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RESEARCH

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Biochar amendments improve soil functionalities, microbial community and reduce Pokkah boeng disease of sugarcane

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Abstract

Background Biochar is mainly used to enhance soil fertility, microbial activity, improve plant performance and reduce disease incidence.

Methods A greenhouse experiment was designed to investigate the response of biochar on rhizosphere soil chemical properties, enzyme activity, microbial communities, and sugarcane Pokkah boeng disease (PB). Two sugarcane varieties Zhongzhe 9 (Z9) and ROC22, susceptible/resistant to PB, were cultivated and treated with: no biochar, 15 t ha^{-1} biochar, and 30 t ha^{-1} biochar.

Results The amendment of 30 t ha^{-1} of biochar (B2) significantly improve soil pH by 1.50% and 9.61% compared with that of B1 and B0, followed by 0.51% increase by 15 t ha^{-1} of biochar (B1) compared with that of control (B0). The application of 15 t ha^{-1} biochar significantly increased available phosphorus (AP) and ammonium nitrogen (NH_4^+–N) by 209.93 mg kg^{-1} and 12.1 mg kg^{-1}, while the application of 30 t ha^{-1} of biochar significantly increased 241.04 mg kg^{-1} of available potassium (AK) ($P < 0.05$). Furthermore, biochar application increased the activities of soil acid phosphatase (S-ACP), urease (S-UE), and sucrase (S-SC). Alpha diversity analysis showed that the addition of biochar significantly altered the variety and abundance of rhizosphere microorganisms ($P < 0.01$) and increased the relative abundance of beneficial microorganisms *Rhodanobacteraceae*, *Stachybotryaceae*, *Agaricacea*, *Talaromyces*, *Nectriaceae*, *Sistotrema*, and *Bacillus* ($P < 0.01$). There was a significant decrease in the relative abundance of the soil pathogen *Fusarium* ($P < 0.01$).

Conclusion These findings suggested that the application of 15 t ha^{-1} biochar could bring desirable variations in soil functionalities, modulate soil microbial community by increasing soil health and reduce the disease index of PB.

Keywords Biochar, *Fusarium*, Microbial community, Pokkah boeng disease, Rhizosphere

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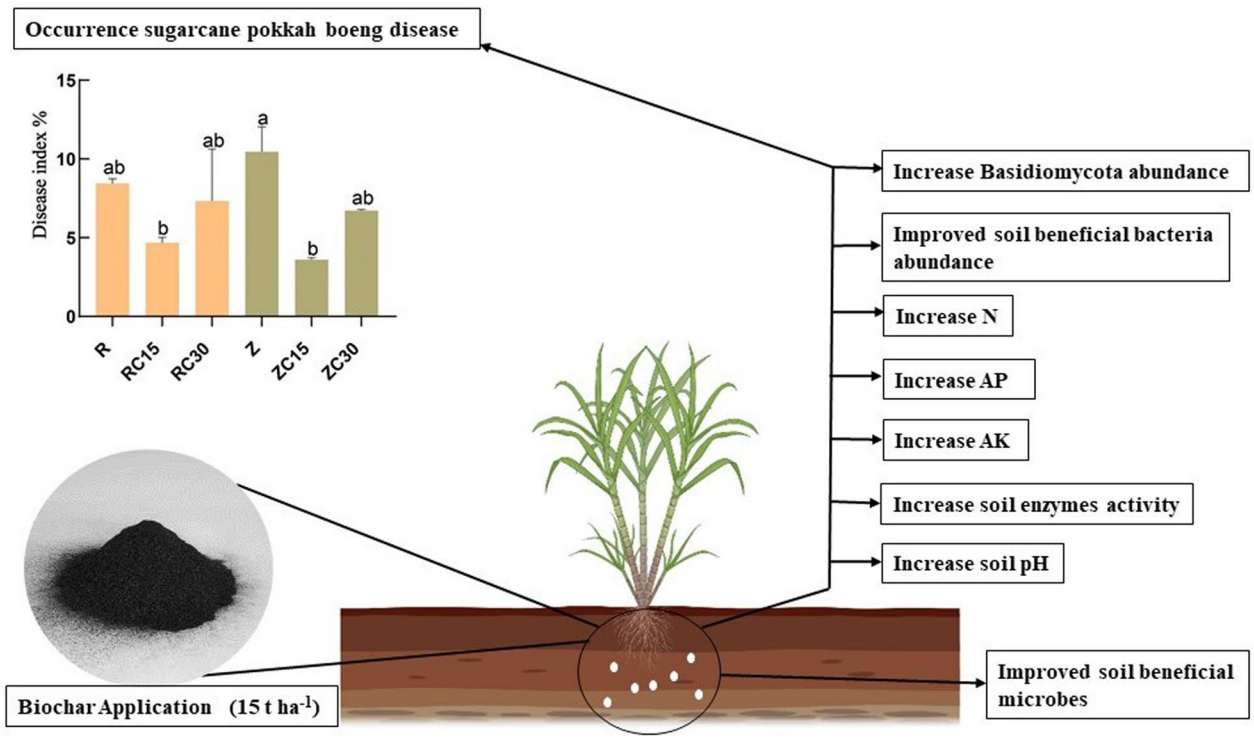
Graphical Abstract



Background

Sugarcane (*Saccharum officinarum* L.) is a significant sugar crop globally. China ranks third in sugar production worldwide, having 90% of sugar production from Southern China [1]. More than 60% of its production is from southern china, specifically the Guangxi Province. Besides on the huge market, sugarcane industry still pose a threat and the sugarcane yield is affected by many factors, including temperature, water, nutrition, weeds, pests, diseases, etc. [2]. Pokkah boeng (PB) is one of the prevalent sugarcane airborne fungal disease caused by *Fusarium spp* [3]. The pathogens of PB are different species of the genus *Fusarium*, such as *Fusarium sacchari*, *F. verticillioides*, *F. proliferatum* [4, 5]. PB disease can be associated with multiple factors, like resistance varieties, rainfall and soil quality as the most important environmental influences [6]. Currently, chemical control of PB is mainly used, but it could cause some environmental pollution [7]. Therefore, the main goal of current research is to develop more ecologically friendly control methods.

Biochar is environmental friendly and has high carbon content, usually produced by the pyrolysis of biological residues (e.g., wood, poultry waste, crop residues, etc.) at high temperatures and pressure [8, 9]. Recently, biochar has been used for three primary purposes: climate change mitigation [10], efficient and cost-effective waste management [11], and as an amendment to enhance soil health and increased crop yield [12, 13]. Biochar changes the rhizosphere microbial community structure and abundance, promotes plant growth, and improves resistance to plant pathogen including fungal disease [14–19]. The biochar derived from citrus raw material was able to mitigate grey mold (airborne) diseases in tomatoes and peppers [20]. It has been reported that biochar has the ability to reduce the rate of root lesions disease caused by the soil borne pathogens *Fusarium oxysporum* f. sp. *asparagi* and *F. proliferatum* [21]. There are enough evidences that biochar's improve the soil health and affect plant diseases resulting in the reduction of disease incidence and effectively suppressed airborne and soil borne pathogens, e.g., *Fusarium* spp., *Phytophthora* spp. [14, 21–23]. Soil microbiome is the collective term for all microorganisms in the soil and their host environment [24]. Soil microbiome are directly involved in plant nutrient acquisition and soil nutrient cycle and indirectly change the available nutrients to plants from the soil [25–27]. The plants using the microbiome complexes in the soil to protect itself from pathogens and enhance its resistance to the diseases

by altering the microbial communities [28]. Therefore, based on previous studies, biochar may improve plant disease resistance by enhancing the abundance and activity of beneficial microorganisms, including mycorrhizal fungi, modifying soil quality in terms of nutrient availability and abiotic conditions (e.g., liming effect), etc. [14].

