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Biochar

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Effects of biochar on sugarcane growth and rhizosphere microecosystem under reduced nitrogen fertilization: a 5-year field experiment study

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Abstract

Previous studies have shown that reduced nitrogen application combined with biochar amendment can promote sugarcane growth and improve soil quality; however, their long-term effects on the rhizosphere microenvironment and microbial community in continuously cropped sugarcane remain unclear. A five-year field experiment was conducted with two treatments: basal fertilizer (BF) and basal fertilizer combined with biochar (BF-BC). After fertilization in the first year, and with no additional fertilizers applied in the subsequent years, relevant indicators at the end of the 5-year period were measured. The results showed that, compared with the control BF treatment, the BF-BC treatment significantly increased sugarcane plant height, stem diameter, and leaf nitrogen balance index (NBI) by 10.81%, 25.79%, and 33.90%, respectively, and resulted in significant reductions in total root volume and average root diameter by 31.06% and 21.53% ($P < 0.05$). Simultaneously, the rhizosphere soil pH and total potassium (TK) content increased significantly by 17.74% and 79.21%, whereas soil organic matter (SOM), organic carbon (SOC), total phosphorus (TP), available potassium (AK), and exchangeable calcium ions (E. Ca^{2+}) decreased significantly by 37.67%, 39.64%, 21.20%, 47.29%, and 12.11%, respectively ($P < 0.05$). Despite receiving no additional fertilization following the initial application, the BF-BC treatment still exhibited significant advantages in promoting sugarcane fine root growth, enhancing rhizosphere soil carbon sequestration, and improving fertilizer use efficiency. Additionally, the BF-BC treatment significantly increased the abundance of beneficial rhizosphere bacteria such as *Leptospirillum*, *Terrimonas*, *Actinobacteriota*, *Sphingobacteriia*, *Chitinophaga*, *Cyanobacteriia*, and *Lechevalieria* ($P < 0.05$). Furthermore, the differentially expressed metabolites in the sugarcane rhizosphere were significantly enriched in major metabolic pathways, including steroids and steroid derivatives, fatty acyl groups, purine nucleotides, imidazole pyrimidines, sphingolipids, organic oxygen compounds, indoles and their derivatives, carboxylic acids and derivatives, and benzodioxoles. Importantly, the BF-BC treatment effectively reduced CO_2 emissions from the soil. In conclusion, the sugarcane root system, surrounding soil, and microorganisms form a complex, interconnected symbiotic ecological network. Thus, even after five years without fertilization, reduced nitrogen combined with biochar application still positively influenced sugarcane root and aboveground biomass growth. This finding suggests that biochar

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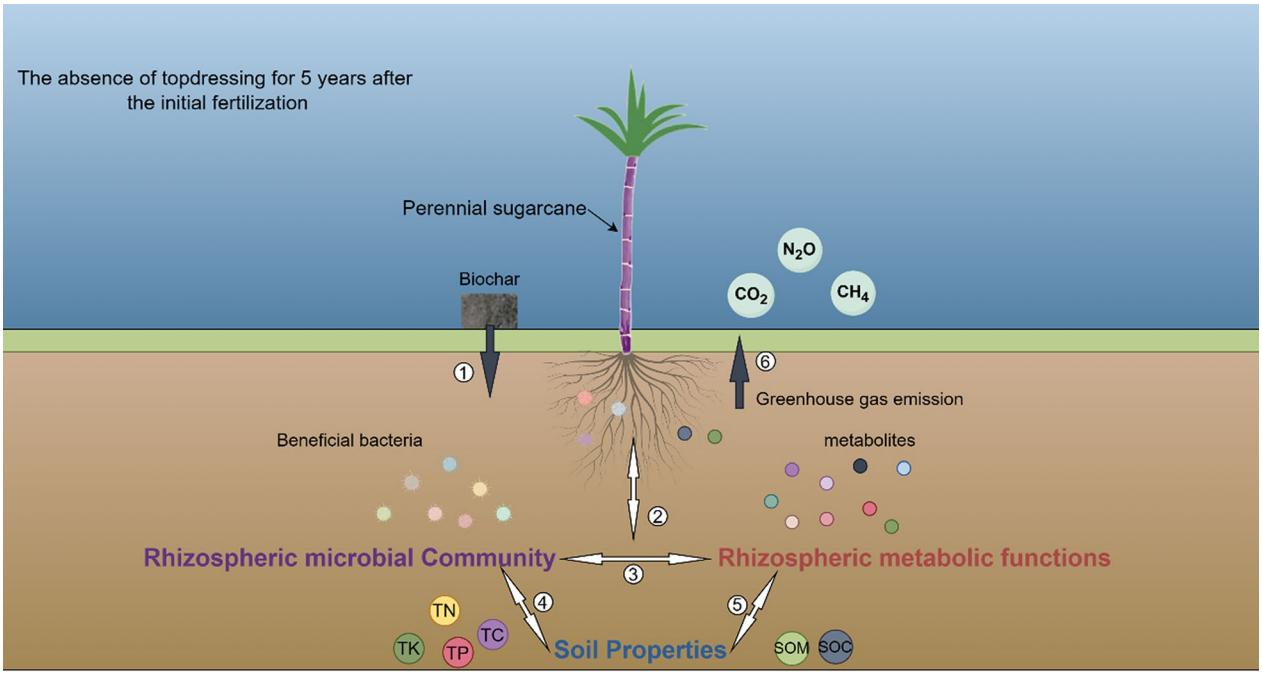
co-application enhances long-term soil fertility. This study provides a reference for fertilization practices and soil improvement in the cultivation of sugarcane and other crops.

Keywords Biochar, Durative agriculture, Long-term field experiment, Sugarcane, Rhizosphere microenvironment, Microorganisms and metabolites, Soil fertility maintenance

Graphical Abstract



① The application of biochar increases the soil's carbon sequestration and nutrient release capacity.

② The endophytic bacteria in the root system and their secreted metabolites affect the rhizosphere microenvironment.

③ ④ ⑤ The interaction between rhizosphere soil characteristics, microorganisms, and metabolites promotes the absorption of nutrients by roots and promotes the growth of sugarcane.

⑥ The interaction between roots, soil, and microorganisms can affect greenhouse gas emissions.

1 Introduction

1.1 Sugarcane production and nitrogen fertilization challenges

Sugarcane is the most important sugar crop in China. Approximately 90% of sugar in China is produced from sugarcane, which is widely planted in southern China (Zhang et al. 2018). Due to sugarcane's high fertilizer demand, a large proportion of the nitrogen fertilizer applied cannot be fully absorbed and utilized during cultivation. Of this unabsorbed portion, a large proportion of nutrients is commonly lost due to leaching, volatilization and soil fixation (Feng and Zhu 2017; Khan et al. 2022). In addition, over-use of nitrogen fertilizer can also cause agricultural non-point source pollution (Lu et al. 2024; Meena et al. 2023). With the increasing number of planting years of sugarcane, changes occur in the rhizosphere microenvironment of the sugarcane field, such as soil acidification, imbalances in soil nutrients and microbial communities, as well as issues related to rhizosphere microbial metabolic activity and greenhouse gas emissions. These changes ultimately may damage the quality and yield of sugarcane (Chalco Vera et al. 2020; Lin et al. 2023).

1.2 Microbial communities and soil–plant systems

While microbial interactions predominantly occur at the micro-level, their cumulative impact on soil health

and crop productivity is profound and well-documented. Microbial communities drive critical biogeochemical processes—such as nutrient cycling (e.g., nitrogen fixation, phosphorus solubilization), organic matter decomposition, and pathogen suppression—that directly influence soil fertility and plant resilience (He et al. 2023; Lv et al. 2023).

Although individual microbial activities may appear transient or compensatory, the functional redundancy and metabolic diversity within these communities ensure ecosystem stability over time. For instance, comparative studies reveal that organic farming systems with greater soil microbiome diversity achieve measurably higher crop yields (Lv et al. 2023; Ye et al. 2019), whereas excessive synthetic fertilizer application destabilizes microbial communities, resulting in progressive soil degradation over time (Dan et al. 2022). Furthermore, microbial-mediated carbon sequestration and root exudate modulation can alter soil structure and carbon storage capacity, demonstrating their macro-scale agronomic and environmental significance (Sheng and Zhu 2018). Thus, despite micro-level dynamics, microbial communities act as keystone regulators of soil–plant systems, with measurable impacts on agricultural sustainability and climate resilience.

1.3 Biochar as a soil amendment

Biochar (BC) is a carbon-rich material produced by the slow pyrolysis of biomass residues—such as agricultural and forestry waste, potential energy crops, and other biomass residues—under high temperatures in anaerobic or oxygen-limited conditions. This process transforms the biomass into biochar with low degradability (Edussuriya et al. 2023). After adding biochar to the soil, it can improve soil structure. Its unique physicochemical properties can enhance soil health and improve crop uptake and utilization of nutrients (Pang et al. 2022). Biochar can increase the pH of acidic soils, affecting the availability of plant nutrients (Hossain et al. 2020).

Additionally, adding biochar can promote root growth and increase root numbers, reduce heavy metal stress, and increase soil SOM content (Chen et al. 2024). Research has also shown that co-application of biochar and nitrogen fertilizer enhances maize's absorption and utilization of nitrogen (Peng et al. 2021; Ren et al. 2021).

1.4 Biochar's role in sugarcane cultivation

According to the literature, biochar has a low nitrogen content and its nitrogen mostly exists in a stable form, making it difficult for sugarcane to directly absorb and utilize it (Pang et al. 2022). Conventional fertilization in agricultural production needs to be mixed with other fertilizers such as urea and organic nitrogen fertilizers to meet the nutrition needs of crops(Gui et al. 2024).

Pang and colleagues found that moderate application of nitrogen fertilizer combined with appropriate biochar application during sugarcane cultivation could improve the supply of available nitrogen in the root zone soil, promote targeted transformation and efficient utilization of soil nitrogen in sugarcane continuous cropping fields. However, the application of biochar alone significantly reduced soluble organic carbon, total soluble nitrogen, ammonium nitrogen, nitrate nitrogen, and nitrification potential, as well as markedly decreased sugarcane plant height, stem diameter, nitrogen balance index, and single-stem weight (Pang 2019; Pang et al. 2022; Zhang 2020).

Studies by Aluthge and Wu et al. have demonstrated that biochar application can significantly improve sugarcane soil quality, optimize the structure and function of microbial communities, and inhibit the growth of harmful bacteria, thereby promoting healthy sugarcane growth (Aluthge et al. 2025; Wu et al. 2024). Additionally, biochar treatment effectively mitigates the negative impacts of harmful elements on sugarcane biomass by regulating soil nutrient dynamics and enhancing microbial community functionality (Aluthge et al. 2025; Farid et al. 2022). Similarly, research by Ferreira et al. further confirmed that during the early growth and development stages of sugarcane, biochar treatment not only enhances soil water retention capacity and stimulates microbial activity but also optimizes metabolic processes in sugarcane leaves, significantly improving root growth and quality (Ferreira et al. 2024).

In addition, biochar has demonstrated significant efficacy in ameliorating acidified agricultural soils and regulating nitrogen transformation processes (Huang et al. 2023; Mangottiri et al. 2019, and Pang et al. 2022). From an economic perspective, its application contributes to reducing substantial material input costs required for soil remediation (Balaganesh P. et al. 2021). The combined implementation of biochar with reduced nitrogen fertilization effectively mitigates economic losses caused by inappropriate nitrogen fertilizer application (Balaganesh et al. 2022; Meena et al. 2023; Pang et al. 2022), while concurrently enhancing overall farmland productivity. This integrated approach provides an economically viable solution for sustainable agricultural development, exhibiting considerable application potential in agricultural economic systems through its dual benefits of cost optimization and productivity enhancement (Jaffar et al. 2024; Rahman et al. 2021).

1.5 Biochar's impact on microbial communities and soil dynamics

Incorporating biochar into soil can also enhance the diversity of microbial communities in the rhizosphere. For instance, when biochar is combined with nitrogen fertilizer in sugarcane soil, it can positively impact the richness and diversity of bacterial communities (Pang et al. 2022). This includes a notable increase in the abundance of nitrifying bacteria (*Nitrospira*) and nitrogen-fixing bacteria (*Rhizobacter*). Moreover, the joint application of biochar and nitrogen fertilizer significantly influences soil microbial activities, particularly in the production of secondary metabolites like lipids and organic acids, as well as in carbon metabolism (Pang et al. 2022; Ren et al. 2022; Xia et al. 2023a).

Research has shown that nitrogen reduction combined with biochar can impact plant root exudation and the processes of soil microbial metabolisms, including the pools of amino acids, sugars, and organic acids (Song et al. 2020). After the addition of biochar to the soil, the interaction between biochar and soil will go through a series of processes over time. This includes variations in mineral dissolution, surface oxidation, and mechanical crushing properties compared to those of fresh biochar (Bandara et al. 2022).

As biochar persists in the soil over time, its physical and chemical characteristics, such as pH value, surface morphology, specific surface area, element composition, aromaticity, and ion exchange capacity, will undergo alterations (Liu and Chen 2022). For instance, with prolonged presence in the soil, soluble salts and organic compounds within the biochar dissolve and leach, potentially leading to shifts in the surrounding pH levels (Liu and Chen 2022).

While numerous studies have highlighted the influence of biochar on microbial activity in soil, there remains limited understanding regarding the impact of biochar's cumulative residence time in soil on microbial activity (Bandara et al. 2021; Nguyen et al. 2018; Pang et al. 2022; Zou et al. 2024). Several studies have also investigated shifts in soil microbial structure (Wu et al. 2024; Xiang et al. 2023). Interestingly, it has been observed that biochar does not significantly alter the soil microbial community structure after 2 to 3 years of residence, although the underlying mechanisms for these effects remain unclear (Nguyen et al. 2018).

1.6 Biochar's role in greenhouse gas mitigation

Biochar represents an eco-friendly product and a burgeoning fertilizer for carbon sequestration and emission reduction. Monitoring CO_2 emissions in sugarcane fields holds significant scientific and practical importance for addressing climate change and advancing sustainable agriculture (Liu et al. 2023). Quantifying CO_2 fluxes enables the assessment of agroecosystem contributions to greenhouse gas dynamics, particularly from soil respiration, organic matter decomposition, and fertilizer application (Kwon et al. 2024).

