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Research article

Biochar-based rhizosphere engineering for enhanced CH_4 removal in landfill cover soil

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ABSTRACT

Rhizosphere engineering represents a promising strategy for augmenting methanotrophic activity in landfill cover soil (LCS). However, the mechanistic interplay between plant-biochar combinations and their collective influence on the rhizosphere-mediated regulation of methane (CH_4) biofiltration processes remains inadequately elucidated. This study systematically investigated the efficacy of vegetated LCS systems by integrating three herbaceous plant species with two distinct biochar types, evaluating CH_4 removal efficiency across three operational phases: Phase I (continuous CH_4 input), Phase II (fluctuating CH_4 input), and Phase III (resumed continuous CH_4 input). During Phase I, vegetation played a pivotal role in shaping rhizosphere microbial communities, with *Solanum americanum*-planted LCS demonstrating superior methanotrophic activity, attributable to its favorable metabolite profile. Swine manure-derived biochar (MBC) significantly enhanced CH_4 removal efficiency compared to maize straw-derived biochar (SBC). However, MBC-amended soils exhibited diminished resistance to CH_4 starvation during Phase II, a phenomenon correlated with reduced availability of root-derived metabolites. Instead, rhizosphere microbial communities with enhanced nutrient-importing capacities demonstrated greater retention of methanotrophic activity. The dominance of rhizosphere-mediated effects was disrupted by CH_4 input fluctuations in Phase II, underscoring the vulnerability of plant-driven systems to variable CH_4 supply. However, biochar emerged as a critical factor in restoring methanotrophic activity upon reintroducing continuous CH_4 input in Phase III. Notably, MBC proved more effective than SBC in elevating soil organic carbon content while concurrently reducing ammonia nitrogen concentrations in the rhizosphere, thereby fostering a more robust recovery of CH_4 removal capacity. This study underscores the synergistic potential of plant-biochar co-application in enhancing the resistance and resilience of soil microbial communities and methanotrophic activities within engineered ecosystems. These findings provide critical insights into optimizing rhizosphere engineering strategies for sustainable CH_4 mitigation in landfill environments.

1. Introduction

As the third-largest contributor to anthropogenic methane (CH_4) emissions, landfills are responsible for approximately 50 Tg of CH_4 released annually on a global scale (Wang et al., 2024b). Landfill gas (LFG), generated during both the operational phase and for decades post-closure (Parsaeifard et al., 2020), represents a significant environmental challenge. A substantial proportion of CH_4 emissions originates from small or aging landfills, particularly those without gas collection infrastructure or with suboptimal capture efficiency. To mitigate these fugitive CH_4 emissions in situ, the most economically feasible solution lies in the deployment of bio-reactive systems utilizing landfill soil covers.

Numerous studies have explored the CH_4 oxidation behavior of methane-oxidizing bacteria (MOB) in landfill cover soils (LCS), identifying critical influencing factors such as CH_4 and oxygen (O_2)

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concentrations, soil type, physicochemical properties, soil amendments, and climatic conditions (La et al., 2018). Notably, vegetation—whether naturally established or artificially introduced during landfill remediation—plays a pivotal role in modulating methanotrophic activity within LCS. Research has consistently demonstrated that vegetated soil covers exhibit significantly higher CH_4 oxidation capacities compared to non-vegetated covers, with the degree of enhancement varying across plant species (Chai et al., 2011; Ndanga et al., 2016; Bian et al., 2019). The beneficial effects of vegetation are primarily attributed to improved soil aeration, enhanced nutrient availability, root-mediated O_2 secretion, regulation of soil moisture content (MC) through transpiration, and the stabilization of soil structure by root systems (Reichenauer et al., 2011; Ndanga et al., 2016; Bian et al., 2020). Despite these advancements, existing studies have predominantly focused on abiotic improvements, largely neglecting the role of rhizosphere-mediated effects.

The rhizosphere effect refers to the enrichment of microbial biomass and activity in the soil surrounding plant roots, coupled with the selective recruitment of specific microbial communities (López et al., 2023). Microbial populations in the rhizosphere can be nearly 100 times more abundant than those in non-vegetated soils (Kulkarni et al., 2024). These microorganisms play a critical role in soil remediation and plant growth promotion through diverse mechanisms, including siderophore production, phytohormone synthesis, phosphate solubilization, biological nitrogen fixation, antibiotic production, and the secretion of lytic enzymes (Joshi et al., 2023). The composition of rhizosphere microbiomes is highly dynamic, varying significantly with plant species, geographical location, climatic conditions, and land management practices (Zhalnina et al., 2018). The assembly of these microbiomes is governed by two key rhizosphere chemistry processes (Venturi and Keel, 2016): (1) the stimulation of microbial proliferation by rhizodeposits and root exudates, and (2) the detection and response to low molecular weight compounds derived from plants or microbes, which trigger cellular responses. Zhalnina et al. (2018) emphasized the role of substrate preferences in microbial colonization within the rhizosphere. For example, Chen et al. (2023) demonstrated that citric acid secreted by sour molds enhances the growth and metabolic activity of MOB in rhizosphere soil. Similarly, Trivedi et al. (2020) reported that amino acid auxotrophic bacteria thrive in root exudates enriched with specific amino acids. Furthermore, rhizosphere effects can indirectly influence microbial community composition and activity by modulating soil redox potential and nutrient dynamics (Hartmann and Six, 2023; Jiang et al., 2023). Despite the well-documented significance of the rhizosphere in shaping microbial communities and functions, its role in regulating methanotrophic activity in LCS remains poorly understood.

Rhizosphere engineering has emerged as a promising strategy for enhancing soil quality, crop productivity, and soil remediation (Xiang et al., 2022). Dessaux et al. (2016) emphasized that all three components of the rhizosphere, including soil, plants, and microbial populations, can be strategically manipulated to achieve targeted outcomes. Among soil amendments, biochar has demonstrated significant potential for promoting CH_4 removal in engineered landfill soil cover (Yargicoglu and Reddy, 2017). Biochar promotes MOB growth and activity by improving soil aeration, supplying micronutrients, and increasing microbial habitat availability (Huang et al., 2021). Furthermore, biochar can indirectly influence CH_4 oxidation by modulating plant physiology or synergizing with plant-root interactions. It enriches soil nutrient content, enhances water and nutrient retention, and boosts plant biomass and grain yield (Yang et al., 2024). Furthermore, biochar mitigates the bioavailability of harmful substances, reducing their impact on plants (Zhou et al., 2021). It also significantly enhances microbial activity in rhizosphere soils, facilitating the recovery of bacterial communities in contaminated soils, strengthening microbiome symbiosis, and improving microbial metabolic functions (Li et al., 2020). Studies have shown that biochar addition generally reduces CH_4 emissions in vegetated soils (Wang et al., 2023a), suggesting a synergistic effect between biochar and plants in enhancing soil CH_4 oxidation.