Biochar has distinct effects on soil physiochemical properties and microbial communities, but there was lack of reports on the application of biochar in sugarcane field to improve soil physiochemical properties and enhance the sugarcane ability to resist the PB disease. The objective of this study was to elucidate the impact of biochar in sugarcane rhizosphere chemical properties, enzyme activities, rhizosphere microbial communities and its response to the PB disease of sugarcane. We hypothesized that biochar soil amendment could alter the chemical properties, soil enzyme, soil microbiome and lead to the reduction of PB disease of the sugar cane.

Materials and methods

Experimental site and management

The greenhouse experiment was planned at Agriculture research station Fusui County, Chongzuo City, Guangxi, China (107°47′17.812′′E, 22°31′7.093′′N). In 2021, the average high and low temperatures of Fusui County was 27 and 18 ℃, respectively, and the annual total precipitation was 867.8 (mm). Two sugarcane varieties Zhongzhe 9 (Z9) and ROC22 that were susceptible/resistant to PB were selected for the current experiment. Biochar used in this experiment was made from palm residues and pyrolysed at 500 ℃ produced locally [29]. The experiment was consist on six treatments, Z (Control; Z9 without biochar); ZC15 (Z9 with 15 t ha^{-1} of biochar); ZC30 Z9 with (30 t ha^{-1} of biochar); R (Control; ROC22 without biochar); RC15 (ROC22 with 15 t ha^{-1} of biochar); RC30 (ROC22 with 30 t ha^{-1} of biochar). The experiment was conducted in randomized complete block design (RCBD) with factorial arrangement. Three biological replicates were set in each group, randomly distributed and six rows were planted in each experimental plot, with a length of 5 m, row spacing of 1.2 m, and plant spacing of about 0.25 m. Each experimental plot was separated by 2 m protective rows, covering an area of 30 m^2. Biochar should be plowed into the ground and mixed uniformly at a depth of 20 cm before sowing, regularly irrigated with a water sprayer, and weeding was carried out thoroughly.

Sample collection

Rhizosphere soil was collected in the middle of July 2021 at the jointing stage. The sugarcane roots were dug out carefully and loosely bound root was shaken off and tightly adhering soil masses were collected [30]. The fresh rhizosphere soil was immediately pass through 20-mesh sieves to remove impurities. For DNA extraction about 6 g of rhizosphere soil was weighted in 2-mL sterilized centrifuge tubes and stored in refrigerator at – 80 ℃. The remaining soil was allowed to dry naturally in the room, away from direct sunlight for measuring the physicochemical properties and enzyme activities.

Measurements of chemical properties of soil and enzyme activities

The rhizosphere soil samples were sieved through a 40-mesh, and analyzed for pH, AP, AK, NH_4^+-N, and NO_3^--N, as well as soil acid phosphatase (S-ACP), soil urease (S-UE), and soil sucrase (S-SC) activities using Solarbio kit (Beijing Solarbio Science & Technology Co., Ltd, China).

Soil DNA extraction, PCR amplification and high-throughput sequencing

Using the FastDNA® Spin Kit for soil, the total DNA of the sugarcane rhizosphere soil was extracted from 0.5 g of fresh soil after being passed through a 20-mesh sieve (MP Biomedicals, Irvine, CA, USA) as per manufacture instructions. The extracted soil DNA was sent for sequencing to Shanghai Meiji Biomedical Technology Co., LTD. Bacterial DNA was amplified using the 515 F/907 R primer set (515F: 5′-GTGCCAGCMGCCGCG GTAA-3′, 907R: 5′-CCGTCAATTCMTTTRAGTTT—3′), which targets the V3–V4 region [31]. Fungal DNA was amplified using ITS1 F/ITS2 R primer set (ITS1F: 5′—CTTGGTCATTTAGAGGAAGTAA-3′, ITS2R: 5′—GCTGCGTTCTTCATCGATGC—3′) for fungi IT1 region [27]. Sequencing results and follow-up analysis were analyzed on the online platform of Majorbio Cloud Platform (www.majorbio.com). Brief description of PCR amplification and sequencing analysis are given in (Additional file 1: Figure S1).

Disease severity assessment

The incidence of PB in each experimental plot was examined in June and July 2021, which is the peak season for PB. The method of Wang et al. [2] was used for the classification of PB disease severity assessment by the formula as described below:

$$\text{Disease incidence} = \text{number of infected plants} / \text{total number of plants} \times 100,$$

while the disease index was calculated as according to the severity of infection %. Like (c1 = 5%, c2 = 15%, c3 = 15 to 35%, c4 = 35–67.5%, c5 = 100%) and the disease index was calculated using the following formula:

$$\text{Disease index} = 5 \times (\text{ncl} + 2\text{nc1} + 5\text{nc3} + 10\text{nc4} + 20\text{nc5}) \big/ \text{n infected plants.}$$

Statistical analysis

This study used PCoA analysis, Alpha diversity, Ternary plot, Kruskal–Wallis rank sum test, and analysis via LEfSe on the Majorbio cloud platform (www.majorbio.com) to investigate the variations of rhizosphere microbial community induced by biochar application. Two-way ANOVA (V × B) was performed using SPSS 19, Graph Pad prism 8.0 to plot histograms.

Results

Effect of biochar on soil chemical properties and enzyme activities

The contents of AK, AP, NH_4^+-N, and NO_3^--N were not significantly ($P < 0.05$) affected by sugarcane varieties, while sugarcane varieties showed significant performance on soil pH (Table 1). Thus ROC22 reported significant 1.93% increase in the pH of soil compared with that of Z9 sugarcane cultivar. However, the significant ($P < 0.05$) changes in soil chemical properties were noted with biochar-treated plots. Therefore, significant higher value of soil pH was 6.95 and the content of soil available K, P, NH_4^+-N, and NO_3^--N were 214.6 mg kg^{-1}, 188.6 mg kg^{-1}, 9.71 mg kg^{-1}, and 47.3 mg kg^{-1} were determined in the rhizosphere soil of sugarcane by B2 treatment (Table 1). Further, the amendments of biochar and the interactive response of B × V showed significant changes in soil pH, and the content of available K and P.

The application of biochar in ROC22 resulted in higher S-UE, S-SC, and S-ACP activities (Table 2). Significant ($P < 0.05$) changes were observed in enzyme activities in the rhizosphere soil of sugarcane. The highest amount of S-SC 36.2 U g^{-1} was observed in Z9. Similarly biochar also significantly improved the soil enzyme activities (Table 2). The significant 48,434 U g^{-1} of S-ACP was noted in the rhizosphere soil of sugarcane. Further, the interactive response of B × V was significant on S-SC, and S-ACP of rhizosphere. The results demonstrated that biochar application significantly increased S-UE, S-SC, and S-ACP activities.