This data-driven approach supports the optimization of farming practices, such as precision nutrient management, conservation tillage, and irrigation efficiency, to minimize emissions while enhancing soil carbon sequestration (Smith et al. 2007). Additionally, accurate emission inventories underpin climate policy frameworks, facilitating carbon credit certification and compliance with international agreements like the Paris Agreement. Furthermore, understanding CO_2 exchange mechanisms in sugarcane systems refines global carbon cycle models and promotes the development of low-carbon bioenergy supply chains, aligning agricultural productivity with ecological resilience and net-zero targets (Liu et al. 2018, Zhang et al. 2020).

Furthermore, the aging of biochar can mitigate soil greenhouse gas emissions of CO_2 and NO_2, although it does not notably affect CH_4 emissions (Feng et al. 2022). At present, there is relatively little research on the greenhouse gas emission reduction effect of biochar on sugarcane field soil.

The short-term research by our team showed that, in the monoculture system of sugarcane, reducing nitrogen application in conjunction with biochar addition can promote sugarcane growth and enhance soil sustainability. It significantly affects the properties of rhizosphere soil and the composition of bacterial communities, especially the nitrifying and nitrogen-fixing bacteria (Liu 2023; Pang et al. 2022; Zhang 2020). Although the short-term study has shown positive effects of reducing nitrogen application combined with biochar, the long-term impacts are still unclear, and the effect of nitrogen reduction combined with biochar on the sugarcane rhizosphere microenvironment and microecology without any fertilizer for a long time is still unclear. The purpose of this study was to explore the effect of the superposition of biochar retention years in soil, and the effects of nitrogen reduction combined with biochar on the microbial community structure and metabolites of rhizosphere soil and roots of perennial sugarcane, and to elucidate the interaction mechanism between rhizosphere soil properties, rhizosphere microbial community composition and metabolites. Therefore, in a field where sugarcane is continuously planted, the first year of application of basal fertilizer and biochar treatment was implemented, and for the next long-term (5 years) period, there was no additional fertilizer supplement. Changes in rhizosphere soil properties,

rhizosphere bacterial communities, and metabolites were studied, and a co-occurrence network between rhizosphere soil properties, rhizosphere bacterial members, and rhizosphere metabolites was unveiled.

2 Materials and methods

2.1 Experimental location, materials, and design

In the sugarcane fields of the National Sugarcane Research Center in Cangshan District, Fuzhou City, Fujian Province, China (26°08'N, 119°23'E), long-term field experiments were conducted. The experimental area falls under a subtropical monsoon climate, with an average annual temperature of 20 °C and an average annual precipitation of 1368.91 mm. The study focused on the main sugarcane variety, Xin Tai Tang No.22 (ROC22). The maximum soil water holding capacity in the test area was 33.6%. The biochar used in the experiment was produced by Timeske Biotechnology (Shanghai) Co., LTD., through the pyrolysis of 6-year-old bamboo (from Jiangshan, Zhejiang Province) at 500–600 °C, exhibiting structural characteristics (aromatic carbon skeleton, micropore development) with residual nutrient-retention potential (Table 1).

The experiment, conducted from March 2018 to 2023, was designed with two treatments at a 50% reduced nitrogen level. These treatments included: (1) *BF* application of basal fertilizer (urea plus P_2O_5 and KCl) only, (2) *BF-BC* co-application of basal fertilizer with biochar. Biochar, produced through a high-temperature carbonization process, has a low nitrogen content (Maniraj et al. 2023). Consequently, it is insufficient to serve as the sole basal fertilizer for sugarcane cultivation over a five-year period. Therefore, additional basal fertilizers are necessary to maintain the normal growth and physiological needs of sugarcane. Our research team has discovered that the sole application of biochar exerts short-term inhibitory effects on sugarcane growth (Liu 2023; Pang 2019; Pang et al. 2022), manifesting as significant reductions in plant height, stem diameter, and single-stem weight. To investigate the feasibility of combining biochar with basal fertilizer in the fifth year for enhancing sugarcane growth parameters (including plant height, stem diameter, and root system development), optimal growth maintenance under continuous cropping must first be ensured. This prerequisite necessitates the provision of sufficient nutritional resources, particularly nitrogen and carbon sources, to achieve sustainable cultivation. Nitrogen plays a crucial role in the physiological and biochemical processes of plants (Ye et al. 2019). Basal fertilizer is used to maintain the basic normal growth and physiological level of sugarcane, providing an indispensable source of N for plant growth, participating in the synthesis of proteins, nucleic acids, and chlorophyll, promoting energy metabolism, enhancing nutrient absorption capacity, and improving the stress resistance of plants (Cheng et al. 2022). The application amount of field biochar in this experiment was determined based on previous experimental results and relevant literature support (Pang 2019; Yang et al. 2024), combined with the stability characteristics of biochar and long-term experimental requirements.

This study established control and treatment groups within distinct randomized blocks, according to a previous report (Pang et al. 2022). Within each block, three replicate plots were randomly arranged. Each plot was planted with 56 sugarcane cane sets (4 rows × 14 plants per row) (Fig. S1). In detail, each treatment consisted of 3 subplots, with each subplot comprising 4 rows spaced 1.2 m apart and 8.0 m in length. The subplot area was 38.4 m^2. A 0.5 m—1.0 m wide isolation strip was placed between each subplot, with a biochar treatment set within a 4.8 m × 1.2 m "sub-subplot". According to the fertilizer application rates (Table 2), the basal fertilizer was uniformly mixed with the soil in each subplot. For the BF-BC treatment, biochar was then added into the top 10 cm soil layer within the designated sub-subplot area. The soil and biochar were mixed thoroughly, and the mixture was subsequently covered with soil (Pang 2019). This depth corresponds to the primary root zone

Table 1 Characteristics of soil and biochar (Pang et al. 2022)

Factor	Soil	Biochar
pH	5.50	10.13
SOM (g/kg^{-1})	17.92	–
TC (g/kg^{-1})	10.77	52.4
TN (g/kg^{-1})	1.22	5.14
DOC (mg/kg^{-1})	–	598.39
TSN (mg/kg^{-1})	–	43.92
NH_4^+–N (mg/kg^{-1})	4.77	3.68
NO_3^-–N (mg/kg^{-1})	1.01	3.7
AP (mg/kg^{-1})	24.45	–
AK (mg/kg^{-1})	78.97	–
E. Ca^{2+} (g/kg^{-1})	0.72	–
E. Mg^{2+} (mg/kg^{-1})	102.11	–
E. Na^{2+} (mg/kg^{-1})	187.21	–
Specific surface area (m^2/g^{-1})	–	136.65
Hole volume (cm^3/g^{-1})	–	0.08
Aperture (mm)	–	24.48
TCa (g/kg^{-1})	–	11.62
TMg (g/kg^{-1})	–	3.23
TNa (g/kg^{-1})	–	38.74
TK (g/kg^{-1})	–	112.79
AK (g/kg^{-1})	–	15.2
Ash (%)	–	19.4

Table 2 Fertilization amount in field experiment

Treatment	Ground fertilizer				First dressing	Second dressing	Total pure nitrogen
	Urea kg/hm^2	P_2O_5 kg/hm^2	KCl kg/hm^2	Biochar kg/hm^2	Urea kg/hm^2	Urea kg/hm^2	N kg/hm^2
BF	135	1152	518.4	0	135	67.5	155.25
BF-BC	135	1152	518.4	8640	135	67.5	155.25

Urea (containing effective N 46%), P_2O_5 (containing effective P_2O_5 12.5%), and KCl (containing effective K_2O 60%) were applied. Three replications were set for each treatment

where sugarcane established extensive root system for nutrient uptake (Liu 2023; Pang 2019; Pang et al. 2022). This practice enhanced early-stage nutrient absorption by sugarcane, thereby maximizing fertilizer utilization efficiency while enabling sustained nutrient release. Additionally, it prevented nutrient depletion in subsequent years caused by excessive sugarcane growth during the initial two-year period (Li et al. 2016).

Each treatment had 3 replicates. On March 1, June 1, and September 1 in Year 2018, 40%, 40%, and 20% of the total nitrogen application were respectively administered. In the first year (2018), basal fertilizer and biochar were applied to soil as initial fertilization for the continuous sugarcane cultivation. Without any additional fertilizers supplemented within five years, relevant indicators were measured at the end of the 5-year period (April 2023).

2.2 Soil sampling and assessment of soil properties

In April 2023, after investigating the aboveground growth performance of sugarcane (e.g., plant height and stem diameter), the entire root system and rhizosphere soil samples (soil closely adhering to roots) were collected for each treatment. Each sample type had three replicates, which were immediately transported to the laboratory. Soil moisture content (SWC) was determined by the drying method. The collected samples were divided into two parts: one was stored at 80 °C for analyzing soil microbial structure and metabolites, and another was air-dried for measuring soil pH value. The physicochemical indicators included total carbon (TC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), ammonium nitrogen (NH_4^+–N), nitrate nitrogen (NO_3^-–N), available phosphorus (AP), and available potassium (AK) in soil (Liu 2023). Exchangeable calcium and magnesium (E. Ca^{2+}, E. Mg^{2+}) in soil were determined using the ammonium acetate-atomic absorption method. Soil organic matter (SOM) and soil organic carbon (SOC) were determined using conventional soil agrochemical analysis methods with potassium dichromate oxidation and external heating method (Pang et al. 2022). Alkali-hydrolyzable nitrogen (AN) was quantified using the alkaline hydrolysis diffusion method (Khan et al. 2022).

2.3 Determination of sugarcane traits

The nitrogen balance index of sugarcane leaves was assessed using a portable nitrogen balance index meter (Dualex 4, Force-A Company, Ile-de-France, France) prior to sugarcane harvest. The average height and diameter of sugarcane stalks were determined using a ruler and vernier caliper, respectively. Root number, root surface area, root volume, and root average diameter were measured using the Epson Expression 12000XL instrument (Seiko Epson Corporation, Nagano-ken, Japan). The root plane image was scanned, followed by analysis using the WinRHIZO root system analysis software. Sugarcane brix levels were measured using a brix meter (PAL-1, ATAGO Scientific Instruments Co., LTD, Tokyo, Japan) during the assessment of sugarcane growth.

2.4 Determination of soil greenhouse gas

The greenhouse gas sampling was conducted using gas collection chambers. Specifically, a gas collection chamber was installed in the central area of each replicated plot for the BF and BF-BC treatments in the field. The chamber consisted of a cylindrical PVC barrel (33.5 cm in height) with a top-opening port connected to a 1 mL pipette tip (serving as a gas channel). The interface was sealed and fixed with 704 silicone sealant, followed by attachment to an external extension tube. The inverted chamber was vertically inserted 5 cm into the soil, leaving the barrel aboveground to form a closed headspace (Jones et al. 2009). After 48 h equilibration, gas sampling was conducted at 7:00 AM and 9:00 AM, respectively. A 50 mL medical syringe was used to extract 50 mL of headspace gas from the chamber, which was then injected into pre-prepared aluminum foil gas sampling bags (Zhu 2022). During sampling, temperatures inside and outside the chamber were recorded using a thermometer, with ambient pressure maintained at 976 Pa. The collected gas samples were analyzed for the concentrations of CO_2, CH_4, and N_2O using gas chromatography within 72 h of collection (GC2010 Plus, Shimadzu, Kyoto, Japan). The soil gas emission flux was calculated according to the formula: $F=\rho\times h\times(P/P_0)\times(T_0/T)\times(dCt/d_t)$, where F represents the gas flux (mg/m^2 h); h represents the height (m)

of the chamber; ρ represents the measured gas density in the standard state; C_t represents the volume concentration (μL/L) of the measured gas in the chamber at time t; t represents the time elapsed since chamber closure; T_0 represents the absolute air temperature (273 K); P_0 represents air pressure (Pa) in the standard state; T represents the absolute temperature (K) at the time of sampling; P represents the pressure (Pa) at the sampling site (Qin et al. 2005).

2.5 High-throughput sequencing for analyzing shifts in soil bacterial populations

The total genomic DNA was extracted from 0.5 g fresh samples according to the DNA extraction kit (Tiangen Biotech Co., LTD., Beijing, China). The quality and quantity of extracted DNA were detected by electrophoresis on 1.8% agarose gel. The concentration and purity of DNA were determined by NanoDrop 2000 ultraviolet–visible spectrophotometer (Thermo Scientific, DE, USA). The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R: (5'-GGACTACHVGGGTWTCTAAT-3'). Both the forward and reverse 16S rRNA primers were tailed with sample-specific Illumina index sequences to allow for deep sequencing. The PCR was performed in a total reaction volume of 10 μL: DNA template 5–50 ng, forward primer (10 μM) 0.3 μL, reverse primer (10 μM) 0.3 μL, KOD FX Neo Buffer 5 μL, dNTP (2 mM each) 2 μL, KOD FX Neo 0.2 μL, and finally ddH_2O up to 20 μL. After initial denaturation at 95 °C for 5 min, the process involved 20 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 40 s, and a final step at 72 °C for 7 min. The amplified products were purified with Omega DNA purification kit (Omega Inc., Norcross, GA, USA) and quantified using Qsep-400 (BiOptic, Inc., New Taipei City, Taiwan, ROC). The amplicon library was paired-end sequenced (2 × 250) on an Illumina NovaSeq 6000 (Beijing Biomarker Technologies Co., LTD., Beijing, China). The data quality control results demonstrated that both the Q20 and Q30 scores achieved average values of 98.8% and 95.5% respectively, meeting the established quality standards.