In summary, vegetation offers multiple benefits for methanotrophic activity in LCS, and biochar-based rhizosphere engineering holds promise for optimizing sustainable CH_4 biofiltration systems. However, to our knowledge, no studies have explored the efficacy of plant-biochar combinations in enhancing methanotrophic activity in landfill soil cover or investigated how such interactions regulate the rhizosphere effect. Moreover, significant daily fluctuations in CH_4 emission rates—ranging from 1.8 to 63.8 μmol m^{-2} s^{-1} due to barometric pressure changes at landfills (Xu et al., 2014)—highlight the need to evaluate the resistance and resilience of rhizosphere communities to such variations. To address these gaps, we constructed vegetated landfill soil covers using soil column reactors. Three representative herbaceous plants and two types of biochar were selected to simulate different plant-biochar combinations. By analyzing differences in rhizosphere effects (e.g., metabolite composition and microbial communities) and associated CH_4 removal efficiencies, this study aims to elucidate how biochar interacts with the rhizosphere effect and mediates the resistance and resilience of rhizosphere communities to significant CH_4 input disruptions.

2. Materials and methods

2.1. Soil and biochar

Soil samples were collected from the upper layer of a closed landfill in Shenzhen, China. The soil was sandy loam, with a pH of 6.77 and an MC of 17 %. Swine manure-derived biochar (MBC) and maize straw-derived biochar (SBC) were procured from a biochar production facility in Henan, China. The feedstocks were subjected to pyrolysis in a controlled furnace under standardized conditions: a nitrogen (N_2) inflow rate of 400 mL min^{-1}, a heating rate of 10 °C min^{-1}, and a peak temperature of 500 °C. Raw biochar was utilized for the experiment. To ensure homogeneity, both the soil and raw biochar were sieved through a 2-mm mesh before further use and analysis. The properties of the two biochar and the original soil are listed in Tables 1 and 2.

2.2. Experimental design

The soil column reactors were constructed by retrofitting PVC plastic drums with a height of 60 cm and an inner diameter of 40 cm. A 10 cm deep gravel layer was placed at the bottom to ensure uniform gas distribution. Soil was then loaded into the reactors to a height of 40 cm. An air inlet port for simulated LFG and an air outlet port for measuring output gas flux were positioned 2 cm above the bottom and 2 cm below the top, respectively. The concentration of CH_4 in the original LFG could reach 60 % (v/v) (Scheutz et al., 2009); however, this concentration decreases as LFG migrates from the underlying waste to the surface soil layer. Based on the gas composition observed at a depth of approximately 40 cm in a previous study (Huang et al., 2020), an input LFG concentration of 20 % (v/v) CH_4 (using N_2 as the balance gas) was adopted for this experiment. The inlet airflow rate for LFG was maintained at 10 mL min^{-1} using airflow controllers, which were calibrated weekly using a standard gas flow calibrator (Gilibrator-2, Sensidyne, USA). The reactor was designed with an open top to simulate natural air infiltration under field conditions and minimize plant disturbance.

Table 1
Physical and chemical properties of biochar.

Items	MBC	SBC
pH	8.17 ± 0.01	7.92 ± 0.16
Moisture content (%)	4.87 ± 0.14	13.33 ± 0.01
Volatile matter (%)	12.84 ± 0.45	40.35 ± 0.05
Ash content (%)	79.36 ± 0.23	26.00 ± 0.04
Fixed carbon (%)	2.93 ± 0.33	19.33 ± 0.06
BET surface area (m^2 g^{-1})	69.8006	46.9339
Pore volume (cm^3 g^{-1})	0.044	0.034
Pore diameter (Å)	24.963	29.332

Table 2
Physicochemical properties of soil in the three phases.