Effect of biochar on the microbial community

Species annotation and alpha diversity assessment

Amplification of V4–V5 region of the 16S rDNA of bacteria resulted 1127,910, and the ITS I region of fungus resulted 1991,590 high-quality sequences, respectively. A total of 8764 bacterial and 4446 fungal OTUs with 97% sequence similarity were obtained. High-throughput sequencing results, the rhizosphere bacterial community was divided into 32 phyla, 84 classes, 193 orders, 287 families, 508 genera, 995 species, and 2030 OTUs. The rhizosphere fungal community was divided into 11 phyla, 35 classes, 81 orders, 168 families, 317 genera, 492 species, and 1103 OTUs. The Shannon index of RC15, RC30 and ZC15, ZC30 were significantly ($P < 0.01$) lower than R and Z, respectively, while that of RC30 was lowest (Fig. 1A, B). Generally, the Shannon and Chao1 index of ZC15 and ZC30 were significantly decreased ($P < 0.05$) compared to Z. In the fungal community, the Shannon

Table 1 Effects of biochar and varieties on the chemical properties (mg kg^{-1}) in the rhizosphere soil of sugarcane field

Varieties (V)	pH	AK	AP	NH_4^+-N	NO_3^--N
ROC22	6.77 ± 0.33^a	191.0 ± 6.2^a	170.8 ± 14.5^a	11.4 ± 0.8^a	46.2 ± 1.0^a
Z9	6.64 ± 0.49^b	182.1 ± 26.2^a	180.3 ± 7.7^a	9.52 ± 0.7^a	45.5 ± 1.9^a
Biochar (B)					
B0	6.34 ± 0.06^c	160.2 ± 18^c	143.5 ± 20.6^b	9.8 ± 1.7^a	46.3 ± 3.4^a
B1	6.84 ± 0.01^b	184.8 ± 16.8^b	194.6 ± 26.1^a	11.8 ± 1.5^a	44.1 ± 1.9^a
B2	6.95 ± 0.02^a	214.6 ± 28.6^a	188.6 ± 16.1^a	9.71 ± 1.0^a	47.3 ± 2.0^a
V	**	ns	ns	ns	ns
B	***	***	**	ns	ns
V × B	***	***	*	ns	ns

Duncan test was adopted for data in the table. Numbers followed by different lowercase letters in a column indicate significant differences ($P \leq 0.05$)

*F values and significance levels at $P < 0.05$; **F values and significance levels at $P < 0.01$; ***F values and significance levels at $P < 0.001$. ns: non-significant

Table 2 Effects of biochar and varieties on enzyme activities (U g^{-1}) in the rhizosphere soil of sugarcane

Varieties (V)	S-UE	S-SC	S-ACP
ROC22	318.5 ± 36.1^a	24.4 ± 2.6^b	41,051 ± 7160^a
Z9	308.9 ± 32.6^a	36.2 ± 3.2^a	34,307 ± 4105^a
Biochar (B)			
B0	299.9 ± 47.3^a	28.6 ± 6.3^a	27,967 ± 8129^b
B1	280.9 ± 41.7^a	31.8 ± 6.3^a	36,637 ± 11602^b
B2	360.4 ± 60.4^a	30.6 ± 8.1^a	48,434 ± 9393^a
V	ns	*	ns
B	ns	ns	*
V × B	ns	**	*

Note: Duncan test was adopted for data in the table. Numbers followed by different lowercase letters in a column indicate significant differences ($P \leq 0.05$)

*F values and significance levels at $P < 0.05$; **F values and significance levels at $P < 0.01$; ***F values and significance levels at $P < 0.001$. ns: non-significant

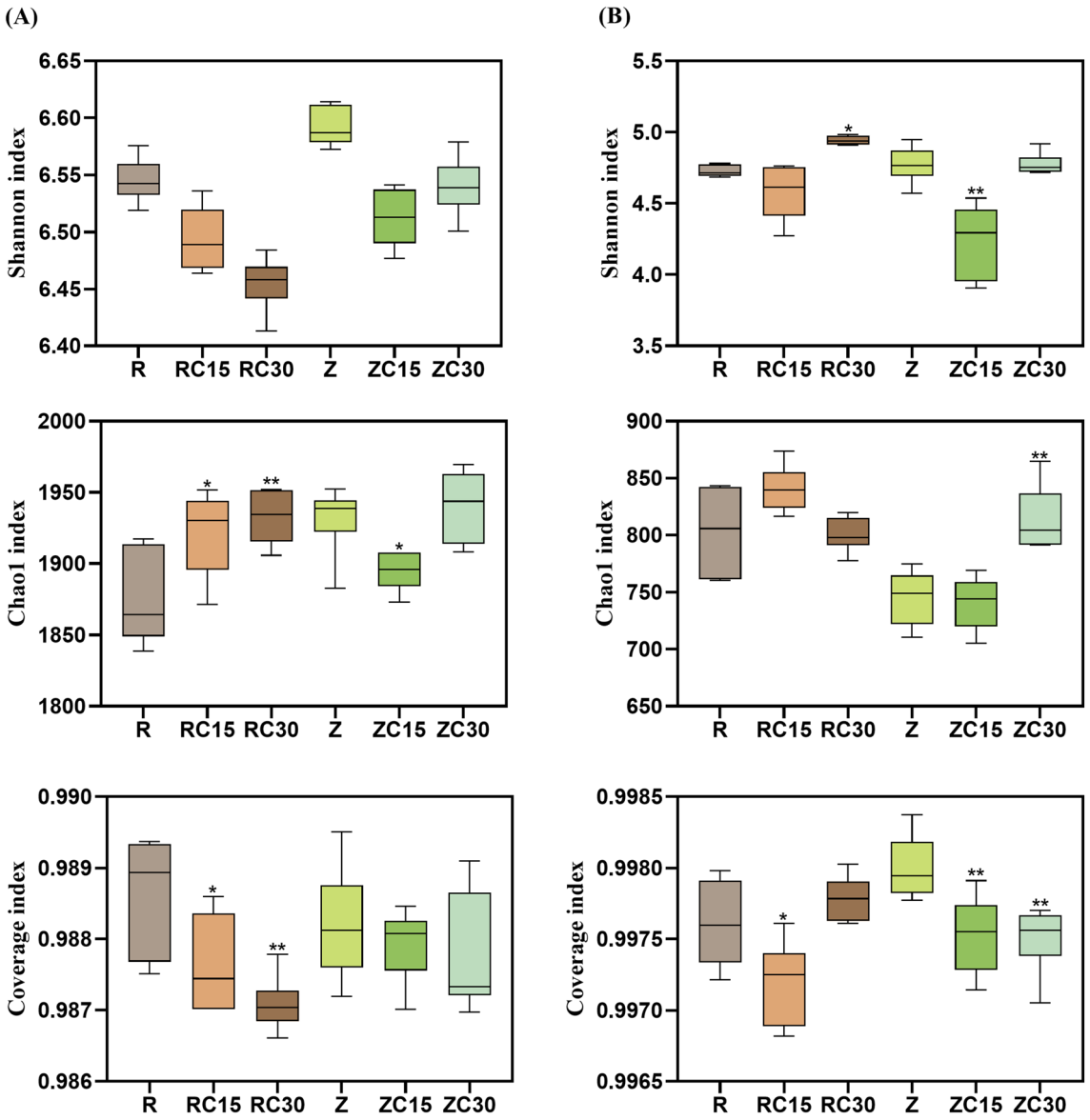


Fig. 1 Alpha diversity indices: Shannon, Chao1 and coverage of soil bacterial communities from different treatments (**A**); Shannon, Chao1 and coverage of fungal communities from different treatment (**B**). * = $P < 0.05$ ** = $P \leq 0.01$. V = Variety, *B* = Biochar, *V* × B = Variety × Biochar

index of RC15 decreased non-significantly compared with R. RC30 was significantly higher than R ($P < 0.05$). The Shannon index of ZC15 was significantly lower compare to Z and ZC30. The Chao1 of the RC15 and RC30 was non-significantly increased. The Chao1 index of Z and ZC15 was lower than ZC30. The microbial community coverage of all the samples was greater than 95%, demonstrating that the sequencing results could genuinely reflect the rhizosphere microbial community. The results suggests that biochar application altered soil microbial community and diversity. However, various sugarcane varieties showed varying responses to biochar application in terms of microbial diversity (Fig. 1A, B).