2.6 metabolite extraction and analysis of soil metabolomics

A 50 mg sample was accurately weighed, and 1,000 μL of extract containing an internal standard (methanol: acetonitrile: H_2O, 2:2:1 (v/v), with an internal standard concentration of 20 mg/L) was added. The mixture was vortexed for 30 s, followed by the addition of steel balls and processed using a 45 Hz grinding instrument for 10 min. The mixture was then subjected to ultrasonic treatment for 10 min in an ice water bath. After standing at − 20 °C for one hour, the sample was centrifuged at 4 °C and 12,000 × g for 15 min. The supernatant (500 μL) was transferred to an EP tube, dried with a vacuum concentrator, and reconstituted with 160 μL of extract [acetonitrile: H_2O, 1:1(v/v)]. The resulting mixture was centrifuged, and 120 μL of the supernatant was transferred to a 2 mL injection bottle. A quality control (QC) sample was prepared by mixing 10 μL of each sample. The samples were analyzed using an Acquity UPLC HSS T3 column (1.8 μm, 2.1 mm × 100 mm) coupled to an Acquity I-Class PLUS system with a Xevo G2-XS QToF (Waters, MA, USA) mass spectrometer. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The mass spectrometer was operated in MSe mode, and data were acquired using the MassLynx V4.2 software. The acquisition cycle consisted of dual-channel data acquisition with low collision energy (2 V) and high collision energy (10–40 V) simultaneously. The scanning frequency was 0.2 s. The ESI ion source parameters were as follows: capillary voltage, 2500 V (positive ion mode) or − 2000 V (negative ion mode); cone hole voltage, 30 V; ion source temperature, 100 °C; desolvation gas temperature, 500 °C; and desolvation gas flow rate, 800 L/h. For every 5 to 10 samples, a QC sample was analyzed by mixing all sample extracts. The Spearman correlation coefficient (≥ 0.8) of the expression levels between QC samples and the RSD value (the proportion of peaks with RSD ≤ 30% accounting for ≥ 0.6) were calculated to evaluate repeatability and instrument stability.

The raw data were processed using Progenesis QI software for peak extraction, alignment, and other data processing operations. The software was used in conjunction with the online METLIN database and a self-built database (Baimaike) for metabolite identification. Theoretical fragment identification was performed simultaneously. The mass deviations for parent ions and fragment ions were within 100 ppm and 50 ppm, respectively. Quantitative data were obtained using a proprietary method.

2.7 Data analysis

For analysis of the sugarcane characteristics and rhizosphere soil properties, the index variances between control and treatment groups were analyzed by F-tests. Data were conducted using Data Processing System 9.01 software and presented as Mean ± SEM, with statistical significance set at $P < 0.05$. In figure legends, asterisks denote significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. In the table legends, alphabetical markers (a, b) indicate significant differences. Venn plots were used to visualize the number of common and unique species between each

sample. The alpha diversity of the microbial phylum was assessed using the richness (ACE) and diversity (Shannon) indices, calculated through the Hundred Mile Cloud analysis. The R software (version 4.3.1) was used for visualization. Using R packages including vegan, ggplot2, ggrepel, and psych, we generated RDA plots, network analysis diagrams, Manhattan plots, and MapTree diagrams to analyze the correlations between bacterial community composition and soil properties, as well as the differences in abundant genera and metabolites among bacterial communities across different groups. These visualizations quantify the explanatory power of multivariate environmental factors on community structure (R^2 ranging 0–1), while PERMANOVA evaluates the contribution of environmental factors to community dissimilarities (explained variance 0–1). Additionally, we assessed correlations between distance matrices by calculating Mantel correlation coefficients (−1 – 1 range). Non-metric multidimensional scaling (NMDS) based on Bray–Curtis distance algorithm combined with PERMANOVA were employed to investigate bacterial community composition differences among samples (Pang et al. 2022).

3 Results

3.1 Synergistic effects of nitrogen reduction and biochar amendments on sugarcane characteristics and rhizosphere soil properties

Nutrient quantities added through biochar and basic fertilizer in the soil are shown in Table 1 and Table 2 (Pang et al. 2022). Sugarcane growth characteristics were measured prior to harvest. The results revealed that key agronomic traits in the BF-BC treatment showed significant improvements compared to the control BF treatment. Specifically, sugarcane plant height, stem diameter, and leaf nitrogen balance index (NBI) increased by 10.81%, 25.79%, and 33.90%, respectively ($P < 0.05$, Fig. 1). Conversely, total root volume and average root diameter decreased significantly by 31.06% and 21.53% ($P < 0.05$, Fig. 1). Additionally, sugarcane brix levels, total root length and root surface area showed no significant differences among treatments

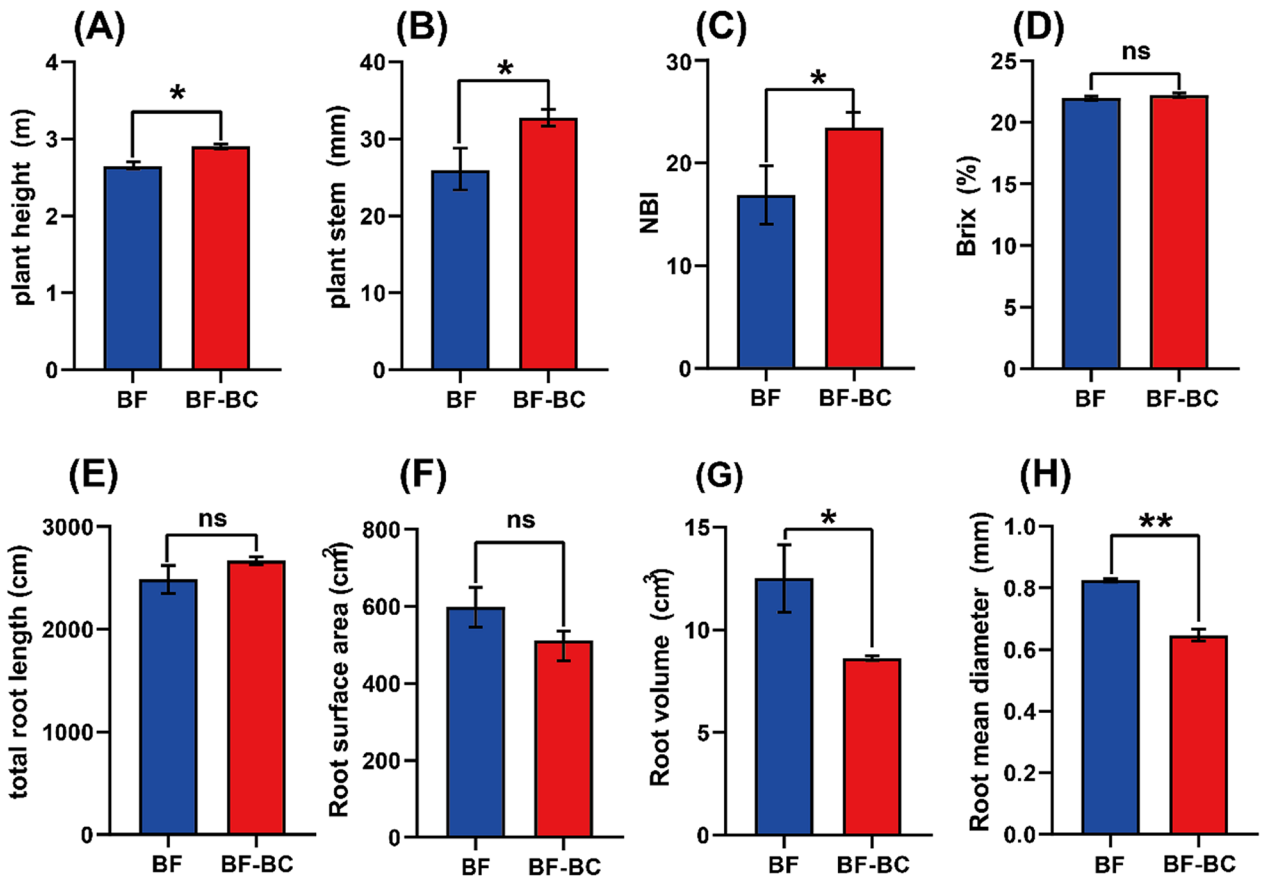


Fig. 1 Comparison of agronomic traits of ratoon sugarcane under basal fertilizer (BF) and basal fertilizer plus biochar (BF-BC) treatments. **A** Plant height; **B** Plant stem; **C** Leaf nitrogen balance index (NBI); **D** Brix; **E** Total root length; **F** Root surface area; **G** Root volume; **H** Root mean diameter. Significant differences between treatments are indicated by: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

($P > 0.05$, Fig. 1). Furthermore, the F-tests revealed that the retention of biochar in soil over time affected perennial sugarcane root growth, promoting the development of new roots and the utilization of nitrogen fertilizer for sugarcane growth (Table S1).

The physical and chemical properties of sugarcane rhizosphere soil were evaluated. Notably, compared to the control BF treatment, the BF-BC treatment group showed significant increases in soil pH and TK content by 17.74% and 79.21%, respectively ($P < 0.05$, Fig. S2 and Table 3). TC, C/N, and NO_3^-–N contents also increased by 34.06%, 44.02%, and 42.95%, respectively, though these differences were not statistically significant ($P > 0.05$, Fig. S2 and Table 3). Conversely, the BF-BC treatment group exhibited significant reductions in soil SOM, SOC, TP, AK, and E. Ca^{2+} by 37.67%, 39.64%, 21.20%, 47.29%, and 12.11%, respectively ($P < 0.05$, Fig. S2 and Table 3). F-test statistical analysis revealed that the BF-BC treatment promoted soil nutrient cycling and the utilization of SOM and SOC, while also increasing soil pH (Table S1). Increased nutrient utilization rates are beneficial for sugarcane growth. Despite the absence of topdressing for 5 years after the initial fertilization, biochar showed significant advantages in soil carbon sequestration and fertilizer use efficiency.

Table 3 Comparison of physical and chemical properties of sugarcane rhizosphere soil

Index	BF	BF-BC
pH	4.98 ± 0.07^b	5.67 ± 0.45^a
SOM (g/kg)	19.86 ± 0.38^a	12.01 ± 0.20^b
SOC (g/kg)	11.18 ± 0.16^a	6.93 ± 0.09^b
SWC (%)	32.79 ± 1.70^a	33.30 ± 1.15^a
TC (mg/kg)	75.84 ± 6.91^a	59.23 ± 11.38^a
TN (g/kg)	0.09 ± 0.01^a	0.09 ± 0.01^a
TP (g/kg)	0.51 ± 0.01^a	0.43 ± 0.01^b
TK (g/kg)	16.60 ± 0.53^b	30.57 ± 0.08^a
C/N	5.69 ± 0.55^a	8.37 ± 1.30^a
NH_4^+–N (mg/kg)	5.55 ± 0.53^a	4.24 ± 0.17^a
NO_3^-–N (mg/kg)	17.32 ± 2.44^a	25.15 ± 5.05^a
AP (mg/kg)	0.28 ± 0.02^a	0.26 ± 0.01^a
AK (mg/kg)	195.24 ± 3.02^a	97.65 ± 0.86^b
AN (mg/kg)	77.19 ± 0.80^a	72.26 ± 0.80^b
E. Ca^{2+} (g/kg)	0.50 ± 0.01^a	0.42 ± 0.01^b
E. Mg^{2+} (g/kg)	0.06 ± 0.002^a	0.06 ± 0.003^a

The samples were from rhizosphere soil treated with only basal fertilizer (BF), soil treated with basal fertilizer plus biochar (BF-BC), respectively. a, b, are letter markers indicating significant differences. *SOC* soil organic carbon, *SWC* soil water content, *TP* total phosphorus, *C/N* carbon-to-nitrogen ratio, *AN* alkali-hydrolyzed nitrogen.

3.2 Effects of nitrogen reduction combined with biochar on the changes of rhizosphere soil and root bacterial community

In the examination of 12 samples, a total of 457,836 high-quality 16S rRNA sequences were collected, yielding 12,716 OTUs/ASVs after clustering and noise filtering. The OTU count per sample ranged from 744 to 1393 (Table S2). The sparse curve displayed a clear asymptote, indicating thorough sampling of the community (Fig. S3). Venn diagram analysis revealed 1912 unique species in the BF treatment and 2111 unique species in the BF-BC treatment. In comparison, BFr (roots from the group treated with basal fertilizer only) exhibited 1558 unique species, whereas BF-BCr (roots from the group treated with biochar plus basal fertilizer) had 1629 unique species. The shared species between rhizosphere soil and root system numbered 184 (Fig. S4). The dominant bacterial phyla in rhizosphere bacteria for each treatment were *Proteobacteria* (26.08% 35.82%), *Acidobacteriota* (8.22% 34.42%), unclassified bacteria (3.21% 20.29%), *Actinobacteriota* (6.12% 13.9%), *Cyanobacteria* (0.21% 14.78%), *Bacteroidota* (1.7% 7.53%), *Myxococcota* (2.77% 4.48%), *Chloroflexi* (1.21% 4.25%), *Gemmatimonadota* (0.47% 5.6%) and *Firmicutes* (0.54% 9.82%) (Fig. 2A). The abundance of *Gemmatimonadota* in rhizosphere soil (under BF and BF-BC treatments) was significantly higher than that in the root system (under BFr and BF-BCr treatments) (Table 4). Non-metric multidimensional scaling (NMDS) analysis showed that BF and BF-BC treatments clustered together; BFr and BF-BCr treatments clustered together. BFr and BF-BCr clusters were significantly separated from the BF and BF-BC clusters (Fig. 2B). Under the experimental conditions, *i.e.*, no additional fertilizer was added after the first fertilization, no significant variations in Abundance-based Coverage Estimator (ACE, indicating abundance of the bacterial community) or Shannon index (indicating diversity of the bacterial community) were detected between BF and the BF-BC treatment group. This may be caused by the high stability and non-toxicity of the biochar to the bacterial species in either the rhizosphere soil or sugarcane roots. Furthermore, no significant changes were observed in the richness and diversity of the bacterial communities in both rhizosphere soil and roots (Fig. 2C, D).

Under the condition of long-term (5 years) absence of any fertilizer supplementation, the rhizosphere microenvironment remained relatively stable, potentially contributing to a consistent composition of bacterial species over long-term evolution.

