associated rhizosphere soils might be attributed to the combined effects of biocrust-associated microorganisms and plant root exudates, creating a more heterogeneous environment that supports a wider range of microbial species [61]. The pairwise comparisons and PerMANOVA results further underscore the significant influence of biocrusts on microbial community structure. The moderate similarity and significant proportion of variance explained between biocrust and non-biocrust pre-planting soils indicate that biocrusts substantially alter the microbial community composition. Similarly, the differences observed between biocrust and non-biocrust rhizosphere soils confirm that biocrusts continue to influence microbial communities even in the presence of crop rhizosphere effects [62]. The PCoA results visually reinforce these findings, showing clear separation between biocrust and non-biocrust samples. Notably, the greater internal variability observed in soil samples without biocrusts, particularly in rhizosphere soils, suggests that biocrusts may have a stabilizing effect on microbial community composition, potentially by providing a more consistent microenvironment [63].

In conclusion, our research underscores the pivotal role of pre-planting biocrust development in shaping the subsequent crop rhizosphere microbiome. The growth of biocrusts prior to planting can profoundly affect the root-associated microbial communities of the crops, leading to context-dependent impacts on soil microbial community structure. This underscores the intricate interplay between biocrusts, plant roots, and soil microorganisms. Understanding these dynamics is crucial for developing sustainable agricultural practices that leverage the ecological functions of biocrusts while maintaining diverse and resilient soil microbial communities [64].

4.3. The Underlying Mechanisms of Biocrust Growth Influence Crop Rhizosphere Microbial Community

Our findings reveal that biocrusts significantly influence crop rhizosphere microbial community through two primary pathways: legacy effects on soil physicochemical properties and pre-planting soil microbial communities. These pathways contribute to the complex interactions between biocrusts, soil properties, and microbial communities in agricultural ecosystems. Biocrusts indirectly influence crop rhizosphere microbiomes by modifying soil physicochemical characteristics, which is consistent with previous studies demonstrating the significant impact of biocrusts on soil properties [65]. Our results suggest that biocrusts enhance nutrient availability and organic matter accumulation, aligning with the findings of such as Xu, Zhang, Shao and Liu [17] and de-Bashan, Magallon-Servin, Lopez and Nannipieri [23].

The significant relationship between pre-planting and crop rhizosphere microbial biodiversity indices suggests that biocrusts shape community diversity patterns that persist through crop growth stages, supporting the concept of historical contingency in microbial community assembly [66]. The persistence of community structure from pre-planting to crop rhizosphere stages underscores the legacy impact of biocrusts on agricultural soil ecosystems, in line with the observations of Lan et al. [67]. The improved model performance when combining soil properties with pre-planting microbial indices suggests a synergistic effect of biocrust-induced changes in soil properties and microbial legacy on crop rhizosphere microbiomes. This integrated effect highlights the complex nature of biocrust–soil–microbe interactions [39,68,69]. These findings have important implications for understanding the role of biocrusts in agricultural ecosystem management and highlight the potential of leveraging biocrust–soil–microbe interactions to enhance soil health and crop productivity. Future research should focus on investigating the temporal dynamics of biocrust influence, examining the differential impacts of various biocrust types, exploring the specific effects on crop growth and yield, and developing practical strategies to utilize biocrusts for improving degraded soils and enhancing agricultural sustainability.

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