Sugarcane rhizosphere microbial diversity and community composition

Venn diagram illustrated the number of unique and shared OTUs in the rhizosphere samples (Fig. 2A, B). In bacterial group a total of 3299 OTUs were shared by all the samples, while 163, 187, 190, 320, 152, and 228OTUs were unique

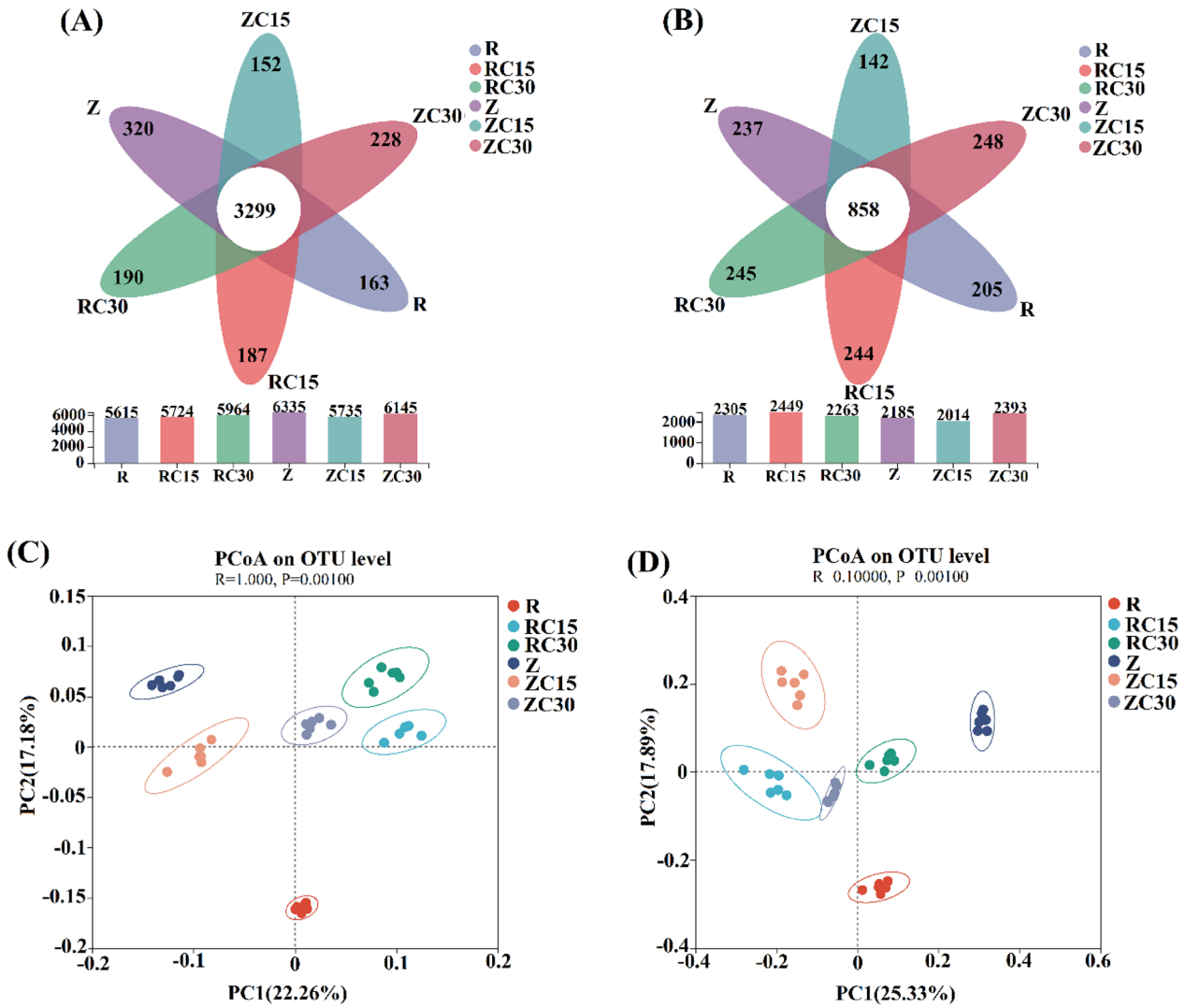


Fig. 2 Venn diagram illustrating the number of shared and unique OTUs in the rhizosphere bacterial and fungal communities (**A**, **B**). PCoA analysis of bacterial and fungal communities (**C**, **D**). The X and Y axes represent the two selected principal axes, and the percentage represents explanatory value of the principal axes for differences in sample composition

to R, RC15, RC30, Z, ZC15, and ZC30, respectively. The number of shared OTUs of fungi in each group were 858, while 205, 244, 245, 237, 142, and 248 OTUs were unique in R, RC15, RC30, Z, ZC15, and ZC30, respectively. Furthermore, the similarities or differences in bacterial and fungal community composition were depicted through PCoA (Fig. 2C, D). For bacterial community, the replicated samples from different group clustered separately on X and Y-axis suggesting greater differences among the groups. PCoA 1 and 2 explained 39.44% of total variation among the groups. Similarly, the fungal communities exhibited a slight similarity between ZC30 and RC15 samples, which clustered close to each other. Further, the two axis explained 43.22% of total variation among the samples and between the different groups.

The rhizosphere bacterial communities in different groups were mainly dominated by Proteobacteria followed by Actinobacteria, Acidobacteria, Chloroflexi and Planctomycetes. However, the abundance of these major bacterial phyla was slightly higher in RC15 and ZC15. The relative abundance of Actinobacteria was increased in RC30 and ZC15, but decreased in RC15. The relative abundance of Chloroflexi was reduced with the amount of biochar applied. Similarly, fungal community of sugarcane rhizosphere mainly comprised Ascomycota, Basidiomycota, Mortierellomycota and unclassified K_Fungi. The relative abundance of Ascomycota was higher in ZC15, lower in ZC30 and enhanced in RC30 as compared