By analyzing the Manhattan plot, we achieved a thorough insight into the impact on bacterial abundance in both the rhizosphere soil and root zone of sugarcane after five years of nitrogen reduction through biochar

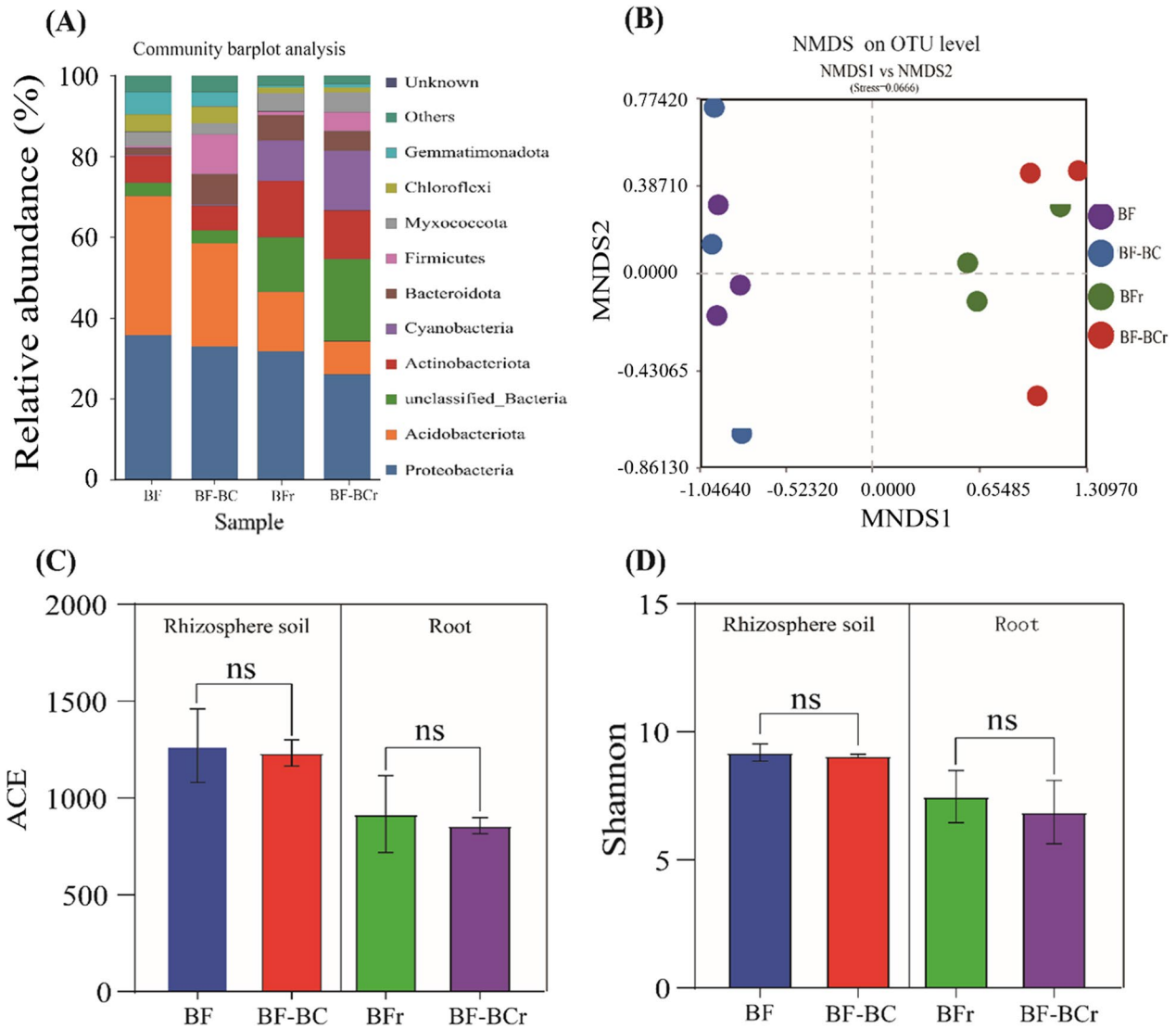


Fig. 2 The effect of nitrogen reduction combined with biochar on rhizosphere bacterial community in the fifth year. Investigation of rhizosphere soil and root bacterial community structure (**A**), non-parametric multidimensional scaling (NMDS) of bacterial community composition (**B**), bacterial richness (**C**), and diversity **D** involve treatments including basal fertilizer application only (BF) and basal fertilizer application with biochar addition (BF-BC)

application. The findings revealed that in comparison to the control group (BF), sole application of basal fertilizer with biochar (BF-BC) significantly enhanced the abundance of *Erysipelatoclostridium*, *Colidextribacter*, *Leptospirillum*, *Sphingobacteriia* bacterium (ID: 2,035,813), WWH38 (ID: 1,387,486), *Actinobacteriota* and bacterium Ellin7530 (ID: 871,857) in the rhizosphere soil ($P < 0.05$). Conversely, it notably decreased the abundance of *Lgnatzschineria*, *Crossiella*, *Fermentimonas*, *Alcaligenes*, *Anaeromyxobacter*, *Fastidiosipila* and *Methyloversatilis* ($P < 0.05$). Furthermore, there was an increase in bacteria within the root system (BF-BCr), including *Faecalibacterium*, *Agathobacter*, *Megamonas*, *Lactobacillus*, *Oscillospiraceae* (UCG_002), *Terrimonas*, *Chitinophaga*, *Prevotellaceae* (Prevotella 9), *Cyanobacteriia*, *Brevundimonas*, *Pseudonocardiaceae* and *Lechevalieria*, which were all significantly elevated ($P < 0.05$). Conversely, *Pleomorphomonadaceae*, *Pedosphaera*, *Rudaea* and *Anaeromyxobacter* were notably reduced ($P < 0.05$; Fig. 3A, B).

Redundancy analysis (RDA) was employed to quantitatively assess the impact of rhizosphere soil physicochemical properties on bacterial community composition. The findings indicated that the primary

Table 4 Rhizosphere soil and root bacterial community structure of Top 10 bacterial phylum relative abundance (%)

Phylum	BF	BF-BC	BFr	BF-BCr
Proteobacteria	0.3929 ± 0.0207[a]	0.3378 ± 0.0565[a]	0.3464 ± 0.0428[a]	0.2681 ± 0.0595[a]
Acidobacteriota	0.3572 ± 0.0498[a]	0.2816 ± 0.0575[a]	0.1543 ± 0.0492[a]	0.0900 ± 0.0323[a]
Unclassified Bacteria (ID: 49,928)	0.0351 ± 0.0029[a]	0.0320 ± 0.0059[a]	0.1453 ± 0.0458[a]	0.2010 ± 0.0624[a]
Actinobacteriota	0.0706 ± 0.0077[a]	0.0639 ± 0.0048[a]	0.1452 ± 0.0108[a]	0.1325 ± 0.0404[a]
Cyanobacteria	0.0022 ± 0.0002[a]	0.0025 ± 0.0008[a]	0.1026 ± 0.0330[a]	0.1418 ± 0.0421[a]
Bacteroidota	0.0181 ± 0.0091[a]	0.0661 ± 0.0549[a]	0.0690 ± 0.0066[a]	0.0511 ± 0.0102[a]
Firmicutes	0.0059 ± 0.0011[a]	0.0879 ± 0.0831[a]	0.0095 ± 0.0047[a]	0.0514 ± 0.0414[a]
Myxococcota	0.0385 ± 0.0005[a]	0.0306 ± 0.0116[a]	0.0463 ± 0.0176[a]	0.0523 ± 0.0185[a]
Chloroflexi	0.0445 ± 0.0083[a]	0.0399 ± 0.0206[a]	0.0146 ± 0.0036[a]	0.0126 ± 0.0027[a]
Gemmatimonadota	0.0579 ± 0.0127[a]	0.0375 ± 0.0133[a]	0.0051 ± 0.0022[a]	0.0093 ± 0.0034[a]

The samples were from rhizosphere soil treated with only basal fertilizer (BF), soil treated with basal fertilizer plus biochar (BF-BC), roots from the group treated with basal fertilizer only (BFr) and roots from the group treated with biochar plus basal fertilizer (BF-BCr), respectively. a, b are letter markers indicating significant differences

SOM organic matter, *TC* total carbon, *TN* total nitrogen, *DOC* soluble organic carbon, *TSN* dissolved total nitrogen, NH_4^+*-N* ammonium nitrogen, NO_3^-*-N* nitrate nitrogen, *AP* available phosphorus, *AK* available potassium. *E.* Ca^{2+} exchangeable calcium, *E.* Mg^{2+} exchangeable magnesium, *E.* Na^{2+} exchangeable sodium, *TCa* total calcium, *TMg* total magnesium, *TNa* total sodium, *TK* total potassium, *Ash* ash content

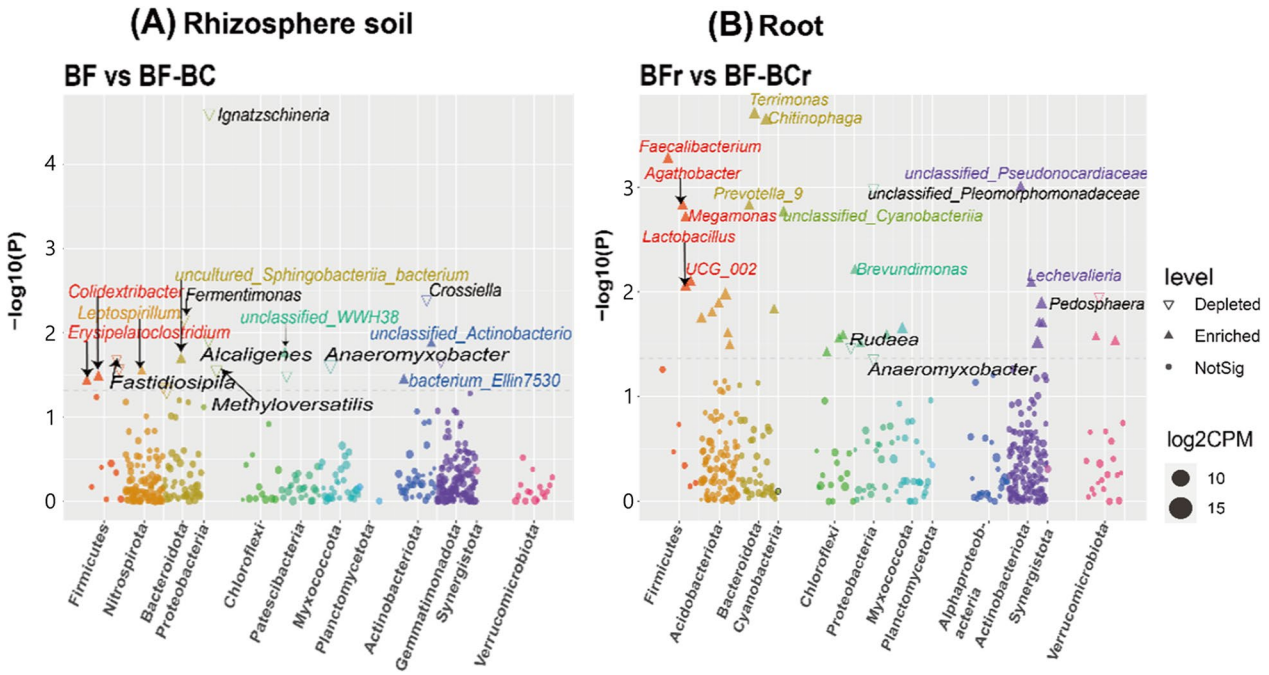


Fig. 3 The Manhattan plot illustrates the composition of bacterial communities in the root systems and rhizosphere soil between BF-BC (r) and BF (r) (the latter was set as control). Each triangle or dot corresponds to a genus. Filled triangles indicate enriched bacteria ($P < 0.05$). Empty triangles indicate depleted bacteria ($P < 0.05$). and dots indicate no significant difference (NotSig)

influencing factors on rhizosphere soil bacterial community composition were TN and AP levels in the soil, followed by AK, SOM, NH_4^+–N, and NO_3^-–N. Positive correlations were observed between *Acidobacteriota* and *Myxococcota* with TN and AP in the soil ($P < 0.05$), while *Bacteroidota* showed a negative correlation ($P < 0.05$). Furthermore, unclassified Bacteria (ID: 49,928), *Actinobacteriota*, *Proteobacteria*, *Gemmatimonadota* and *Chloroflexi* demonstrated positive correlations with soil AK, SOM, NH_4^+–N, NO_3^-–N and soil pH ($P < 0.05$). Soil TC emerged as a significant factor affecting the composition of root-associated bacterial communities, with *Acidobacteriota*, *Gemmatimonadota* and *Proteobacteria* showing positive

correlations with soil TC, NO_3^-–N, AK, SOM, TN ($P < 0.05$), although they showed negative correlations with NH_4^+–N in the soil ($P < 0.05$, Fig. 4A).

Through network analysis, we assessed potential interactions between soil factors and bacterial community composition at the genus level. In the rhizosphere soil treated with BF and BF-BC, bacterial taxa like *Oxalobacteraceae*, *Parasutterella*, *Muribaculaceae*, *Alistipes*, *Reyranella*, *Acidobacteriales* bacterium (ID: 1,978,231), *Bryobact*, *Bacteroidales* bacterium (ID: 2,030,927), *Oscillospiraceae*, *Parabacteroides* and *Micropepsaceae* displayed significant positive correlations with key physicochemical properties including soil SWC, TP, SOM, SOC, NH_4^+–N, E. Ca^{2+}, AN, AK, E. Mg^{2+}, TN, TC, soil pH, NO_3^-–N and AP ($P < 0.05$). Conversely, bacterial *Acidobacteriae* (Subgroup 2), uncultured eubacterium WD298 (ID: 133,604), *Bacillus*, *Conexibacter*, *Dyella*, *Helicobacter*, *Lachnospiraceae*, *Proteobacterium*, *Diplorickettsiaceae*, *Holophaga* sp. (ID: 188,475), *Acidobacteriales*, *Pajaroellobacter*, *Gaiellales* and *Gemmatimonadaceae* exhibited significant negative correlations with key physicochemical properties including soil TP, SOC, AN, AK, NH_4^+–N, AP, TN, TC, SOM, E. Ca^{2+} and soil pH ($P < 0.05$, Fig. 4B and Table S3). These results were consistent with the permutation test analysis, indicating significant correlations between bacterial genera associated with carbon, nitrogen, sulfur compounds, and nitrifying bacteria genera with the aforementioned key soil factors ($P < 0.05$).