	pH			MC			NH_4^+ (g kg^{-1})			NO_3^- (g kg^{-1})			TOC (g kg^{-1})			SO_4^{2-} (g kg^{-1})		
Phases	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
MBC	6.65± 0.21	6.95± 0.07	7.05± 0.07	0.21^{b}± 0.01	0.22^{ab} ±0.01	0.26^{a}± 0.01	1.32^{a}± 0.17	1.06^{ab} ±0.08	0.90^{b}± 0.04	0.79± 0.04	0.93± 0.06	1.03± 0.14	0.81^{b}± 0.02	1.09^{a}± 0.06	1.24^{a}± 0.06	0.46^{c}± 0.05	0.61^{b}± 0.01	0.81^{a}± 0.03
SA/RS	6.60± 0.14	7.00± 0.28	6.95± 0.07	0.17^{b}± 0.02	0.21^{ab} ±0.01	0.24^{a}± 0.01	1.32^{ab} ±0.16	1.23^{b}± 0.12	1.66^{a}± 0.06	0.51± 0.03	0.67± 0.0	0.80± 0.15	0.92^{b}± 0.12	0.93^{b}± 0.04	1.34^{a}± 0.13	0.44^{b}± 0.03	0.63^{a}± 0.05	0.72^{a}± 0.03
SA/NS	6.85^{b} ±0.21	7.20^{ab} ±0.14	7.55^{a}± 0.07	0.19^{b}± 0.01	0.23^{a}± 0.01	0.25^{a}± 0.01	1.61± 0.11	1.47± 0.05	1.54± 0.04	0.52^{b}± 0.01	0.74^{ab} ±0.17	1.03^{a}± 0.08	0.78^{b}± 0.10	1.19^{a}± 0.08	1.38^{a}± 0.01	0.56± 0.14	0.65± 0.05	0.75± 0.06
MBC_SA/RS	6.65^{b} ±0.21	7.25^{ab} ±0.07	7.55^{a}± 0.35	0.21± 0.02	0.22± 0.03	0.26± 0.01	1.61^{a}± 0.11	1.48^{a}± 0.02	1.21^{b}± 0.09	0.71^{b}± 0.05	0.78^{b}± 0.11	1.04^{a}± 0.02	1.32^{b}± 0.16	1.52^{b}± 0.04	1.93^{a}± 0.08	0.40± 0.08	0.50± 0.08	0.45± 0.06
MBC_SA/NS	6.65± 0.35	6.65± 0.21	7.20± 0.28	0.20± 0.04	0.23± 0.01	0.26± 0.03	1.60± 0.11	1.53± 0.05	1.37± 0.11	0.55^{b}± 0.04	0.67^{b}± 0.05	0.89^{a}± 0.09	1.02^{b}± 0.03	1.12^{ab} ±0.08	1.35^{a}± 0.11	0.40^{b}± 0.04	0.46^{ab} ±0.08	0.63^{a}± 0.05
MBC_NC/RS	6.80± 0.14	7.20± 0.14	7.30± 0.28	0.16± 0.01	0.22± 0.02	0.33± 0.07	1.56± 0.21	1.27± 0.06	1.44± 0.05	0.64± 0.08	0.71± 0.12	0.84± 0.09	1.36± 0.05	1.15± 0.09	1.04± 0.14	0.35^{b}± 0.06	0.60^{b}± 0.09	0.92^{a}± 0.08
MBC_NC/NS	6.60± 0.14	6.95± 0.07	6.75± 0.35	0.17± 0.01	0.19± 0.01	0.20± 0.04	1.56± 0.05	1.59± 0.19	1.83± 0.03	0.53± 0.09	0.73± 0.08	0.77± 0.06	0.96± 0.13	1.04± 0.19	1.05± 0.04	0.43^{b}± 0.05	0.66^{ab} ±0.22	1.03^{a}± 0.04
MBC_PC/RS	6.75± 0.21	7.00± 0.00	7.20± 0.14	0.17^{b}± 0.02	0.20^{ab} ±0.03	0.25^{a}± 0.02	1.68± 0.04	1.72± 0.15	1.45± 0.13	0.60± 0.11	0.70± 0.11	0.95± 0.15	1.37± 0.13	1.24± 0.12	1.46± 0.09	0.43± 0.05	0.46± 0.05	0.46± 0.07
MBC_PC/NS	6.65± 0.21	6.80± 0.28	6.95± 0.07	0.22^{ab} ±0.00	0.26^{a}± 0.02	0.19^{b}± 0.01	1.45± 0.01	1.40± 0.20	1.78± 0.12	0.49^{c}± 0.01	0.71^{b}± 0.03	0.92^{a}± 0.03	0.78^{b}± 0.21	1.04^{ab} ±0.06	1.36^{a}± 0.12	0.47± 0.05	0.61± 0.11	0.68± 0.06
SBC_SA/RS	6.55± 0.21	6.75± 0.35	7.10± 0.28	0.21± 0.03	0.24± 0.04	0.27± 0.02	1.51± 0.11	1.37± 0.18	1.36± 0.12	0.51± 0.08	0.54± 0.16	0.76± 0.04	1.14± 0.05	0.98± 0.09	1.05± 0.14	0.46± 0.05	0.39± 0.03	0.66± 0.14
SBC_SA/NS	6.60^{b} ±0.28	6.55^{b}± 0.07	7.35^{a}± 0.21	0.18± 0.02	0.22± 0.04	0.25± 0.03	1.50± 0.33	1.57± 0.04	1.72± 0.05	0.47^{b}± 0.03	0.54^{ab} ±0.05	0.65^{a}± 0.05	0.89± 0.08	0.93± 0.14	1.15± 0.20	0.47^{b}± 0.05	0.65^{ab} ±0.13	0.74^{a}± 0.05
SBC_NC/RS	6.45^{b} ±0.07	6.85^{a}± 0.07	6.85^{a}± 0.07	0.23± 0.04	0.26± 0.00	0.26± 0.04	1.59^{a}± 0.10	1.11^{b}± 0.14	0.84^{c}± 0.14	0.59± 0.13	0.72± 0.03	0.73± 0.15	1.42^{a}± 0.05	1.21^{b}± 0.09	0.94^{c}± 0.03	0.52± 0.01	0.62± 0.09	0.69± 0.05
SBC_NC/NS	6.70± 0.14	6.90± 0.00	6.90± 0.14	0.17± 0.02	0.15± 0.01	0.22± 0.04	1.41± 0.12	1.43± 0.08	1.55± 0.17	0.49^{b}± 0.04	0.62^{ab} ±0.09	0.80^{a}± 0.03	0.92± 0.14	0.94± 0.04	1.12± 0.08	0.48± 0.11	0.56± 0.09	0.77± 0.07
SBC_PC/RS	6.70^{b} ±0.14	7.30^{ab} ±0.28	7.45^{a}± 0.21	0.16^{b}± 0.02	0.25^{a}± 0.04	0.29^{a}± 0.01	1.66^{ab} ±0.03	1.46^{b}± 0.17	1.81^{a}± 0.02	0.62± 0.04	0.66± 0.16	0.89± 0.04	1.26^{b}± 0.11	1.39^{ab} ±0.20	1.81^{a}± 0.04	0.49^{b}± 0.06	0.77^{b}± 0.13	1.09^{a}± 0.05
SBC_PC/NS	6.85± 0.49	6.95± 0.21	6.80± 0.14	0.18± 0.01	0.20± 0.04	0.25± 0.01	1.58^{b}± 0.07	1.67^{ab} ±0.12	1.88^{a}± 0.07	0.46^{b}± 0.06	0.61^{b}± 0.04	0.80^{a}± 0.01	0.90^{b}± 0.13	1.11^{ab} ±0.04	1.22^{a}± 0.03	0.48± 0.09	0.56± 0.08	0.64± 0.08
Original soil	6.77±0.15			0.17±0.02			0.83±0.05			0.40±0.03			0.30±0.04			0.41±0.03		

Notes: Lowercase letters a, b & c mean significant differences at $P < 0.05$ in comparing each term among the three phases.

During gas emission flux measurements, however, the reactor was sealed with a cover, and clean air was continuously aerated into the chamber at a rate of 10 mL min^{-1} through an inlet port positioned 2 cm above the soil layer. A diagram of the experimental setup is given in Fig. 1.

To simulate vegetated soil cover, three herbaceous plant species were selected based on field investigations at local landfills: *Solanum americanum* (SA), *Nephrolepis cordifolia* (NC), and *Praxelis clematidea* (PC). For each species, three plants in their vigorous growth stage were transplanted into soil amended with 15 % (v/v) of either MBC or SBC. MBC and SBC were chosen as representative biochar derived from biosolids and lignocellulosic wastes, respectively, which are rich in minerals and carbon (Weber and Quicker, 2018). This resulted in six treatment groups: MBC_SA, MBC_NC, MBC_PC, SBC_SA, SBC_NC, and SBC_PC. The 15 % (v/v) biochar application ratio was based on previous findings demonstrating its significant enhancement of CH_4 removal in LCS (Huang et al., 2020). Additionally, two control groups were established: one (MBC) consisting of bare soil amended with 15 % (v/v) MBC, and the other (SA) consisting of SA-planted soil without biochar amendment. SA was chosen as the control due to its strong blight resistance and adaptability to diverse climates (Lin et al., 2023). At the time of transplantation, all three herbaceous species were in their early vigorous growth stage, with average heights of 20–30 cm. More descriptions for the three species can be found in the supporting information (SI).