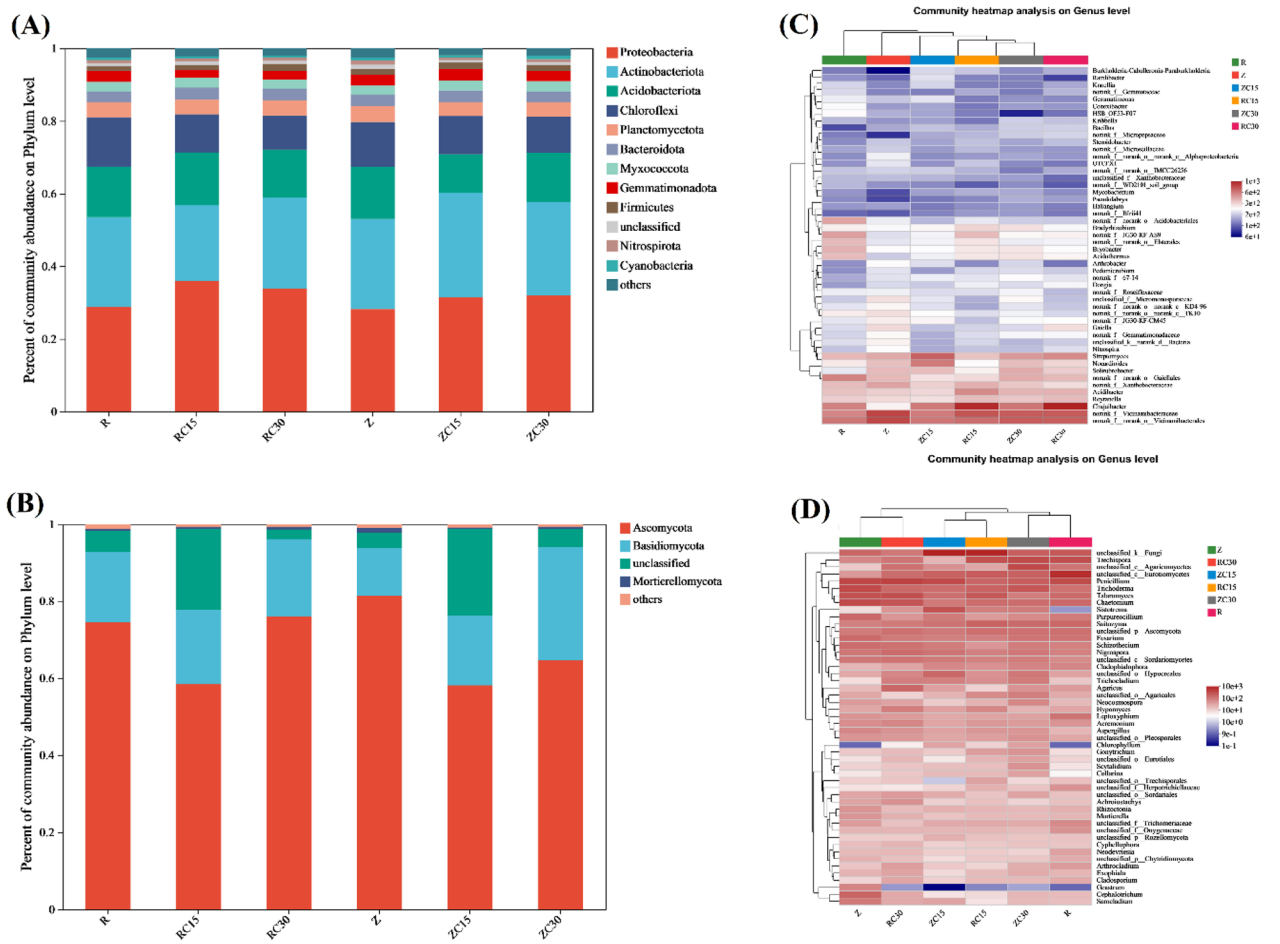


Fig. 3 Species composition analysis: relative abundance of major bacterial and fungal phyla in the rhizosphere soil of different treatments (**A**, **B**); heat map diagrams of different treatments with community composition (**C** bacterial) and (**D** fungal)

to R and Z. The relative abundance of Basidiomycota was highest in RC30 and ZC30 (Fig. 3A, B).

The heat map correlation was carried out on top 50 species in the samples with different treatments. Bacterial community composition treated with the similar amount of biochar in RC30, ZC30 and RC15, ZC15 had similar compositions, and were distinguished from the control R and Z groups (Fig. 3C). Fungal community composition with RC15 and ZC15 had similar composition and could be separated from other treatment (Fig. 3D). The distribution patterns of each species in context to each treatment are presented through ternary plot analysis (Additional file 1: Figure S1).

Analysis of species differences

LefSe analysis was used to understand relative differences between the treatments. Figure 4A shows that the relative abundance of Proteobacteria (phylum and its class Gammaproteobacteria) were enriched in RC15. Xanthomonadales (order and its family Rhodanobacteraceae, genus *Chujaibacter*) was enriched in RC30. Acidobacteriota (phylum and its class Vicinamibacteria, genus *Vicinamibacterales*) was enriched in Z. Gammaproteobacteria (class and its order Xanthomonadales, family Rhodanobacteraceae) was enriched in the ZC15. Phylum proteobacteria were enriched in ZC30 (Fig. 4B).

Figure 4C shows that Eurotiomycetes had significantly higher abundance in R. RC15 mainly contain unclassified_k_Fungi. Ascomycota (phylum and its family Trichocomaceae, genus *Talaromyces*; class Sordariomycetes, family Agaricaceae, genus *Agaricus*) was significantly enriched in RC30. Similarly, Ascomycota (phylum and its genus *Penicillium*) in Z was significantly enriched. Incertae-sedis was significantly enriched in ZC15. Basidiomycota and Agaricomycetes (from phylum to genus) had significantly higher relative abundance in ZC30 than in the other two groups (Z, ZC15). The differences among the treatments were tested by Kruskal–Wallis rank sum and subordinate level and the results are presented in (Additional file 1: Figure S2).