Furthermore, root-associated (BF-BCr) bacteria including *Koribacteraceae Candidatus Koribacter* (ID: 658,061), *Roseiarcus*, *Rickettsiales* (SM2D12), *Mucilaginibacter*, *Terracidiphilus*, uncultured eubacterium WD298 (ID: 133,604), *Acidicapsa*, *Acetobacteraceae*, *Steroidobacter* sp. (ID: 549,818), uncultured forest soil bacterium (ID: 169,009), *Ktedonobacteraceae*, *Acidobacteria* bacterium (ID: 171,953), *Devosiaceae*, *Xanthobacteraceae*, *Polyangiaceae*, *Acidobacterales*, *Mesorhizobium* and *Duganella* demonstrated a significant positive correlation with soil AK, SOC, AN, SOM, E. Ca^{2+}, TP, TK and TC ($P < 0.05$). Conversely, *Labrys*, *Actinospica, Polyangiaceae*, *Bacteroides*, *Massilia*, *Catenulispora*, *Microscillaceae, Pajaroellobacter*, *Granulicella* and *Dyella* exhibited a significant negative correlation with soil pH, TC, AN, AK, E. Ca^{2+}, SOM and SOC ($P < 0.05$, Fig. 4B and Table S4).

To forecast the functions of rhizosphere soil and root-associated bacteria, KEGG annotated sequence data was used to match and identify unique protein gene and enzyme names from the collected samples. Picrust 2 analysis indicated that a majority of protein sequences in the samples were linked to metabolism (78.73%–78.97%), genetic information processing (6.34%–6.89%), environmental information processing (6.01%–6.58%), organismal systems (1.44%–1.64%) and cellular processes (3.54%–3.84%) (Fig. S5).

3.3 Effects of nitrogen reduction combined with biochar on rhizosphere soil and root microbiome metabolism

By analyzing the principal components of the samples (including quality control samples), the study provided preliminary insights into the overall metabolic differences among sample groups and the variability within each sample group. The results revealed that in the fifth year after treatments with only basal fertilizer (BF) and basal fertilizer combined with biochar (BF-BC), the total metabolic variability in sugarcane roots exceeded those in rhizosphere soil bacteria (Fig. 5A). Analysis of the composition of metabolites (Top100) showed that the rhizosphere predominantly accumulated compounds such as fatty acyls, glycerophospholipids, organonitrogen compounds, indoles and derivatives, prenol lipids, steroids and steroid derivatives, benzene and substituted derivatives, carboximidic acids and derivatives, glycerolipids, and organic oxygen compounds; glycerophospholipids and organonitrogen compounds were found in higher concentrations in the rhizosphere soil compared to the roots, while the roots exhibited higher levels of indoles and derivatives, as well as organic oxoanionic compounds, compared to the rhizosphere soil (Fig. 5B).

In the differential metabolite analysis, a total of 376 compounds were identified as having statistically significant differences among different treatments ($P < 0.05$), and all detected metabolites were subjected to HMDB classification analysis. Utilizing the MapTree plot provided a comprehensive understanding of the microbial metabolites in the rhizosphere soil and root regions of sugarcane after five years of nitrogen reduction combined with biochar application. In BF and BF-BC treatments, the microbial pathways in rhizosphere soil bacteria and roots mainly included organic nitrogen compounds, organic oxygen compounds, fatty acyls, glycerophospholipids, carboxylic acids and their derivatives, benzopyrans, steroids and steroid derivatives, brassinosteroids, and purine nucleosides, with flavonoids and glycerolipids also involved in root metabolism.

Results indicated that in the BF-BC treatment, the levels of metabolites in rhizosphere soil such as LPA(18:2(9Z,12Z)/0:0), 1-Octen-3-ol, undecylic acid, 4alpha-carboxy-5alpha-cholesta-8,24-dien-3beta-ol, 22-alpha-hydroxy-campest-4-en-3-one, obtusifoliol, hesperetin-7-O-glucoside, dephospho-CoA, dodecaprenyl diphosphate and 3,4,5-tricaffeoylquinic acid significantly decreased, while metabolites such as hexadecanedioate acid, docosatrienoic acid, stearidonic acid, (9Z,11E)-(13S)-13-hydroperoxyoctadeca-9,11-dienoic acid, nateglinide and 6-pentadecyl salicylic acid, delta

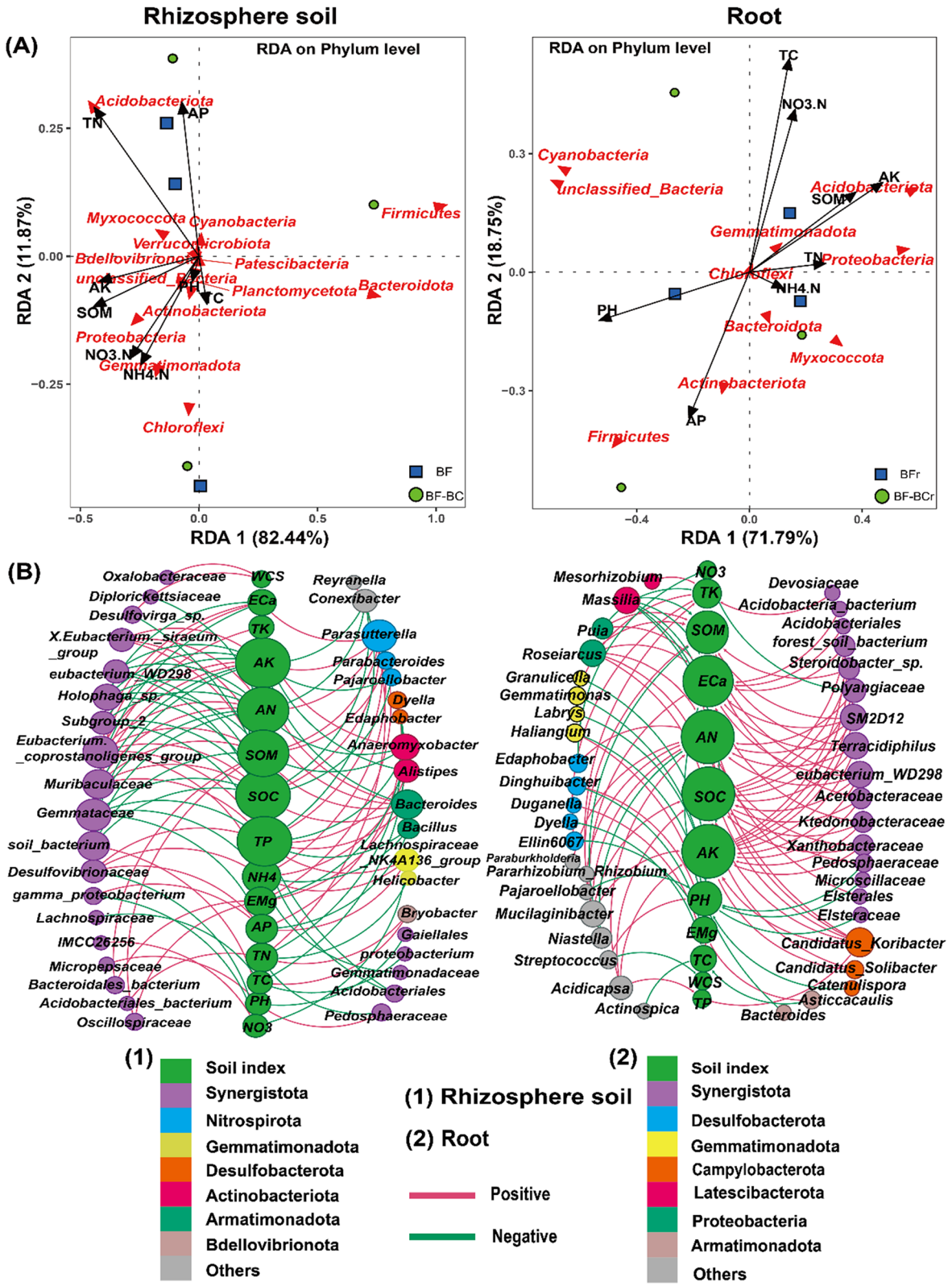


Fig. 4 Examination of the relationship between rhizosphere bacterial community composition and soil biochemical characteristics. **A** Redundancy analysis (RDA); **B** Correlation network analysis. Positive correlations are denoted by red lines. Negative correlations are represented by green lines

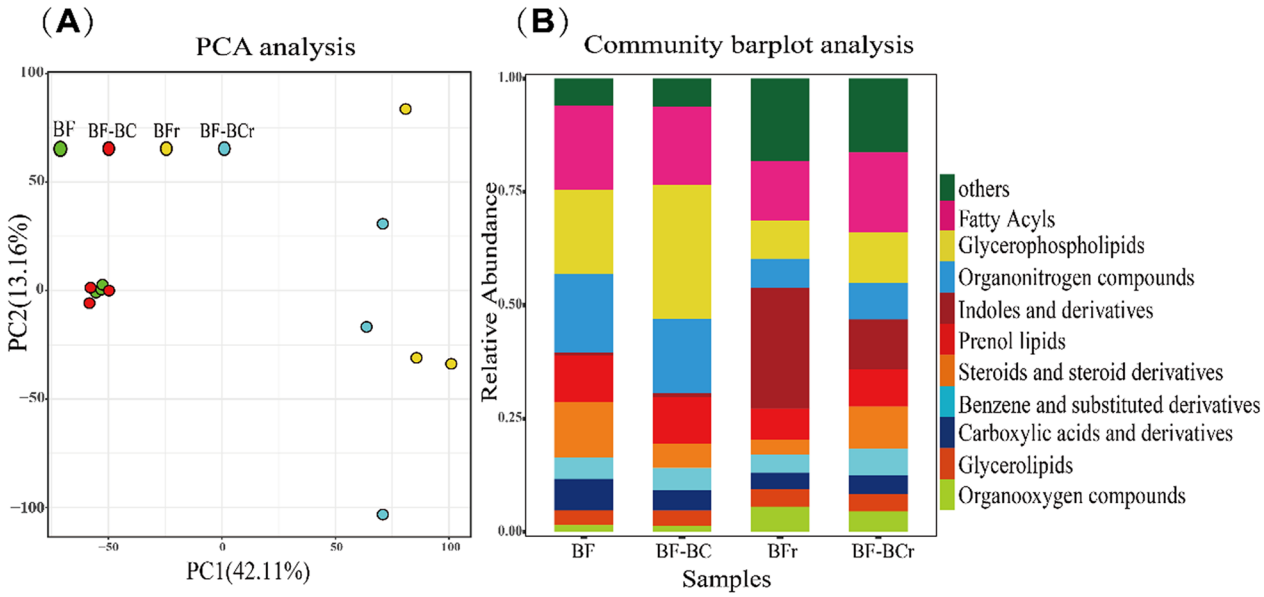


Fig. 5 Principal component analysis (PCA) was conducted on samples treated with only basal fertilizer (BF) and basal fertilizer combined with biochar (BF-BC), along with the main classification of metabolites (Top100) in each sample. **A** The degree of variation between samples in the group, PC1 and PC2 denote the first and second principal; **B** The composition of metabolites (Top100) in each sample

8,14-sterol, nerol and 3-(4-lsopropylphenyl) propanal showed a significant increase in levels ($P < 0.05$, Fig. 6A and Table S5).

In root (BF-BC) metabolites, the levels of several metabolites such as 4beta-(hydroxymethyl)-4alpha-methyl-5alpha-cholest-7-en-3beta-ol, hesperetin-7-O-glucoside, gentisic acid, baccatin III, gibberellin A20, 3,4,5-tricaffeoylquinic acid, wogonoside, methyl octanoate, L-leucine, phenylalanyl-cysteine, L-methionine, L-gamma-glutamyl-L-alanine, and S-(formylmenthyl)glutathione significantly decreased ($P < 0.05$), while others like 4alpha-carboxy-5alpha-cholesta-8,24-dien-3beta-ol, 22alpha-hydroxy-campest-4-en-3-one, 1-octen-3-ol and neomethymycin showed a significant increase in roots compared to rhizosphere soil ($P < 0.05$). Additionally, the levels of metabolites such as 3-beta-hydroxy-4-beta-methyl-5-alpha-cholest-7-ene-4-alpha-carbaldehyde, adenosine 5'-monophosphate, kanamycin B, leukotriene E4, capsianoside IV, 4,8 dimethylnonanoyl carnitine, undecylic acid, hexadecanoic acid, palmitic acid, (9Z)-octadecenoic acid, N-acetylmannosamine, N-((R)-pantothenoyl)-L-cysteine, furanodienone, and diaziquone also significantly increased ($P < 0.05$, Fig. 6B and Table S5).

This study investigated the correlation between rhizosphere soil and root system metabolites and bacterial communities in BF and BF-BC treatments. In the rhizosphere soil, metabolites such as terpin, undecanoic acid, heptyl acetate, 4-hydroxy-2,2'-bipyrrole-5-carbaldehyde, dihydrosphingosine, ethyl decanoate, alpha-methylstyrene, thesiuside, pyrrolidonecarboxylic acid, propynoate, C16 sphinganine, and PS[18:1(11Z)/16:0] were found to be positively correlated with bacterial species including *Sphingomonas*, *Rhodanobacter*, *Alphaproteobacteria*, *Nitrosomonadaceae* (MND1), *Gaiellales* and *Gemmatimonas* ($P < 0.05$). Conversely, metabolites monopalmitin, phthalic acid mono-2-ethylhexyl ester, PC[o-18:1(9Z)/18:2(9Z,12Z)] and PS[16:1(9Z)/18:1(9Z)] were negatively correlated with bacterial species *Rhodanobacter*, *Alphaproteobacteria*, *Nitrosomonadaceae* (MND1), *Gaiellales* and *Gemmatimonas* ($P < 0.05$, Fig. S6A).