The reactors were housed in a semi-open laboratory with a sheltered roof. The local annual average temperature and relative humidity in Shenzhen were 23 °C and 74 %, respectively, as reported by the Meteorological Bureau of Shenzhen Municipality. The experiment commenced in July 2023 and concluded in January 2024, spanning a total of 180 days. To account for the impact of CH_4 concentration fluctuations on methanotrophic activity, the study period was divided into three phases. In Phase I (first two months), LFG was supplied continuously to the soil covers. In Phase II (middle two months), LFG supply cycled weekly: one week on, one week off. In Phase III (last two months), LFG supply was resumed to a continuous mode, consistent with Phase I. During Phase I and Phase II, the soils were irrigated with 250 mL of water twice weekly, whereas in Phase III, the watering frequency was reduced to once per week. All transplanted plants survived and exhibited healthy growth throughout the 180-day experiment. No visible signs of senescence, disease, or nutrient deficiency were detected.

2.3. Sampling and measurements

The dynamic chamber method was employed to measure the output gas flux. After sealing the headspace, fresh air was continuously flushed into the reactors. Once the outlet airflow stabilized and its rate was quantified using the gas flow calibrator, two air samples were collected from the outlet air ports. The CH_4 concentration in these samples was analyzed using a gas chromatograph (GC-7890B, Agilent Technologies, USA). The average concentration from the two samples was then used to calculate the outlet CH_4 flux ($Flux_{out}$) and CH_4 removal efficiency, following equations (1) and (2):

$$Flux_{in\ or\ out} = C_{CH4} \times FR/A \quad (1)$$

$$\text{Gas removal efficiency} = (Flux_{in} - Flux_{out}) \ / \ Flux_{in} \quad (2)$$

Here, C represents the gas concentration (mg m^{-3}) in the inlet or outlet air, FR denotes the inlet or outlet airflow rate (m^3 d^{-1}), as measured by the gas flow calibrator, and A is the surface area of the soil cover. A total of 4, 2, and 3 measurements were successfully conducted during Phase I (days 1, 10, 20, and 31), II (days 76 and 111), and III (days 133, 152, and 180), respectively. Prior to experimentation, all reactors underwent a two-week pre-incubation period with continuous CH_4 feeding to exclude microbial adaptation from the experimental phases. During Phase II, gas sampling and measurements were performed on two representative days at the end of two separate weeks following the resumption of continuous LFG supply. Soil samples were collected monthly in each phase, including days 1, 36, 76, 116, 152, and 180. Briefly, the root was gently removed from the bulk soil. Then, it was manually shaken to obtain those soils easily peeled off from the roots, which is defined as non-rhizosphere soil (NS). Afterward, a sterilized brush was used to collect the soil adhered to the root surface, which was rhizosphere soil (RS). For comparison, the soils at a depth of 0–10 cm were collected and mixed as the control soil samples for the MBC group. All RS and NS samples were measured for their pH, MC, TOC, ammonia (NH_4^+) nitrogen, nitrate nitrogen (NO_3^-), and sulfate (SO_4^{2-}) content. Triplicate RS and NS subsamples were analyzed for pH and MC, while duplicate subsamples were used for TOC and ion analyses. The methods were detailed in SI. Soil properties were characterized by experimental phase using averaged values from days 1 and 36 (Phase I), days 76 and 116 (Phase II), and days 152 and 180 (Phase III).

2.4. Rhizosphere microbial and metabolite analysis

Representative RS and NS samples in each phase (days 31 for Phase I, day 76 for Phase II, and day 152 for Phase III) were selected for 16S rRNA gene sequencing. Detailed methodologies, including DNA extraction, PCR amplification, Illumina MiSeq sequencing, and sequencing data processing, are provided by Guangdong Magigene Biotechnology Co., Ltd. (https://www.magigene.com/). The raw sequencing data have been uploaded to the NCBI Sequence Read Archive database (Accession Number: PRJNA1234060). Only representative RS

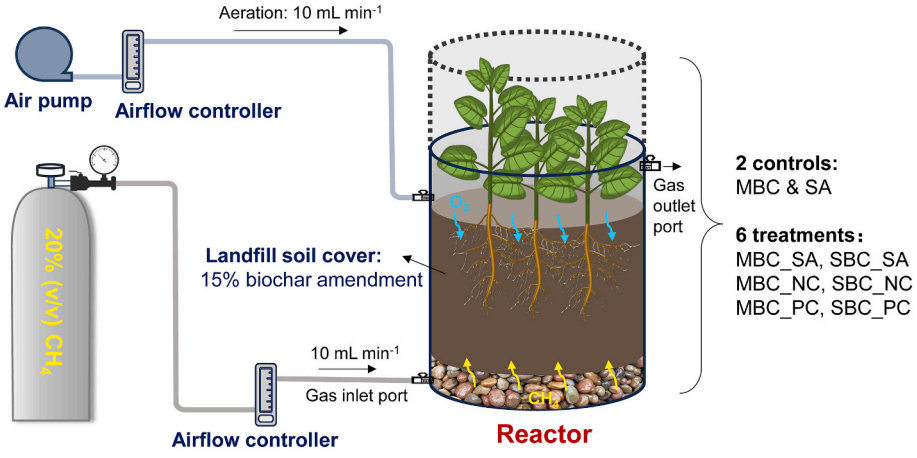

Fig. 1. A diagram of the experimental setup.

and NS samples (prepared in triplicate) from Phase I (day 31) were selected for metabolite composition analysis when the rhizosphere effect was most significant in shaping microbial composition, as discussed in section 4.3. The metabolite analysis method is described by Guangdong Magigene Biotechnology Co., Ltd, which can be found in SI.