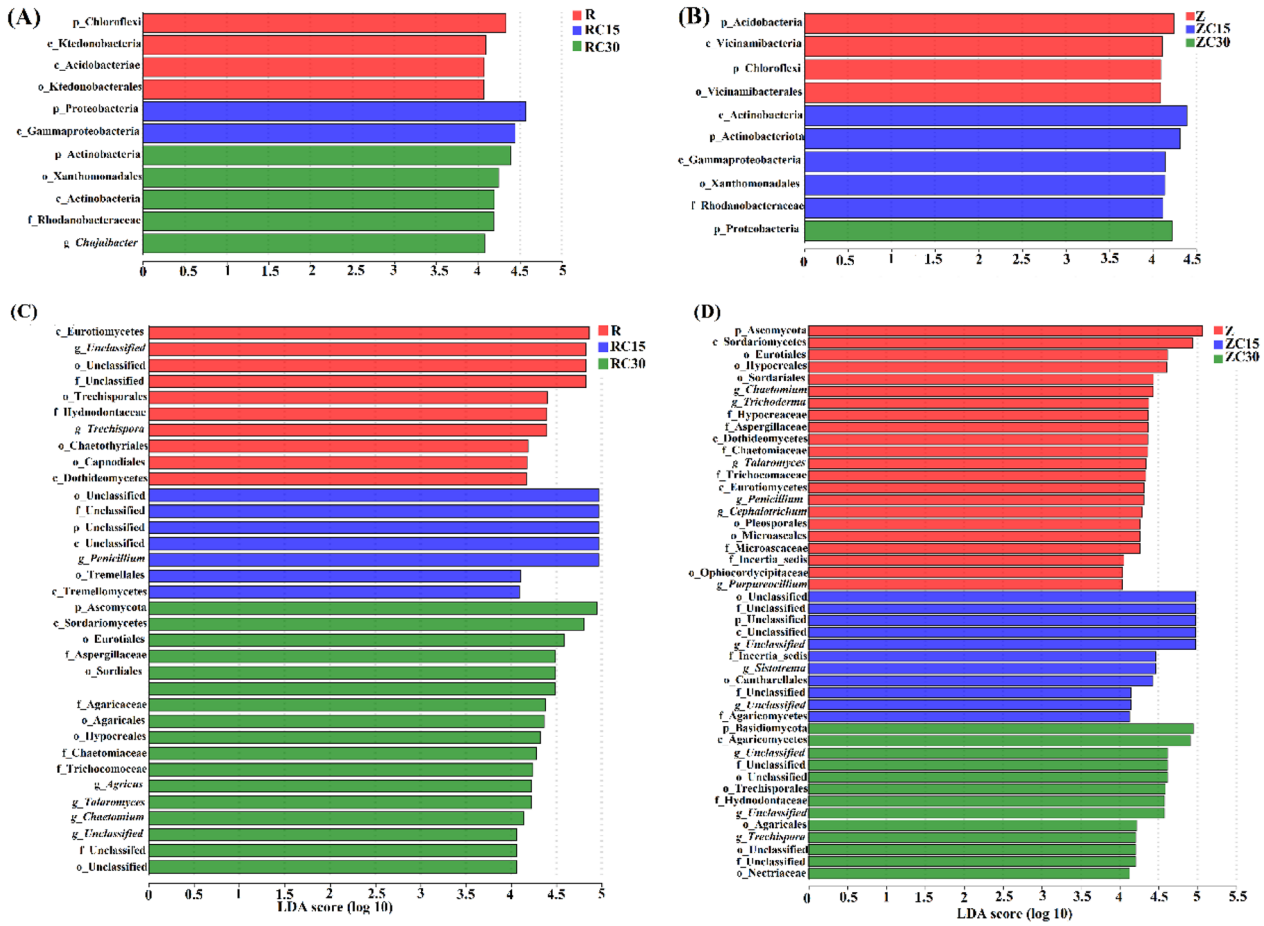


Fig. 4 LEfSe analysis of the sugarcane rhizosphere samples among different treatments on phylum level (**A** bacterial, **B** fungal); LEfSe analysis of the sugarcane rhizosphere samples among different treatments on genus level (**C** bacterial, **D** fungal). The higher the LDA (least discriminant analysis) score, the greater the influence of species abundance on the difference effect

Redundancy and network correlation analysis

RDA analysis illustrated that the first two axis explained 20.83%, 10.41% and 19.92% and 9.79% of the total variation among the bacterial and fungal communities, respectively (Fig. 5A, B). The analysis revealed that the key factors in shaping bacterial community structure were AP, AK, NH_4^+-N, S-SC, and S-ACP, while AP, S-SC, and S-ACP were key factors in shaping fungal communities. AK and S-ACP had more influence on bacterial and fungal communities' distribution in the present study.

In bacterial communities, correlation analysis showed that soil AK was positively correlated with Proteobacteria, Methylomirabilota, and MBNT15 (Fig. 5C), and soil AP was positively correlated with Proteobacteria and Myxococcota. Further, Myxococcota and Fibrobacterota were linked with S-SC in soil. While, in fungal community, the AP and NH_4^+-N showed negative correlation with fungal communities (Fig. 5D), while S-UE was significantly positive correlated with Kickxellomycota, and S-ACP with Basidiomycota and Mucoromycota.

Figure 6A displays the co-occurrence network diagram between the environmental factors and bacterial phyla. The results showed a negative correlation of *HSB_OF53-F07* (Chloroflexi) with AP and S-ACP while *Pedomicrobium* (Proteobacteria) with the content of NH_4^+-N. Figure 6-B shows the co-occurrence network between environmental factors and fungal phyla. *Agaricus* (Basidiomycota) was positively correlated with AK. *Exophiala* (Ascomycota) and *Trechispora* (Basidiomycota) with S-ACP, Gonytrichum (Ascomycota) with S-ACP and AP, *Scytalidium* (Ascomycota) with S-ACP and S-SC, and *Exophiala* (Ascomycota) with S-UE. *Neocosmospora* (Ascomycota) and *Cephalotrichum* (Ascomycota) were negatively correlated with NH_4^+–N and AK. *Purpureocillium* (Ascomycota) was negatively correlated with S-ACP.

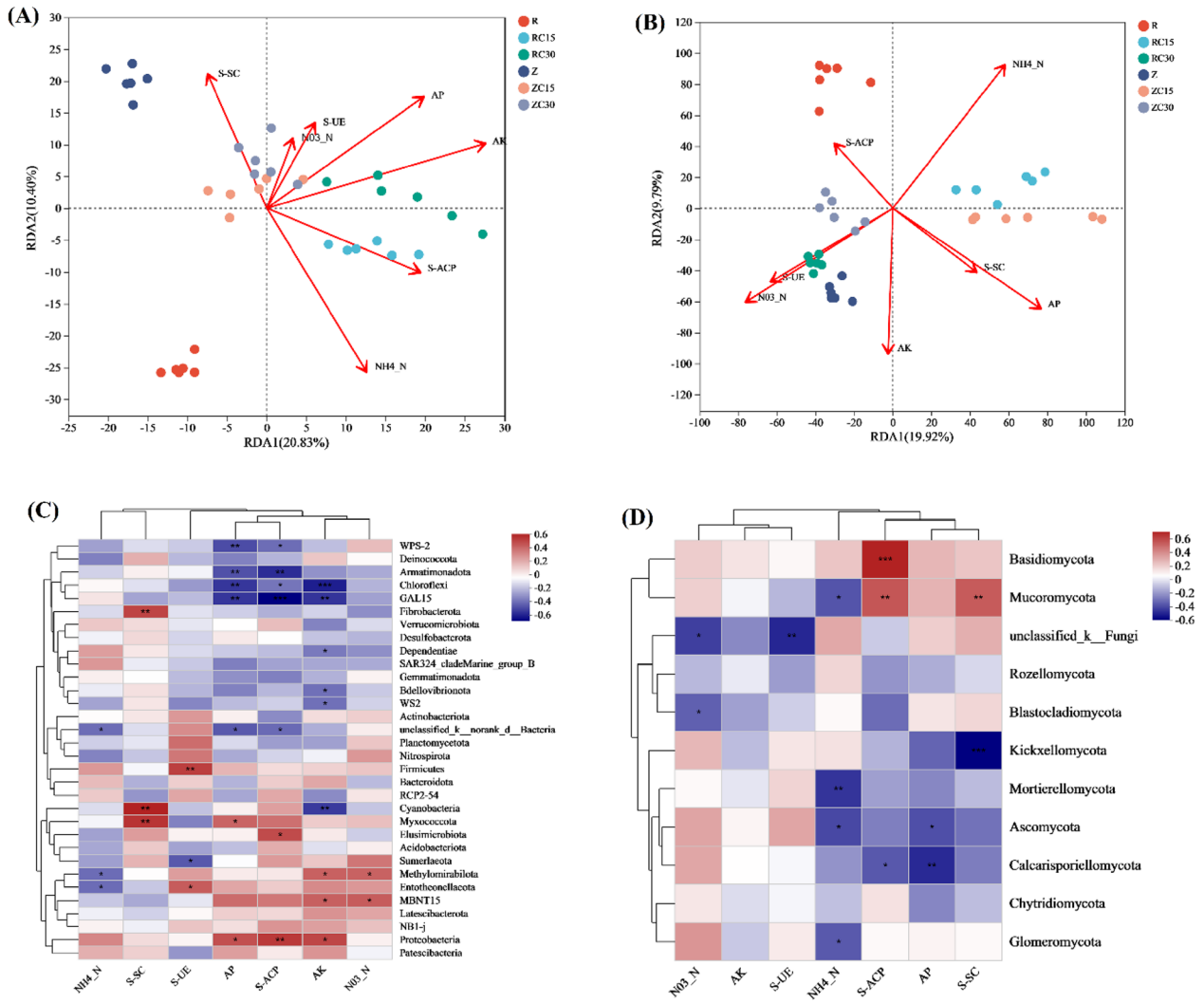


Fig. 5 RDA analysis between the rhizosphere bacteria, fungi community and soil environmental factors in the sugarcane field, respectively (**A** and **B**). The red arrow represents the quantitative environmental factors, and the length of the arrow of environmental factors represents the influence degree (explanatory quantity) of environmental factors. Correlation analysis between soil environmental factors and bacteria (**C**), and correlation analysis between soil environmental factors and fungal communities (**D**). The legend on the right is the color range of different R values, * $P \le 0.05$, ** $P \le 0.01$, *** $P \le 0.001$

Biochar reduces Pokkah boeng disease index

Figure 7 represents the disease index for PB. The disease index of PB was lower in RC15 and RC30 than in the R, and was significantly lower in RC15 ($P < 0.05$). Compared to Z, the disease index of PB was lower in ZC15 and ZC30 and significantly lower in ZC15 ($P < 0.05$). In inference, biochar application reduced PB disease index.