Within the root system, bacterial species *Vicinamibacterales*, *Gemmatimonadaceae*, *Conexibacter*, *Puia*, *Ktedonobacteraceae* and *Bradyrhizobium* were found to be positively correlated with metabolites such as stearic acid, Ser-Gln-Leu-Lys, 9,12,15-octadecatrien-1-ol, palmitic amide, monopalmitin, PG(16:0/0:0)[U], protolimonoid, 9,10-dihydroxy-12,13-epoxyoctadecanoate, 4-hydroxybenzoyl choline, phthalic acid mono-2-ethylhexyl ester, PC[o-18:1(9Z)/18:2(9Z,12Z)], PG(13:0/14:0), lysoPE 18:2, ampelopsin A, 2-descarboxy-betanidin, 2-acetamido-4-(D-alanylamino)-2,4,6-trideoxy-D-mannopyranose, PE[18:1(11Z)/18:1(11Z)], 3.alpha.-mannobiose, 1-linoleoylglycerophosphocholine, and MG(18:1(9Z)/0:0/0:0)[rac] ($P < 0.05$). However, bacteria *Acidobacteriae* (Subgroup 2), *Micromonosporaceae*, *Elsterales*, *Dyella* and *Acidobacteriales* showed negative

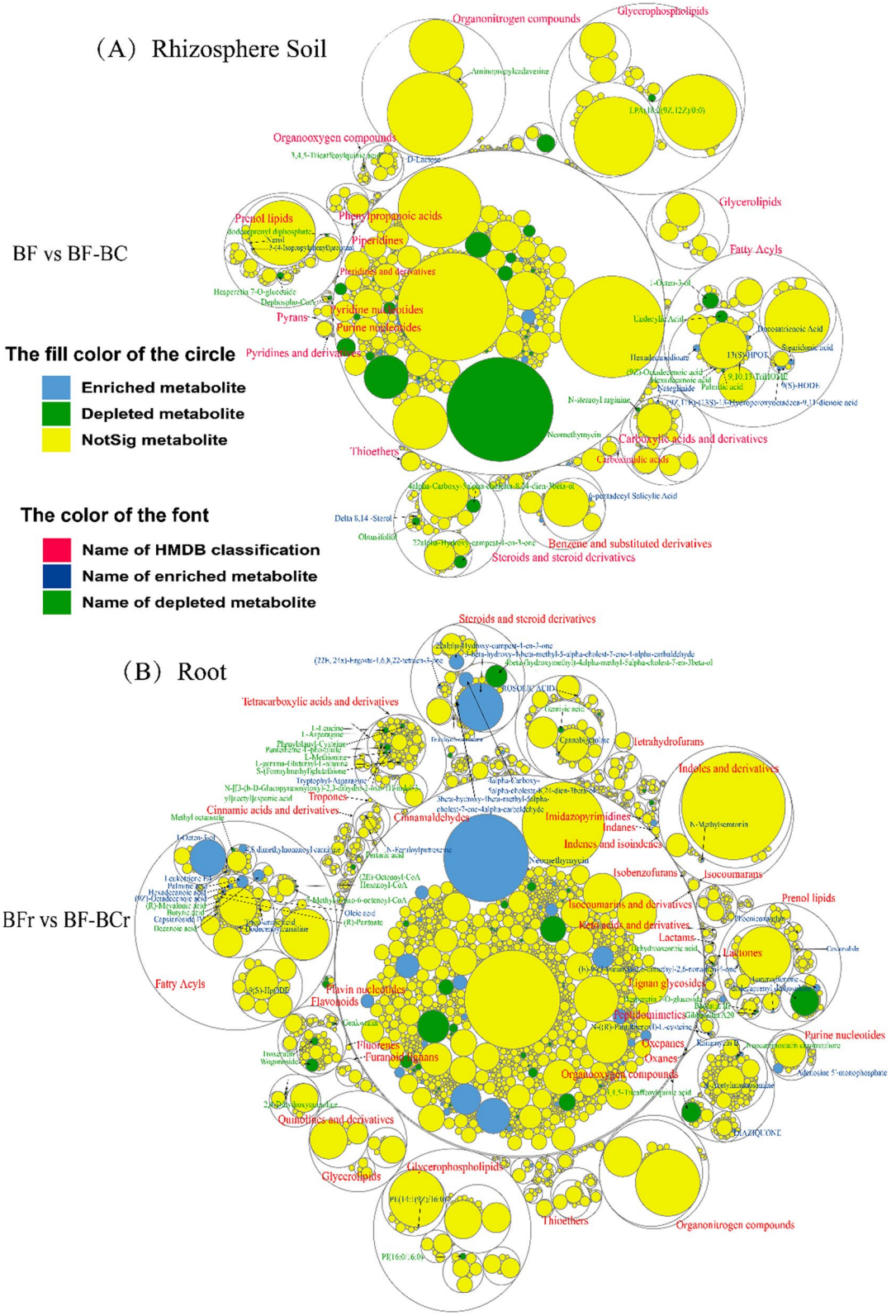


Fig. 6 The MapTree plot of differential metabolites in the rhizosphere soil and root regions. **A** Differential metabolites of rhizosphere soil; **B** Differential metabolites of roots. Each large circle represents the HMDB classification name in red font. Filled blue circles represent upregulated enriched metabolites with names in blue font ($P < 0.05$). Green circles represent downregulated depleted metabolites with names in green font ($P < 0.05$). Yellow circles represent no significant difference (metabolite names in yellow font)

correlations with metabolites indoleacetaldehyde, dihydro-isorescinnamine, protolimonoid, 13(S)-HpODE, 16alpha-hydroxyestrone, 9,10-dihydroxy-12,13-epoxyoctadecanoate, PG(16:0/0:0)[U], bergenin, dihydrosphingosine, PG(19:0/17:0), undecanoic acid, terpin, PE[18:1(11Z)/18:1(11Z)], lysoPE 18:2, and Ser-Gln-Leu-Lys ($P < 0.05$, Fig. S6B).

Furthermore, across the entire rhizosphere, bacterial species *Xanthobacteraceae*, *Vicinamibacterales* and *Burkholderiaceae* (Burkholderia Caballeronia Paraburkholderia) demonstrated positive correlations with metabolites neomethymycin, 2-acetamido-4-(D-alanylamino)-2,4,6-trideoxy-D-mannopyranose, 2-descarboxy-betanidin, 3,3',4,5'-tetrahydroxy-trans-stilbene, MG(18:2(9Z,12Z)/0:0/0:0)[rac], D-mannosamine, honyumine, 1-linoleoylglycerophosphocholine, indoleacetaldehyde, dihydro isorescinnamine, and gingerglycolipid B ($P < 0.05$, Fig. S6C). These findings highlight the complex interactions between metabolites and bacterial communities within the rhizosphere and root system, suggesting potential roles for these interactions in plant growth and development.

3.4 Combined effects of biochar and nitrogen reduction on the interactions between soil properties, bacterial communities and metabolites

In the rhizosphere, the interaction of nitrogen reduction combined with biochar can lead to significant changes in soil properties, impacting nutrient availability and soil SOM content. These alterations can influence the production and utilization of rhizosphere microbial metabolites, with interplays among soil properties, bacterial groups, and metabolites playing a crucial role in the soil carbon cycle.

Research findings suggest that these changes are attributed to the close associations between metabolites and bacteria in rhizosphere soil and roots. For instance, soil TK showed a positive correlation with metabolite PG (19:0/17:0) and the bacterial genus *Mesorhizobium* ($P < 0.05$). Furthermore, soil SOC and SOM exhibited positive correlations with metabolites such as hesperetin-7-O-glucoside, staphyloferrin B, kinamycin D, safflower yellow, as well as bacterial taxa including *Acidicapsa*, *Roseiarcus*, *Acetobacteraceae*, *Puia*, *Mucilaginibacter*, *Terracidiphilus*, *Candidatus Koribacter* (ID:658,061), *Xanthobacteraceae*, *Ktedonobacteraceae*, *Candidatus Solibacter* (ID: 332,162), *Acetobacteraceae* and uncultured forest soil bacterium (ID: 169,009) ($P < 0.05$; Fig. 7A, B).

Soil pH showed positive correlations with bacteria such as *Micropepsaceae*, *Pedosphaeraceae*, soil bacterium (ID: 164,851), and metabolites IPC 18:0; 2/20:0;0. Notably, it was positively linked with *Gaiellales* and metabolites like thesiuside, PG (14:0/16:1(9Z), N,N-dimethyl-safingol, N-methylnicotinate, propynoate, pyrrolidonecarboxylic acid ($P < 0.05$). Soil TP exhibited positive associations with bacteria including *Parasutterella*, *Desulfovibrionaceae*, *Lachnospiraceae* (*Lachnospiraceae* NK4A137 group), *Muribaculaceae*, *Alistipes*, *Bacteroides*, as well as metabolite PG(13:0/14:0), while displaying negative correlations with *Gemmataceae*, *Anaeromyxobacter*, *Bacillus*, *Conexibacter*, *Acidobacteriae* (Subgroup 2), uncultured eubacterium WD298 (ID: 133,604) and metabolite PA(P-16:0e/18:2(9Z,12Z)) ($P < 0.05$).

Moreover, soil AK and AN exhibited positive associations with bacteria such as *Muribaculaceae*, *Parasutterella*, *Bacteroides*, [Eubacterium] siraeum group (ID: 39,492), *Ruminococcaceae* ([Eubacterium] coprostanoligenes group), while soil AN was negatively correlated with metabolites PA(P-16:0e/18:2(9Z,12Z)) and bacteria including *Anaeromyxobacter*, *Holophaga* sp. (ID: 188,475), soil bacterium (ID: 164,851), *Gemmataceae*, *Conexibacter*, uncultured eubacterium WD298 (ID: 133,604), *Acidobacteriae* (Subgroup 2) ($P < 0.05$, Fig. 7C).

3.5 Effect of biochar addition on N_2O, CH_4 and CO_2 emissions from sugarcane soil

The sugarcane soil significantly influences greenhouse gas emissions as its characteristics and biological activity impact the production and liberation of these gases. In the fifth year, soil greenhouse gases were measured. The N_2O, CH_4 and CO_2 emissions values from the BF-BC treated sugarcane field soil without fertilizer in the fifth year are presented in Fig. 8. The emissions of N_2O, CH_4, and CO_2 under BF treatment conditions were 0.0131 mg/m^2·h, 0.0056 mg/m^2·h, and 172.0712 mg/m^2·h respectively. The emissions of N_2O, CH_4, and CO_2 in BF-BC group were 0.0115 mg/m^2·h, 0.0116 mg/m^2·h, and 137.1786 mg/m^2·h, respectively. The emission of CO_2 was inhibited in the BF-BC treatment group ($P > 0.05$), which may be caused by the formation of complexes composed of SOM on the soil surface, leading to stabilization of the carbon derived from biochar. This is consistent with the better preservation and longer release results achieved by C fertilizer. The reduction in carbon dioxide emissions also effectively slowed down soil acidification, providing a richer carbon source for microbial growth. The complex reduced the release of labile components from biochar by providing steric hindrance; these labile components could otherwise serve as a nutrient source for soil bacteria. In the fifth year of this study, the bacterial community within aged biochar particles may differ from that present in fresh biochar particles, potentially exerting an influence on CO_2 emissions. Furthermore, the presence of a reduction in N_2O release may be attributed to the enhanced abundance of nitrifying bacteria facilitating

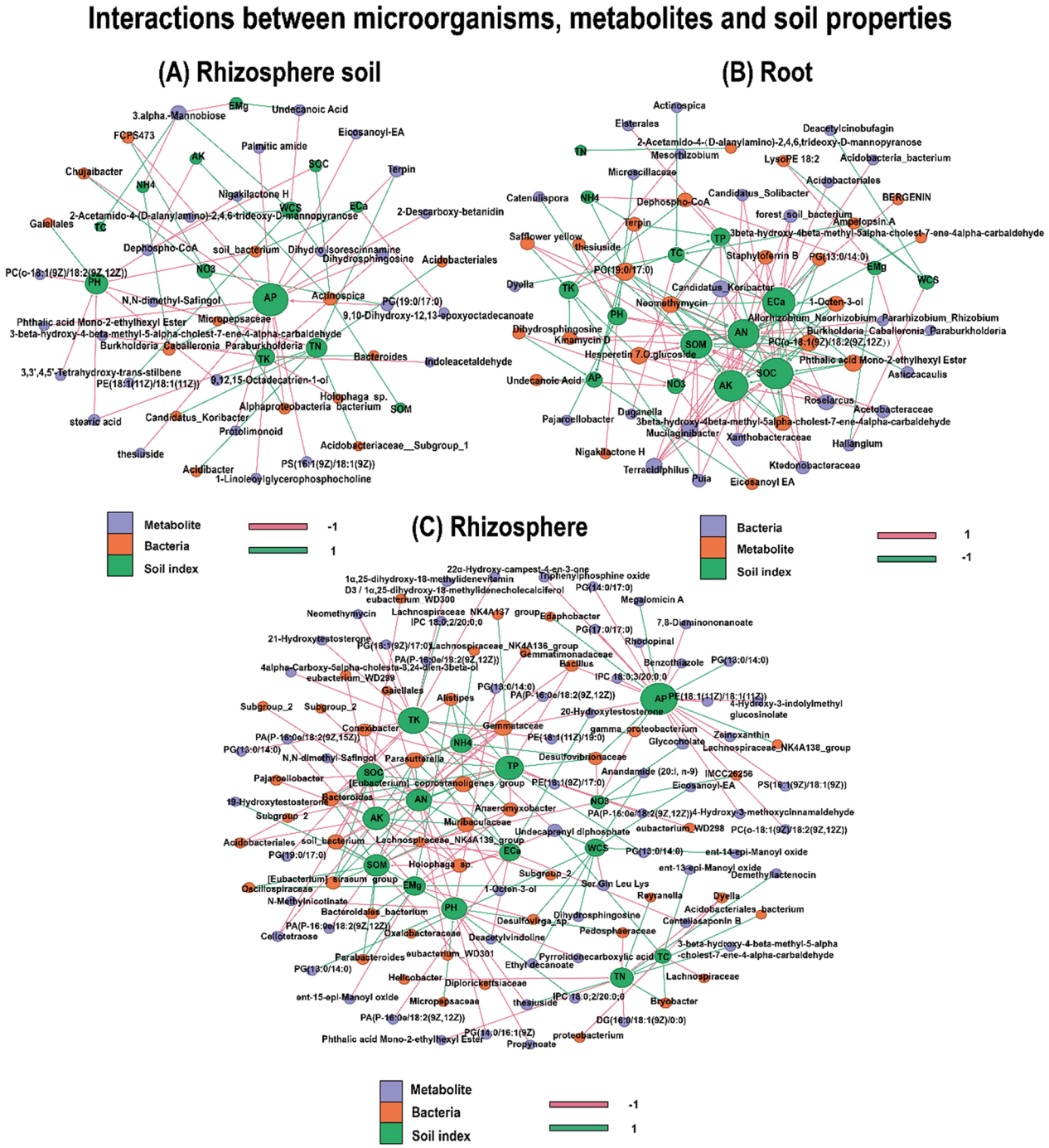


Fig. 7 Correlation network analysis of rhizosphere soil properties, bacterial groups, and metabolites. The proportion of identified groups is represented by the node size. **A** Rhizosphere soil; **B** Root system; **C** Rhizosphere (soil plus root system). Positive correlations are represented by "1". Negative correlations are represented by "1" ($P < 0.05$)

denitrification (Fig. 3). Simultaneously, the escalating trend in CH_4 emissions can be ascribed to the influence of biochar on soil conditions (Fig. 2). Consequently, the utilization of biochar exhibits immense potential for carbon sequestration and emission mitigation in agricultural soils.