2.5. Statistical data analysis

One-way ANOVA test was performed by SPSS software version 26 (IBM, USA) to evaluate the significance of differences in CH_4 removal efficiencies and soil properties across experimental groups, with post-hoc comparisons conducted using Dunnett T3 and Duncan. The General Linear Model method was utilized to examine the main and interaction effects of biochar and plant on CH_4 removal efficiencies. Alpha diversity indices, including the Chao and Shannon indices, were computed using the R programming language. Differences in alpha diversity among multiple groups were assessed using the Kruskal-Wallis rank sum test. Heatmap analysis, based on sample distances, was generated using the *vegan* package in R, with hierarchical clustering performed via the *hclust* function. Distance-based redundancy analysis (db-RDA) was carried out using the *vegan* package, utilizing Bray-Curtis distances to evaluate community composition patterns. Functional gene prediction was conducted using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States). Operational taxonomic units (OTUs) derived from 16S rRNA sequences were normalized, and Kyoto Orthology (KO) profiles were generated using the PICRUSt algorithm. Metagenomic functional annotation was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) ortholog database (http://www.kegg.jp/).

3. Results

3.1. Soil properties

The soil properties for RS and NS across the three experimental phases are summarized in Table 2. A slight pH increase was observed in most treatment groups across all three phases, though values remained within the optimal range (5.5–8.5) for MOB growth and activity (Scheutz et al., 2009). MBC-amended RS exhibited relatively higher pH than MBC-amended NS in later stages, while no such difference occurred between SBC-amended RS and NS. Furthermore, MBC-amended soils generally maintained higher pH than SBC-amended soils. This pattern aligns with biochar's known ability to elevate soil pH through soluble alkaline mineral release (Huang et al., 2025). The greater ash content in MBC suggests more abundant mineral reserves, whose dissolution may be enhanced by acidic root exudates in the rhizosphere (Zhalnina et al., 2018), explaining MBC's more pronounced pH effects.

MC generally increased throughout the experimental period, except for SBC_NC. While irrigation temporarily increased soil MC, these gains were largely offset by evaporative water loss and plant transpiration. The sustained MC increase was primarily attributed to water production through methanotrophic activity (Huang et al., 2025). On average, MC was higher in RS than NS for most biochar-amended groups. This observation aligns with the greater microbial biomass and enhanced biochemical activity typically associated with RS.

In Phase I, biochar significantly enhanced the TOC content of RS, increasing it from 0.30 to 1.32–1.37 g kg^{-1} for MBC-amended soils and 1.14 to 1.42 for SBC-amended soils (Table 2). The TOC content continued to rise in Phase II, reaching 1.52 g kg^{-1}, and further increased to 1.93 g kg^{-1} in Phase III for MBC_SA/RS. In contrast, TOC content slightly declined for MBC_NC/RS and remained relatively stable for MBC_PC/RS. For SBC-amended soils, the TOC content of SBC_PC/RS exhibited a significantly higher value in Phase II and a robust increasing trend across all three phases, similar to the trend observed for MBC_SA/RS. Conversely, the other two plant-SBC combinations showed declining trends. Higher TOC content is known to benefit microbial growth and activity (Fang et al., 2018). The significantly higher TOC content in the rhizosphere of MBC_SA and SBC_PC suggests stronger carbon assimilation and retention activities in these soils.

Methanotrophs require nitrogen for their metabolic activities, but nitrogen cycling and its byproducts can, in turn, influence methanotrophic activity (He et al., 2019). Overall, MBC was more effective than SBC in increasing NO_3^- content in both RS and NS, particularly in SA-planted soils. For NH_4^+, a 25 % decrease was observed in MBC_SA/RS and a 15 % decrease in MBC_SA/NS. In contrast, the other two plant-MBC combinations showed a slight reduction in RS but an increase in NH_4^+ content in NS. Similar trends in NH_4^+ content were observed for SA-planted soils amended with SBC. In NC-planted soils, NH_4^+ content decreased significantly in RS but increased slightly in NS. For PC-planted soils, NH_4^+ content increased to the highest levels in both RS and NS across all groups from Phase I to Phase III. These findings suggest that biochar may stimulate distinct nitrogen cycling processes, indirectly affecting methanotrophic activity in different planted soils.

3.2. Methane removal efficiency

The CH_4 removal efficiencies for all reactors are plotted in Fig. 2. In Phase I, the lowest removal efficiencies were observed in the two control groups, averaging 48 % for MBC and 54 % for SA. Both MBC and SBC amendments enhanced CH_4 removal efficiencies, but the extent of improvement varied by plant species. MBC significantly increased CH_4 removal efficiency for SA to 88 %, but its effects were less pronounced for NC and PC. Under SBC treatment, SBC_SA exhibited slightly higher CH_4 removal efficiency than SBC_NC and SBC_PC, though the differences were not statistically significant ($P > 0.05$). During Phase II, when LFG supply was intermittent, CH_4 removal efficiencies decreased for all groups, with the most significant decline observed in MBC-amended soils. In contrast, SBC-amended soils maintained relatively stable CH_4 removal efficiencies, particularly for NC and PC treatments. Additionally, the control SA and all MBC-amended planted groups exhibited lower performance stability, as indicated by greater standard deviations in CH_4 removal capacity. In Phase III, the resumption of continuous LFG supply restored CH_4 removal efficiencies to 54 %–75 % for MBC-amended planted groups, compared to 47 %–54 % for SBC-amended groups. Notably, the ranking of CH_4 removal efficiency for MBC-planted treatments in Phase III mirrored that in Phase I.

3.3. Rhizosphere microbial community structure

3.3.1. Microbial diversity

The structure of rhizosphere microbial communities arises from complex interactions and feedback mechanisms among plant roots, microorganisms, and the physicochemical environment of the soil. The Chao and Shannon indices, which serve as indicators of microbial diversity, are plotted in Fig. 3(a). In Phase I, nearly all RS samples exhibited greater microbial diversity than NS and non-planted soil (MBC), regardless of biochar type or plant species. These findings align with previous studies demonstrating that vegetated soils harbor significantly more plant-root-associated microorganisms than bare soils (Kulkarni et al., 2024). Biochar treatments generally led to a decrease in the Shannon index for SA/RS but an increase for SA/NS. Additionally, both RS and NS under MBC treatment displayed higher Chao and Shannon indices compared to those under SBC treatment. However, the differences between biochar treatments were less pronounced for NC and PC-vegetated soils. Notably, MBC_SA/RS and MBC_SA/NS exhibited the highest Chao indices among all RS and NS samples.