The OTU representative sequence was compared to the Unite database to understand better the differences in the relative abundance of *Fusarium* in the rhizosphere. The relative abundance of rhizosphere *Fusarium* in RC15 and RC30 decreased compared to R, but having no significant difference (Fig. 8A). The relative abundance of rhizosphere *Fusarium* in groups ZC15 and ZC30 decreased as compared to Z, and the results in group ZC15 were highly significant ($P < 0.01$). Biochar significantly reduced the relative abundance of *Fusarium*. Application of biochar at the rate of 15 t ha^{-1} had the highest effect, as evidenced by the lowest relative abundance of *Fusarium* in the RC15 and ZC15 groups. Compared to other treatments, the relative abundance of rhizosphere *Bacillus* increased with biochar application and the highest abundance was observed in RC30 ($P < 0.05$; Fig. 8B). The results showed

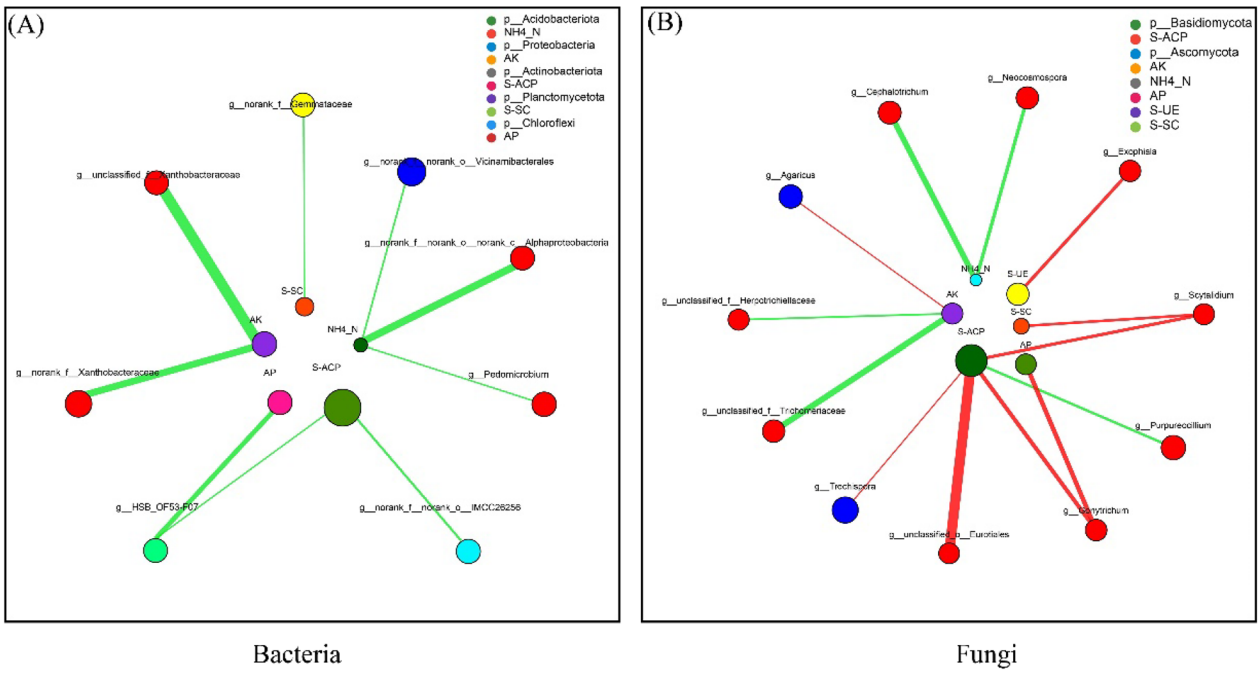


Fig. 6 Correlation network analysis of rhizosphere bacterial (**A**) and fungal (**B**) communities with soil environmental factors in the sugarcane field. The size of nodes represents the abundance of species, and different colors indicate different species. Red line represents positive correlation and green represents negative correlation; the thickness of the line indicates the size of the correlation coefficient

that biochar application could significantly increase the relative abundance of rhizosphere *Bacillus* ($P < 0.05$).

Discussion

Biochar application is one of the most popular approaches in agriculture production [32]. Soil fertility and available nutrients are closely associated with the growth and development of crops [33]. Despite extensive research on sugarcane rhizosphere activities in relation to sugarcane cultivars or organic/inorganic amendments, the exact effect of combination of both particularly using biochar is still unclear. The current study highlights the effect of two different sugarcane cultivars along with different rates of biochar on sugarcane rhizosphere microbial communities and soil chemical properties. Additionally, field surveys were undertaken for Pokkah boeng disease to get insights into effect of biochar addition on the disease incidence. The results showed that biochar application at a rate of 15 t ha^{-1} could significantly increase AP and $NH4^{+}$-N; while increasing AK contents at a rate of 30 t ha^{-1}. The results are in line with previous studies that the application of biochar to soil increased soil AP, $NH4^{+}$-N, and AK levels [34–36]. Different rate of biochar have distinct effect on soil nutrients that could be directly related to the amount of biochar and crop varieties. Soil enzymes are thoroughly correlated to soil microorganisms, fertility, and crop yield [37, 38]. Application of biochar increases the enzyme activities of S-ACP, S-UE, and S-SC with varying levels (Table 2). S-ACP plays a crucial role in the soil P cycle and can be used as one of the indicator to evaluate soil fertility [39]. S-SC enzyme catalyze sucrose production of monosaccharides to enhance plant growth and development and is an important indicator to evaluate soil fertility [40]. As a neutral enzyme, urease can decompose organic matter or urea into nitrogen in soil available for plant absorption and use. The current

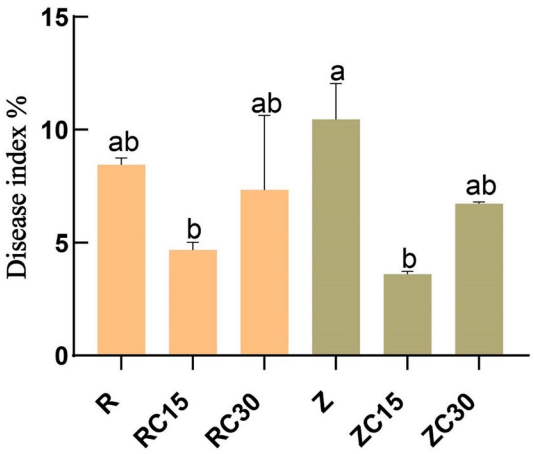


Fig. 7 Disease index of sugarcane Pokkah boeng disease

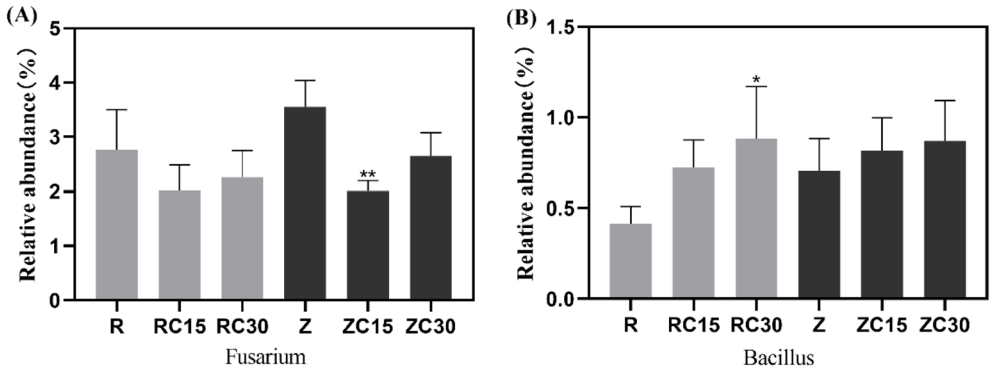


Fig. 8 Histogram of relative abundances of Fusarium (**A**) and Bacillus (**B**) in the rhizosphere of sugarcane field and different treatments