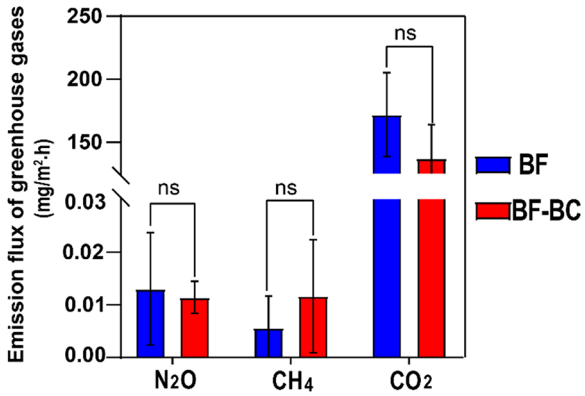


Fig. 8 Effect of biochar addition on N_2O, CH_4 and CO_2 emissions from rhizosphere soil in the BF-BC and BF group. BF: treatment with only basal fertilizer. BF-BC: treatment with basal fertilizer plus biochar ($p < 0.05$)

4 Discussions

This study focuses on the dynamic equilibrium mechanisms of microbial communities, key nutrient elements (such as nitrogen, phosphorus, and potassium), and metabolites, as well as their persistence characteristics under natural conditions without additional fertilization for five years. Compared with other teams' research on the effects of continuous fertilization modes on soil microecology and crop growth (Nan et al. 2023; Nguyen et al. 2018), this study builds upon our team's preliminary short-term research on sugarcane. It further investigates the enhancement of sugarcane yield and sugar accumulation through reduced nitrogen application combined with biochar under long-term (5-year) no-fertilizer-supplement conditions.

The extensive literature has confirmed that biochar exhibits broad-spectrum applicability across various crop species and soil types (Dan et al. 2022; Luo et al. 2017; Ren et al. 2023). This study investigated the effects of nitrogen reduction combined with biochar application on sugarcane growth. The total root length of sugarcane increased by 7.41%, while the root surface area decreased by 11.19%. The root volume significantly decreased by 31.06% ($P < 0.05$), and the average root diameter significantly decreased by 21.53%. This may be achieved by increasing fine root branching. Additionally, studies by Yang et al. demonstrated that biochar addition can cause a greater increase in root length than in root diameter, and promote the growth of new roots (Xiang et al. 2017). Biochar application significantly increased the nitrogen balance index (NBI) and enhanced sugarcane growth ($P < 0.05$). This result is consistent with other studies on gramineous crops, where co-application of biochar and chemical fertilizer significantly enhanced crop biomass yield (Sadaf et al. 2017). Similar results have been observed in other studies, where nitrogen reduction combined with biochar boosted root absorption area, fresh weight, and nitrogen agronomic efficiency in cotton (Cui et al. 2024; Nyambo et al. 2023; Song et al. 2020).

Biochar enhances soil organic matter (SOM) mineralization, evidenced by elevated CO_2 emissions and nutrient release (e.g., NH_4^+, NO_3^-) in short-term studies. This effect extends beyond pH modulation (e.g., acidification reduction) and involves multiple mechanisms, including enhancing microbial habitats, priming effects, improving aggregate structure, and reducing temperature sensitivity (Anderson et al. 2011; Rasul et al. 2022; Singh et al. 2022; Tian et al. 2016). Biochar can improve soil porosity and cation exchange capacity (CEC), fostering microbial activity and enzyme diffusion (Munera-Echeverri et al. 2018). Biochar acts as an electron shuttle, stimulating redox-driven SOM decomposition. Labile carbon in biochar triggers microbial priming, accelerating native SOM breakdown (Rasul et al. 2022). While initial nutrient release is amplified by these interactions, long-term biochar stability may shift microbial communities toward recalcitrant carbon decomposition, moderating mineralization rates (Tian et al. 2016). Thus, the synergy of physicochemical, biochemical, and microbial factors—not solely pH changes—drives mineralization, promoting the release of soil nutrients (Wang et al. 2021a).

Biochar serves as a key tool for promoting nutrient release and carbon storage, thereby enhancing soil fertility and advancing sustainable agriculture. However, our study results show that the TC content in the BF-BC treatment increased by 34.06%, while SOC and SOM content decreased by 39.64% and 37.67% ($P < 0.05$). The former result is consistent with the short-term research conclusion of our team (Pang et al. 2022), while the latter shows the opposite trend, which may be attributed to the accelerated depletion of soil SOM caused by sugarcane growth under long-term fertilizer deprivation. Factors such as pH and water content in the soil environment regulate the interaction network between biochar and microorganisms, influencing the decomposition of dissolved organic carbon (DOC) in biochar (Jaffar et al. 2024). This process promotes the mineralization of soil SOC by providing carbon sources (Li and Wu 2019). While complex aromatic DOC (e.g., derived from biochar) decomposes slowly, influencing the accumulation of soil SOC, the aging of biochar in soil also affects carbon sequestration (Pei et al. 2021), ultimately leading to increased TC content. The addition of biochar accelerates soil SOM mineralization, promoting the release of soil nutrients (Song et al. 2024). The stability of soil SOC is also influenced and controlled by roots (Ren et al. 2022; Song et al. 2024).

Under the condition of biochar application combined with nitrogen reduction at the initial stage, without subsequent fertilizer supplementation for an extended period, the pH and C/N ratio in the sugarcane rhizosphere soil from the BF-BC treatment group increased by 17.7% and 44%, respectively, compared with the control group (BF). This suggests that the nitrogen reduction combined with biochar may lead to altered soil characteristics around the roots, which further promotes sugarcane root growth. Such enhanced root development thereby promotes more efficient mineral nutrient uptake, ultimately allowing plants to absorb nutrients more effectively (Chen et al. 2023; Liu et al. 2024). These results align with the findings from our team's previous short-term sugarcane studies (Chen et al. 2023; Pang et al. 2022).

Further studies indicate that biochar can continue to influence soil metabolism and carbon metabolism over time, potentially serving as a tool to regulate soil nitrogen supply and impact rhizosphere soil microbial communities and root metabolites (Cui et al. 2024). Mia et al. also demonstrated that field aging of biochar reduces N fixation (Mia et al. 2018).

Previous research demonstrated that under one-year nitrogen reduction, biochar application significantly elevated soil pH, TC, and C/N ratio compared to the control (nitrogen reduction alone), while TN and NH_4^+–N remained unchanged (Pang 2019). This study demonstrated that prolonged retention of biochar in soil had no detrimental effects. Compared with short-term observations (Pang 2019), soil pH showed sustained increases, while TN and TC content decreased, leading to a reduced C/N ratio. The long-term retention of biochar in soil may further improve nitrogen fertilizer use efficiency during sugarcane growth by altering soil physicochemical properties and enhancing nutrient retention capacity.

By expanding sampling points (triplicate per treatment) and performing rigorous statistical validation of analytical data, we adopted a multi-point multi-dimensional microenvironment sampling method to enhance sample representativeness. This approach ensured the universality and reliability of the dataset. Through integrated high-throughput sequencing and metabolomic profiling, we systematically characterized the structural diversity of sugarcane rhizosphere bacterial communities along with rhizobacterial metabolites. This comprehensive approach provided deeper insights into the ecological status and functional potential of soil microbiota. When interpreting findings, we analyzed factors potentially affecting long-term outcomes. In subsequent studies on dominant bacterial applications, our team will incorporate additional experimental parameters for extended observation and systematic analysis.

The BF-BC treatment did not induce significant changes in the diversity of bacterial communities in the sugarcane rhizosphere. Consistent with the findings of previous two-year studies (Nguyen et al. 2018), this phenomenon may be attributed to the prolonged natural aging of biochar in the field, which helps maintain a natural equilibrium state in the soil (Li et al. 2020; Nguyen et al. 2018). Meanwhile, the study by Xiang et al. demonstrated a negative correlation between soil bacterial diversity and pH value in biochar-amended soils (Xiang et al. 2023). The lack of significant variation in soil bacterial community diversity could also be explained by the absence of notable changes in soil water content within the sugarcane field (Bandara et al. 2021). Under long-term fertilizer-free conditions, the biochar derived from bamboo in this study contained no biologically toxic elements or compounds, thus causing no harm to the sugarcane root systems or the indigenous soil microbial communities (Li et al. 2020 and Pang et al. 2022). According to Nguyen, TN et al., biochar residence in soil for two years showed no significant effects on soil microstructure, composition, environment, or bacterial community structure (Nguyen et al. 2018).

The study revealed that the relative abundances of *Terrimonas* and *Actinobacteria* showed significant elevation, while the relative abundances of *Mesorhizobium* and *Rhizobacter* increased by 41% and 117%, respectively ($P < 0.05$, Fig. 3). These changes could markedly influence carbon, nitrogen, and phosphorus cycling as well as the decomposition of soil SOM, while also promoting root development and improving aboveground plant growth (Lv et al. 2024; Ran et al. 2023; Yin et al. 2024). The study by Qiu et al. demonstrated that biochar can significantly influence the abundance of *Actinobacteria* and other bacteria, as well as the cycling of soil elements over time (Qiu et al. 2023). In the process of beneficial soil bacterial nitrogen fixation, researchers proved that biochar amendment substantially enhances the abundance of diazotrophs (Liu 2023; Pang et al. 2022). He et al., demonstrated that biochar aging can significantly influence the abundance of nitrogen-fixing bacteria (He et al. 2021); the abundance of nitrogen-fixing bacteria undergoes significant changes, which can enhance plant growth by converting atmospheric nitrogen into bioavailable ammonia, thereby alleviating nitrogen deficiency in crops (Xu and Wang 2023).

Moreover, the study by Chen et al. also demonstrated that biochar increased the abundance of *Leptospirillum* bacteria in soil after three years, which is consistent with the experimental results of this study (Fig. 3). These bacteria are capable of participating in carbon and nitrogen fixation as well as biosynthetic pathways (Goltsman et al. 2013, 2009). These beneficial microorganisms can

provide substantial nitrogen for sugarcane growth and enhance nitrogen uptake efficiency in plant leaves (Zhu et al. 2023). Relevant studies have shown that *Rhizobacter* is significantly correlated with nitrogen content in the aboveground parts of plants (Wang et al. 2023). The combined effects of these factors may explain why the nitrogen balance index (NBI) in sugarcane leaves of the BF-BC group was higher than that of the BF group. Additionally, sugarcane plant height and stem diameter growth are closely associated with these factors (Pang et al. 2022). We found that the abundances of the bacterial phyla *Actinobacteria* and *Proteobacteria* in the BF-BC group were lower than those in the BF group (Fig. 3 and Fig. 4); however, experimental data showed that sugarcane growth indicators (such as plant height and stem diameter) were significantly better than those in the BF group. This finding is consistent with the findings that biochar aged in soil for over three years can influence bacterial community richness by altering soil chemical properties (Chen et al. 2020). The hydrolytic enzymes secreted by *Actinobacteria* accelerated the decomposition of SOM and promoted plant growth (Lv et al. 2024).

However, the abundance of *Sphingobacteriia* in the rhizosphere soil of the BF–BC group increased significantly (Fig. 3). This may be attributed to the five-year ratoon cropping of sugarcane without long-term fertilization, leading to a deficiency in SOM, including aromatic compounds (Hui et al. 2023 and Liu et al. 2021). Sugarcane growth is influenced by diverse microorganisms, among which competitive interactions may occur. This competition can lead to an increase in certain beneficial bacteria while reducing others. Our research demonstrates that aged biochar by the fifth year promotes sugarcane growth through an overall positive microbial community effect, indicating that the newly established microbial consortium reaches a balanced state at this stage, thereby creating more favorable conditions for sugarcane growth. Research on the dynamic effects of microbial communities on soil and crops over extended periods is currently underway in our laboratory.