In Phase II, the Shannon index declined more significantly under MBC treatments than under SBC treatments, suggesting that microbial community structures in MBC-amended soils were less resistant to gas fluctuations. Interestingly, the Shannon index for SA/NS surpassed that of SA/RS in Phase II. Nevertheless, biochar amendments maintained slightly higher microbial diversity in RS compared to NS. By Phase III,

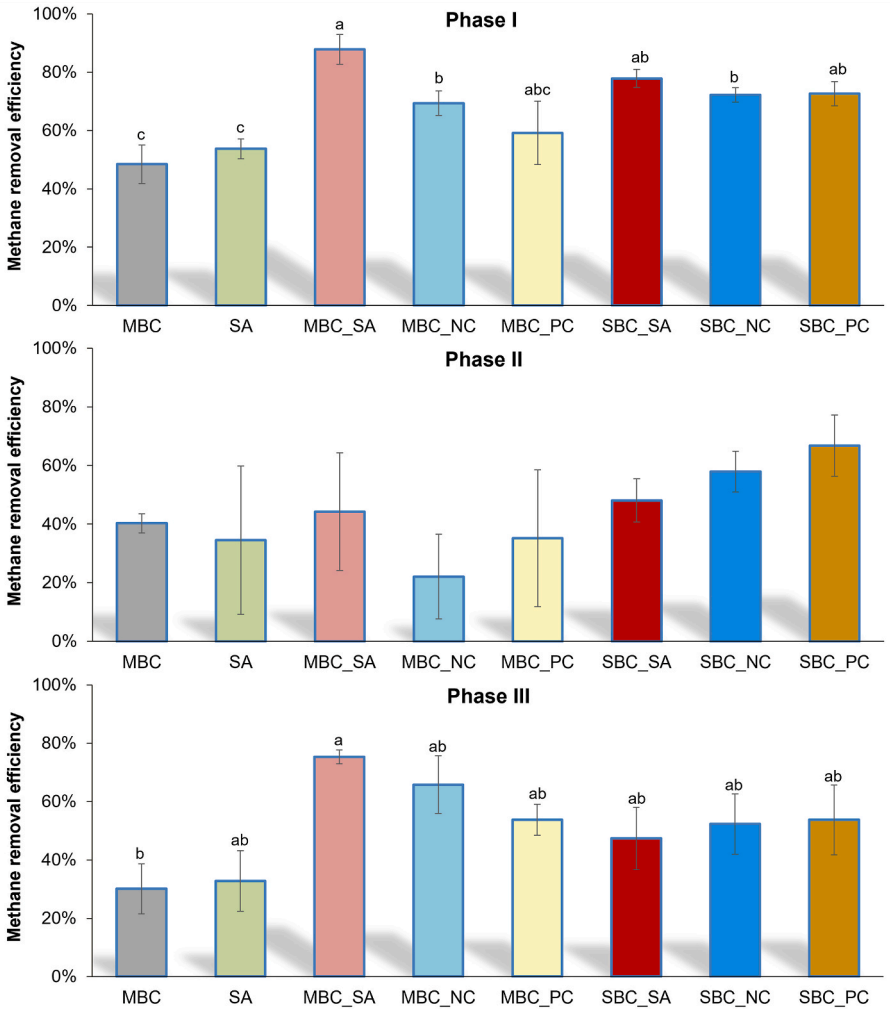

Fig. 2. Methane removal efficiencies in Phase I, II, and III (different lowercase letters indicate significant differences at $P < 0.05$).

the Chao index for RS became lower than that for NS under SBC treatment. Meanwhile, the Shannon index generally increased for all biochar-amended NS but decreased for biochar-amended RS.

As illustrated in Fig. 3(b), the Shannon index did not vary significantly ($P > 0.05$) across the three phases for SA_RS but showed notable differences ($P < 0.05$) between Phase I and Phase III for NC_RS and PC_RS. These results suggest that LFG fluctuations had a greater impact on the α-diversity of NC_RS and PC_RS compared to SA_RS.

3.3.2. *Community composition*

Both plant species and biochar significantly influenced the number of soil genera. As shown in Fig. S1, SA_RS exhibited a greater number of bacterial genera and unique species compared to NC_RS and PC_RS. Furthermore, MBC_SA/RS contained more unique genera than SBC_SA/RS, consistent with the trends observed in the Chao and Shannon indices. The top 15 microbial genera primarily comprised plant growth-promoting bacteria (PGPB), as shown in Fig. 4. Notably, *Bradyrhizobium* (nitrogen-fixing and stress-alleviating) and *Sphingomonas* (abiotic stress tolerance and phytoremediation) were most abundant in Phases I and III, alongside other beneficial genera (*Burkholderia-Caballeronia-Paraburkholderia*, *Candidatus Solibacter*, *Bryobacter*, *Pseudoxanthomonas*, and *Stenotrophobacter*) (Hu et al., 2025; Saini et al., 2023; Youseif, 2018). While cumulative PGPB abundance was initially higher in RS than NS during Phase I, this trend reversed by Phase III, coinciding with the reduced RS microbial diversity.

A total of six MOB genera were identified: *Methylocystis*, *Methylobacter*, *Methylobacterium*, *Methylococcus*, *Methylocaldum*, and *Methylomonas* (Kalyuzhnaya et al., 2015; Zehra et al., 2021). Among these, *Methylocystis* was the dominant methanotroph in all groups, as the abundance of other methanotrophs was extremely low. According to the ternary plot for RS (Fig. S2a), *Methylocystis* was particularly abundant in SA-planted soils compared to NC and PC-planted soils. Additionally, in Phase I, the differences between SA/RS and bare soil under MBC treatment indicated a stronger promotion of *Methylocystis* by plants than by biochar alone (Fig. S2b). MBC further enhanced the growth of *Methylocystis* in conjunction with plant benefits, as evidenced by the higher abundance in MBC_SA/RS compared to SA/RS.

3.4. *Metabolite composition*

Rhizosphere metabolites consist of a diverse array of compounds derived from plant root exudates and microbial secretions (Zhuang et al., 2024). Although over 1200 soil metabolites were identified, only the top 400 most abundant metabolites in rhizosphere soil are plotted in Fig. 5(a). The results reveal that the dominant metabolites were significantly more diverse in SA/RS compared to MBC-amended soils without vegetation. Additionally, both MBC and SBC treatments significantly reduced the concentrations of the majority of rhizosphere metabolites in SA-planted soils. However, the impact of biochar on NC and PC-planted soils varied depending on the biochar type.