study results are supported by Chen et al. [41], who investigated that RO22 and Z9 resulted in higher S-UE activity. The previous study had shown that biochar could catalyze enzyme-catalyzed reactions through the adsorption of reaction substrates and improve the catalytic efficiency of soil enzymes. The molecular structure of urease is complex, resulting in the insignificant effect of biochar on its activity [42]. Therefore, biochar could change the rhizosphere soil chemical properties and enzyme activity and improved soil quality.

Soil microbial community plays a crucial role in maintaining soil function, quality, and ecosystem sustainability [43–45]. Alpha diversity analysis showed that biochar application significantly changed the diversity and richness of rhizosphere microorganisms ($P < 0.05$). Zhang et al. [18] showed that biochar made from corn stover significantly reduced the diversity of bacteria and fungi in black soil. Wang et al. [46] also found that biochar made from rice straw in contaminated soil reduced bacterial diversity. Therefore, we hypothesized that changes in microbial abundance might be caused by the biochar actual composition and soil properties. The genus-level analysis revealed that the distribution difference of the Ternary plot of the bacteria was not very obvious (Additional file 1: Figure S1A, B). Kruskal–Wallis rank sum test (Additional file 1: Figure S2) and LEfSe analysis (Fig. 4 A-D) showed that the bacterial community in the control group without biochar application mainly enriched *Acidobacteriae, Ktedonobacterales, Vicinamibacterales,* etc. *Acidobacteriae* and *Ktedonobacteria* are mainly distributed in waste rock and slag rock [46]. *Vicinamibacterales* could be used to resist the toxicity of heavy metals [47]. It is obvious from the results and disease incidence and Qpcr data that the experimental center soil was contaminated with *Fusarium* and bacteria and lead to the disease appearance in the control treatments. The treatment group with 15 t ha^{-1} biochar was mainly enriched with Rhodanobacteracea. Concurrently, it had also been found that Rhodanobacteraceae had the capacity for denitrification and could perform the biological restoration of contaminated environments through denitrification [48]. *Acidobacteriae* were mainly enriched in the treatment group where 30 t ha^{-1} biochar was applied. Therefore, we suggested that the application of biochar enriched microbial community could effectively improve the soil microenvironment, improve soil quality and inhibit the occurrence of sugarcane fungal diseases.

This study investigated the influence of soil chemical properties on microbial community as biochar could not directly alter the soil microbial community. Results showed a significant correlation between changes in relative abundance of microbes and soil chemical properties (Figs. 5, 6). The rhizosphere microbial community and S-ACP indicated a substantial positive correlation. The present finding are in line with previous research on the impact of straw biochar on the microbial community in the red loam [49]. Beneficial bacteria Rhodanobacteraceae, fungi Stachybotryaceae, Agaricacea, Talaromyces, and Nectriaceae were enriched in the biochar-treated group. AK S-ACP and AK were positively and significantly correlated with the phylum Proteobacteria. Liu et al. [50] reported that the application of biochar increased the relative abundance of Proteobacteria and Bacteroidetes**.** Members of Proteobacteria are mainly Gram-negative bacteria that are mainly involved in the manipulation of soil nutrients.

Biochar amendments change soil physiochemical characteristics that are determinant in the interactions of soil microbiota, interfering directly or indirectly in the suppression of plant disease, caused by pathogens inhabiting the soil. Field investigation of PB showed that the application of biochar significantly reduced the disease index and the relative abundance of *Fusarium* in the sugarcane rhizosphere. Previous studies demonstrated that using biochar effectively relieved diseases caused by plant pathogens transmitted through the soil and the air are mainly

soil and air borne [14]. However, with the increasing disease incidence within the past few years, more attention has been drawn toward the control management strategies of PB disease. The use of resistant varieties is an important management strategy and such varieties can be used as a source of resistance. PB is airborne, and a suitable climate will accelerate the occurrence of the disease. Fusarium is a plants pathogen, endophyte, and saprophytic fungi. Three microorganisms (*Fusarium*, *Sistotrema* and *Bacillus*) associated with the disease were selected from the top 15 dominating species with relative abundance ratio based on the combined analysis of microbial and disease data (genus level). Among them, the relative abundance of *Fusarium* and incidence of PB decreased significantly, showed that higher *Fusarium* in the rhizosphere, presented the higher the incidence of *Fusarium* wilt-diseased [51]. At the same time, biochar application significantly increased the relative abundance of *Sistotrema* and *Bacillus*. Further, Jin [52], reported that root length, surface area, volume, and tip of oak were maximized by *Sistotrema* inoculation. *Bacillus* was used as a biological fertilizer to inhibit plant fungal diseases (such as *Fusarium* wilt disease) [53, 54]. Biochar inhibits the development of PB by considerably reducing the relative abundance of *Fusarium* in soil, hence relative abundance of *Fusarium*-resistant microbes was enhanced, and influencing the colonization and accumulation of *Fusarium* in roots.

Conclusion

Biochar improves soil rhizosphere microorganisms, and reduces the disease index of PB. The application of biochar changes the diversity of soil microbes in the plant's rhizosphere, improved the ability to prevent pathogens, and reduced the growth of fungi that cause Pokkah boeng disease. Additionally, the alterations in the root microbial population (i.e., the enrichment of *Sistotrema* and *Bacillus* and the decrease of *Fusarium*) improved the resistance against sugarcane Pokkah boeng disease. Results concluded that establishing the rhizosphere microbial community by biochar is one of the important ways to control fungal diseases in sugarcane fields. This research study provides technical references for improving the soil environment, realizing sustainable soil utilization, and improving the sugarcane production environment.

Supplementary Information

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Additional file 1: Figure S1. Ternary plot analysis of bacterial and fungal samples **A**, **B**. The size of the circle represents the average relative abundance of the species. **Figure S2.** Kruskal–Wallis rank sum test results of the rhizosphere bacterial and fungal communities in the sugarcane rhizosphere soil samples (**A**, bacterial; **B**, Fungal). The Y-axis represents the species name under a taxonomy level, the X-axis represents the average relative abundance of species in different groups, and the columns with different colors represent different groups. * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$.

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Author contributions
RW, SA, and XZ designed the experiment and wrote the manuscript. XZ and MW performed the experiment. YS, YC, SA and QL helped in data collection. RW, and BH supervised the study and provide funding. All authors contributed to the article and approved the submitted version.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This manuscript is an original paper and has not been published in other journals. The authors agreed to keep the copyright rule.

Consent for publication
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Competing interests
The authors declare no competing of interests.

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