In the present study, compared to the control BF treatment, the BF-BC treatment reduced soil N_2O emissions by 12.21% (Fig. 8). It reduced NH_4^+–N content in soil by 44.70%, while increasing NO_3^-–N content and SWC by 42.95% and 5.6%, respectively. These results indicate that the nitrification activity in BF-BC treated soil was stronger than that in the BF group, and might be slightly higher than the denitrification activity, which may contribute to reduced N_2O emissions (Fan 2023; Han et al. 2023). Our research revealed that under prolonged conditions without fertilizer supplementation, the BF-BC treatment may exert a negative impact on the nitrification and denitrification processes in the soil. In our further study, we will investigate the application of reduced nitrogen combined with biochar along with annual fertilizer addition to enhance soil nitrogen retention and balance nitrification–denitrification processes. However, existing research demonstrates that nitrification processes can accumulate N_2O, and soil N_2O emissions show negative correlations with soil pH and C/N ratio (Jiang et al. 2019; Tan 2017). Soil N_2O emissions are governed by complex interactions among multiple environmental indicators, including soil nutrients, temperature, moisture, and microbial activity (Hiis et al. 2024). The study by Wang et al. demonstrated that aged biochar can reduce N_2O emissions by decreasing the release of unstable components (Wang et al. 2021b). Simultaneously, CO_2 emissions were significantly inhibited in the treatment involving basal fertilizer plus biochar (Fig. 8), accompanied by a notable increase in *Cyanobacteria* (Fig. 3B), thereby enhancing CO_2 fixation capacity (Badger and Price 2003). *Chloroflexi* is also capable of CO_2 fixation via the 3-hydroxypropionate pathway (Wang et al. 2021b). The lack of significant reduction in CO_2 emissions may be attributed to the long-term persistence of biochar in soil, which leads to partial carbon mineralization (Shen et al. 2017). Our research showed that in the fifth year, biochar can still reduce soil CO_2 emissions, as aged biochar promotes soil carbon sequestration (Wang et al. 2021b). Previous studies showed that biochar maintained in the field for one year can reduce soil CO_2 emissions (Pan et al. 2024). The decrease in soil SOC content within sugarcane field soils during the fifth year contributes to the mitigation of CO_2 emissions (Shrestha et al. 2023). Overall, total CO_2 emissions encompass both the decomposition of biochar and the conversion of SOC to inorganic carbon (Sheng and Zhu 2018). The study demonstrates that biochar enhances carbon sequestration capacity by mitigating CO_2 emissions, providing critical guidance for optimizing integrated nitrogen-reduced fertilization strategies in agricultural systems.

Our study revealed notable shifts in methanogenic archaea and methanotrophic bacteria, including *Methanomicrobiales*, *Methylacidiphilales*, *Methylococcales*, and *Methylomirabilales*. Compared to the BF group, the BF-BC group exhibited 83.33% and 71.43% reductions in *Methylococcales* and *Methylomirabilales* abundances, respectively. Relative to the BFr group, *Methanomicrobiales* increased remarkably (from undetectable trace amount to detectable level), whereas that of *Methylacidiphilales* decreased by 92.13% in the BF-BCr group. These microorganisms are all involved in methane metabolism (production or oxidation), with *Methanomicrobiales* and *Methylacidiphilales* primarily functioning as methanogens dependent on strictly anaerobic environments (Browne et al. 2016; Sousa

et al. 2015). *Methylococcales* demonstrates dual metabolic capabilities in both methanogenesis and methane oxidation, enabling adaptation to broader environmental conditions (Rissanen et al. 2023). *Methylomirabilales* employs unique mechanisms for methane oxidation under hypoxic conditions (Ettwig et al. 2010). Microbial community interactions drive methane production towards a new equilibrium state (Huang et al. 2025). Furthermore, the structural characteristics of biochar, particularly its density and pore network architecture, can influence methane emissions through promotion or inhibition effects (Lu and Zong 2018). These combined factors collectively lead to the experimental outcomes observed in this study, where *Methanomicrobiales* exhibited a slight increase in abundance.

Particularly, the 5-year field experiment demonstrated that, compared to the BF control group, the biochar-treated BF-BC group reduced soil CO_2 and N_2O emissions while increasing CH_4 emissions in the fifth year in sugarcane fields (Fig. 8). However, studies on the emission of N_2O, CO_2, CH_4 and other soil nutrient indicators over a period of 1 to 5 years are undergoing in our laboratory and will be analyzed as separate topics.

Rhizosphere metabolites primarily originate from plant roots and soil microorganisms. In this study, compared with the control group BF, the BF-BC treatment group exhibited significant changes in rhizosphere differential metabolites, which is consistent with findings reported in relevant literature that the application of biochar and nitrogen fertilizer can significantly influence crop rhizosphere metabolites (Ren et al. 2023; Xia et al. 2023b). While there was no significant change in polysaccharide content (Table S7), the content of UDP-N-acetylmuramate, a metabolite involved in peptidoglycan biosynthesis and its regulatory mechanism in bacteria, was observed to decrease significantly in the biochar-amended rhizosphere soil (BF-BC).

The results of this study indicate that after a 5-year residence of biochar in BF-BC treated soil, significant changes occurred in rhizospheric lipid compounds (Fig. 7). Relevant studies have demonstrated that the combined application of biochar and microbial fertilizer can enhance plant root lipid metabolism and improve phosphorus availability in rhizosphere soil (Li et al. 2022). Meanwhile, studies by Sui et al. have demonstrated that biochar-based fertilizers have a significant effect on the organic acid content in plant root systems (Jiang et al. 2023). This finding contrasts with another study by Jiang et al., which reported that biochar increased soil phosphate retention (Jiang et al. 2022). Notably, these metabolites showed significant changes in the BF-BC treatment in this study ($P<0.05$, Fig. 6). Root (BF-BCr) exudates (including carbon sources, organic acids, and phenolic compounds) were observed to directly or indirectly participate in SOM mineralization processes through alterations in soil physicochemical properties or microbial activity (Wiesenbauer et al. 2025).

Previous studies have demonstrated that the addition of biochar facilitates the adsorption of aflatoxin B1 in soil (Appell et al. 2023). In this study, compared with the BF group, the BF-BC treatment maintained unchanged aflatoxin levels in the sugarcane rhizosphere soil. However, aflatoxin B1 increased by 2.7% in the root system (BF-BCr), while aflatoxin B2 content in the roots rose by 52%. Furthermore, the abundance of the genus *Pseudonocardia* in the roots of BF-BC (BF-BCr) group showed a significant increase ($P<0.05$), which exhibits inhibitory effects on aflatoxin production (Riahi et al. 2022). It was demonstrated that a positive correlation existed between the concentration of 1-octen-3-ol and the production of aflatoxin B1 (Singh et al. 2020). This increase may be attributed to the significant rise in the content of the root metabolite 1-octen-3-ol ($P<0.05$, Fig. 6 and Table S6).

Additionally, compared with the control BF group, the BF-BC group exhibited reductions in soil AP and TP contents by 5.43% and 21.20%, respectively. In this study, the contents of organic acid metabolites in root exudates (BF-BCr), including L-malic acid, hexanoic acid, and L-lactic acid, increased by 49.7%, 4.4%, and 15.5%, respectively. Under the experimental conditions here, the soil TK content in the BF-BC group increased significantly by 79.39%, while soil AK content decreased markedly by 47.39%. This phenomenon may be attributed to the combined effects of organic acid compounds secreted by sugarcane roots and enhanced microbial activity in the rhizosphere under long-term non-fertilization conditions, which potentially accelerate the uptake of AP and AK from the rhizosphere at a rate exceeding the potassium release capacity of biochar (Wang et al. 2018; Yang et al. 2021).

Existing studies have demonstrated that organic acids secreted by plant roots can enhance the solubility of insoluble phosphates in soil, which enhances phosphorus fertilizer use efficiency and ultimately reduces soil TP content through enhanced phosphorus cycling and mineralization processes (Tian et al. 2019 ; Yang et al. 2021). Moreover, the organic acids secreted by the rhizosphere enhanced the bioavailability of phosphorus in the soil, promoted the release of potassium from minerals, and enhanced its effectiveness (Olaniyan et al. 2022).

While biochar rapidly releases potassium in the short term—readily utilized by soil microbes and plants—its mineralogical structure immobilizes potassium, reducing its migration efficiency in the soil solution (Cong et al. 2020). The elevated soil pH increased adsorption

and fixation of K^+ ions, further slowing potassium release (Wang et al. 2018). These combined effects ultimately led to a significant rise in TK content in the soil. In our study, the discrepancy might be attributed to differences in biochar application duration, as our 5-year long-term field trial potentially accelerated phosphorus mineralization through sustained microbial activity and organic acid-mediated transformations (Zhou et al. 2020).

This study provides a critical theoretical foundation for optimizing cultivation strategies and soil health management through an in-depth analysis of the sugarcane rhizosphere metabolome. Significant changes in rhizospheric metabolites play a pivotal role in promoting microbial activity and root system health in sugarcane (Jeffery et al. 2017; Oladele et al. 2019 ; Smith et al. 2005). The organic acids (e.g., citric acid, malic acid) and phenolic compounds secreted by sugarcane roots not only markedly enhance soil phosphorus activation efficiency but may also suppress soil-borne pathogens through specific metabolite combinations (Liu et al. 2016 and Tan et al. 2025).

These findings suggest that the dual objectives of nitrogen enhancement and biological control could be achieved simultaneously by breeding high-secretion cultivars or applying metabolic precursors (Kumar et al. 2024; Raman et al. 2022; Shair et al. 2021; Xi et al. 2023). The rhizospheric metabolites undergo significant changes, which facilitate the healthy growth of both rhizosphere microorganisms and root systems in sugarcane (Ling et al. 2021; Seehausen et al. 2017; Sullivan et al. 2020). Furthermore, the interactions between metabolites and microorganisms demonstrate potential in mitigating soil contamination risks, highlighting their significant practical value in agricultural ecosystems (Ye et al. 2019). By integrating precision fertilization techniques, we propose a synergistic management model of "metabolic regulation-soil nutrient activation-pathogen suppression," offering a systematic solution from theory to practice for sustainable sugarcane cultivation.

The regulatory efficacy depends on biochar feedstock (e.g., wood vs. crop residue) and pyrolysis conditions, which influence its porosity, pH, and nutrient retention capacity (Edussuriya et al. 2023). For instance, alkaline biochar may enhance acidic soil buffering but could oversaturate neutral soils, disrupting nutrient balance. While biochar improves fertilizer use efficiency (e.g., reducing NH_3 volatilization or NO_3^- leaching), excessive or mismatched application may immobilize nutrients (e.g., N sequestration in carbon-rich biochar) or alter microbial activity, counteracting intended benefits (Pang et al. 2022). Biochar's persistence may stabilize soil structure and carbon sequestration, yet its aging process (e.g., surface oxidation) or interactions with agrochemicals (e.g., pesticide adsorption) could unpredictably modify regulatory thresholds over time (Wu et al. 2024). These limitations underscore the need for context-specific design of biochar-fertilizer systems to optimize regulatory functions without compromising soil resilience.

Our study revealed that, over five years, nitrogen reduction with biochar application significantly altered ratoon sugarcane rhizosphere ecology. As key drivers of soil metabolism, microorganisms directly reflect environmental responses and partially determine metabolite profiles, thereby influencing nutrient cycling. This relationship underscores the importance of soil metabolism-microbiome interactions. The treatment also actively regulated root exudates, reshaping microbial communities and modulating greenhouse gas emissions. Our fifth-year multi-dimensional assessment—covering agronomic traits, soil properties, microbiota, metabolites, and field emissions—confirms that the sustainable fertilization mode (reduced nitrogen plus biochar) demonstrates enhanced viability versus nitrogen reduction alone.

Our team has conducted a series of short- to medium-term scientific assessments on the effects of reduced nitrogen application combined with biochar on sugarcane growth and soil environmental impacts (Liu 2023; Pang 2019; Pang et al. 2022; Zhang 2020). However, there have been no reports on the influence of the five-year natural aging of biochar in field conditions on the rhizosphere micro-ecosystem, including soil microbial diversity and metabolic byproducts. This study focuses on the entire rhizosphere ecosystem which underwent the long-term (5-year) natural rebalancing and assesses the feasibility of long-term reduced nitrogen fertilization combined with biochar application. We collected extensive data, including sugarcane agronomic traits, rhizosphere soil physicochemical properties, bacterial community composition, rhizosphere metabolites, and greenhouse gas emissions from sugarcane fields. The results demonstrate that reducing nitrogen input while applying biochar still positively influences sugarcane root and shoot growth while enhancing long-term soil fertility. As a phased study, this work serves as a valuable supplement to research in this field. At present, we are conducting continuous long-term scientific monitoring of the field experiment outcomes. In our ongoing study, we will include antagonistic effects of bacterial communities, and metabolites into our plan. For example, we will compare and analyze the experimental data of single factors without interference and the data of all coexisting factors. We believe that these studies will provide more accurate guidance for future management of fertilizer combining biochar application in sugarcane cultivation.

5 Conclusions

After five years of field experiments, the results demonstrated that combining nitrogen reduction and biochar application continued to impact the growth of perennial sugarcane roots and aboveground parts, even in the fifth year without any additional fertilizer application, significantly influencing the composition of the sugarcane rhizosphere bacterial community and metabolite content. Beneficial bacteria were conducive to the optimization of rhizosphere microecology, further stabilizing the rhizosphere microenvironment and promoting the growth of sugarcane. The interaction between sugarcane rhizosphere bacteria enhanced nutrient absorption and utilization by sugarcane, improved soil fertility, promoted the renewal of the microbial community, and inhibited rhizosphere pathogens. Additionally, the multiple functional roles of sugarcane rhizosphere metabolites in regulating plant development, enhancing immune function, and responding to environmental stress also changed. In addition, the emission of CO_2 in the rhizosphere soil was effectively reduced by this treatment, while the inhibitory effect on N_2O emission was not obvious. In summary, sugarcane growth and soil environment were positively affected by biochar application. An optimized soil-bacteria-metabolite interaction network was established to promote the synergistic effect among sugarcane roots, soil and bacteria, thus indicating great potential in agricultural soil carbon sequestration and emission reduction.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-025-00519-2.

Additional file1

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Author contributions

Yixian Jia: Data Collection, Visualization, Validation, Software, Methodology, Investigation, Formal Analysis, Data Curation, Writing—Original Draft, Writing—Review & Editing. Lei Zhang: Conceptualization, Project Design & Management, Supervision, Investigation, Formal Analysis, Validation, Proposal of micro ecological network and data verification, Writing—Original Draft, Writing—Review & Editing. Ziqin Pang: Data Collection, Software, Visualization. Lifang Mo: Data Collection, Software, Visualization. Huaying Fu: Management of fields and instruments. Zhaonian Yuan: Funding Acquisition, Resources. Chaohua Hu: Conceptualization, Funding Acquisition, Resources. All authors read and approved the article.

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Data availability

The datasets analyzed in the current study are available from the corresponding authors upon reasonable request.

Declarations

Competing interests

There are no competing interests involved in the article.

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