The accumulated soil metabolites, categorized by superclass, are illustrated in Fig. 5(b). Organic nitrogen compounds were the most

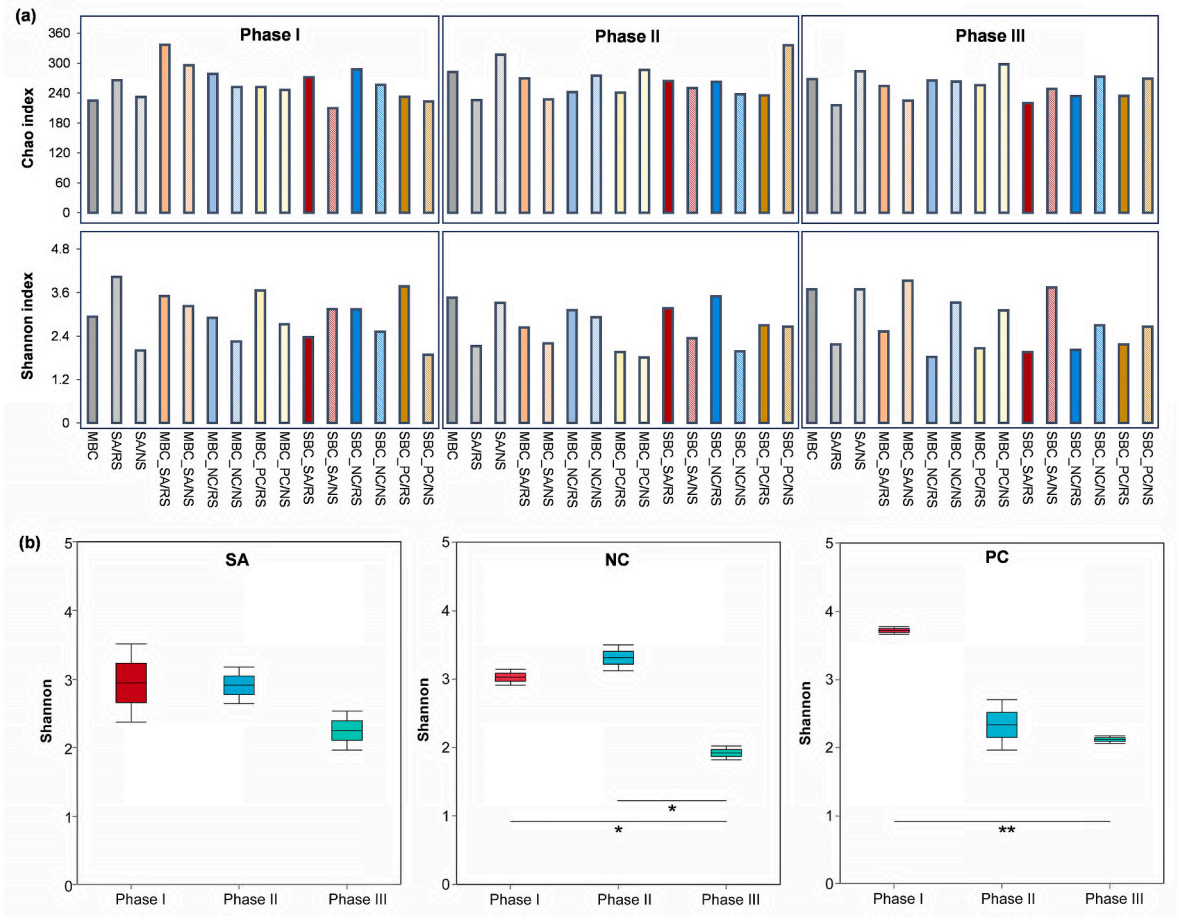

Fig. 3. Comparisons of α-diversity. (a) the Chao and Shannon index and (b) the Krustal-Wallis H test for the Shannon index (genus level) among SA/RS, NC/RS, and PC/RS (* and ** indicate $P < 0.05$ and $P < 0.01$, respectively).

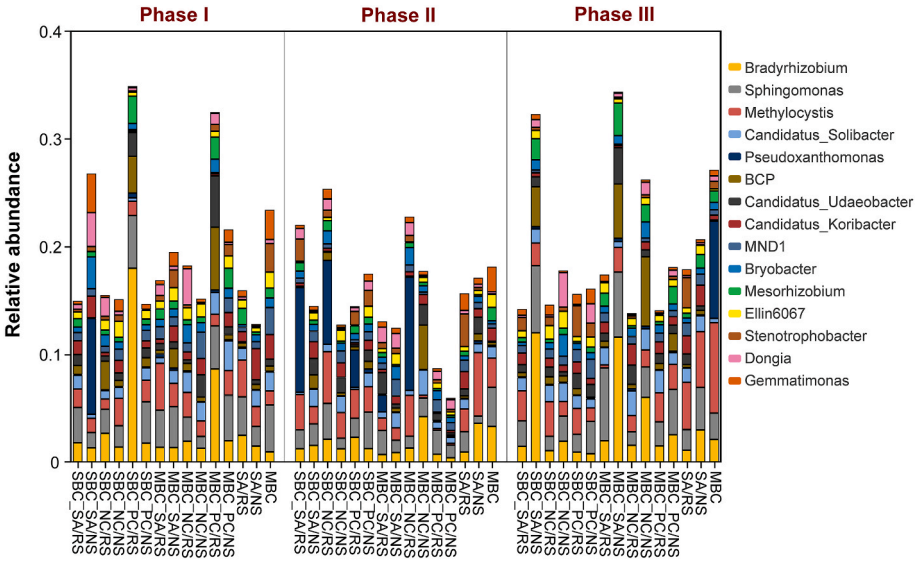

Fig. 4. Abundance of the top 15 genera in different groups.

abundant metabolites across all groups, followed by organoheterocyclic compounds, organic acids and their derivatives, and fatty acids. The majority of organic nitrogen compounds were amines, such as 2-Ethylbutan-1-amine, 2-Methylbutylamine, and 1-Heptanamine, which are produced through the aminization process and serve as a critical nitrogen source for microorganisms (Philip et al., 2021). However, these amines can be further transformed into NH_3 and NH_4^+ via ammonification (Philip et al., 2021). This process may negatively impact methanotrophs due to competition for the methane monooxygenase enzyme site by NH_4^+ or the toxicity of intermediates such as hydroxylamine or

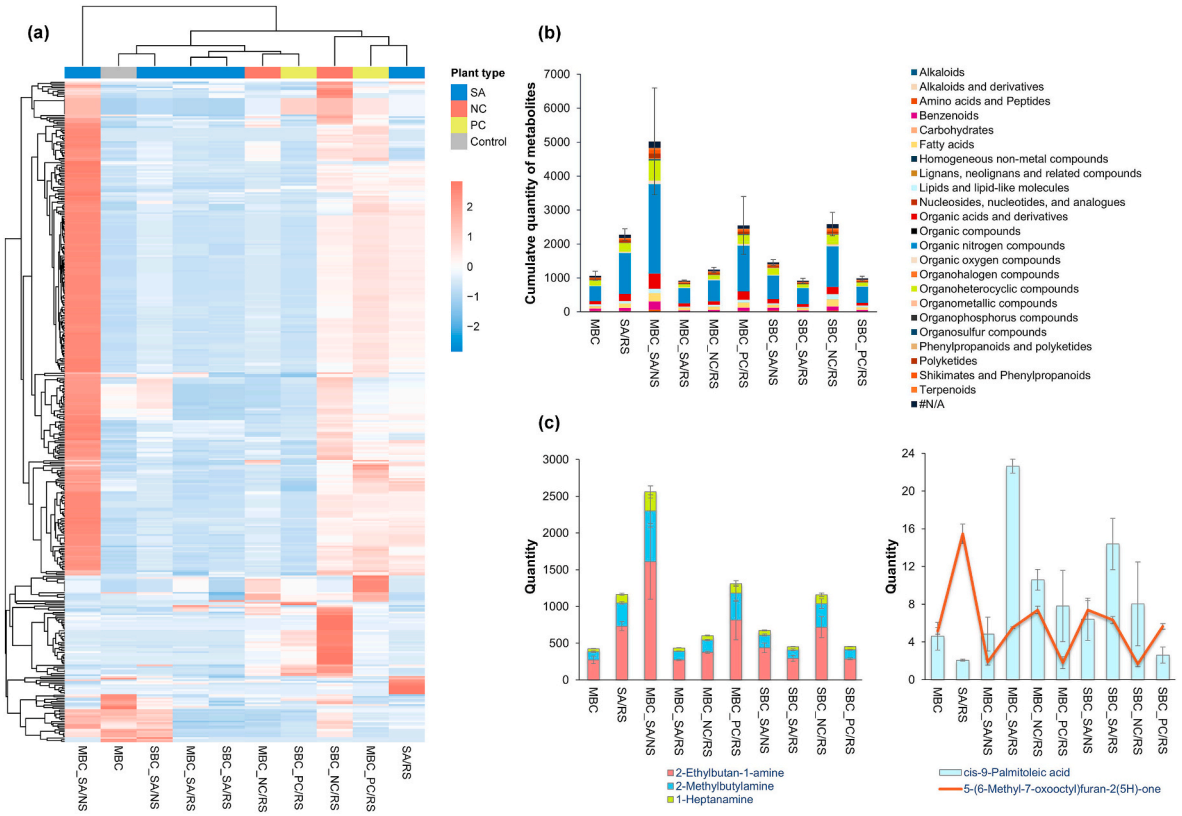

Fig. 5. Soil metabolite composition and quantities. (a) metabolite composition using top 400 abundant metabolites, (b) quantities in the super class, and (c) quantities of specific metabolites.

nitrite during ammonia oxidation.

Biochar has been shown to influence rhizosphere metabolite composition, as observed in previous studies. For example, tobacco stalk-derived biochar primarily altered the abundance of organic acids, amines, and carbohydrates in agricultural soils (Dong et al., 2024). Similarly, rice-straw biochar modified the basic metabolic processes of carbohydrates, lipids, and amino acids in saline-alkali soils (Wang et al., 2024a). In this study, both biochar types effectively reduced amine concentrations in the rhizosphere of SA-planted soils (Fig. 5(c)), though their efficacy varied for the rhizosphere of NC and PC-planted soils. Notably, MBC_SA and SBC_SA, which exhibited higher CH_4 removal efficiencies in Phase I, consistently showed lower levels of amines in the rhizosphere. Additionally, NS exhibited higher metabolite quantities than RS, as demonstrated by comparisons between MBC_SA/NS and MBC_SA/RS, as well as SBC_SA/NS and SBC_SA/RS. This pattern was primarily driven by organic nitrogen compounds, followed by organoheterocyclic compounds, organic acids and derivatives, benzenoids, and fatty acids. The observed metabolite accumulation in NS suggests slower utilization efficiency or turnover rates compared to RS, a phenomenon consistent with prior findings (Zhang et al., 2017).

4. Discussion

4.1. Biochar and plant promotion in CH_4 removal

Biochar significantly enhances microbial CH_4 oxidation in soils, with studies showing 45 % higher CH_4 reduction in SBC-amended paddy fields (Wang et al., 2023b) and improved performance in MBC-amened LCS (Wu et al., 2024). Pore volume is the most critical biochar property governing MOB composition and activity, followed by surface area and cation exchange capacity (Huang et al., 2021). MBC's greater pore volume and surface area better promote soil aeration and gas retention in LCS than SBC, explaining its superior CH_4 removal efficiency in Phase III when biochar influence dominates plant influence as discussed in section 4.4 (Huang et al., 2020). This performance difference is consistent with meta-analytic findings demonstrating biosolids-derived biochar's enhanced efficacy as an agricultural soil CH_4 sink relative to lignocellulosic-derived materials (Jeffery et al., 2016). Vegetation has also been shown to enhance CH_4 removal in LCS (Bian et al., 2019). Combined Phase I and III data revealed that both plants and biochar significantly influenced CH_4 removal efficiency ($P < 0.01$), accounting for 45 % and 38 % of the variation, respectively. However, their interaction contributed substantially less (14.50 %; $P < 0.05$), indicating that optimal synergy requires specific biochar-plant combinations.

4.2. Evolution of microbial composition

In Phase I, species richness and the Shannon index were both higher in RS than NS and non-vegetated soil, demonstrating the rhizosphere's ability to recruit a more diverse microbial community. However, the advantage of species richness in RS diminished in Phase II, suggesting that CH_4 deprivation disrupted the rhizosphere effect. By Phase III, when continuous CH_4 supply resumed, the Shannon index was higher for NS than for RS, though it showed no significant difference from non-vegetated soil. Notably, the Shannon index in all MBC-amended NS was higher than in SBC-amended NS.

As shown in Fig. 6, the rhizosphere microbial composition in Phase I was primarily clustered by plant species, with less influence from biochar type, indicating that plant factors played a more significant role in the early stages. However, the dominance of plant species in shaping rhizosphere microbial composition became less apparent in Phase II during LFG intermittence. In Phase III, microbial composition became more distinctly clustered according to biochar type. These results suggest that, in the absence of CH_4-C supply disturbances, plant-dependent rhizosphere effects primarily determine community structure. However, LFG intermittence disrupted this regulation, and biochar emerged as a

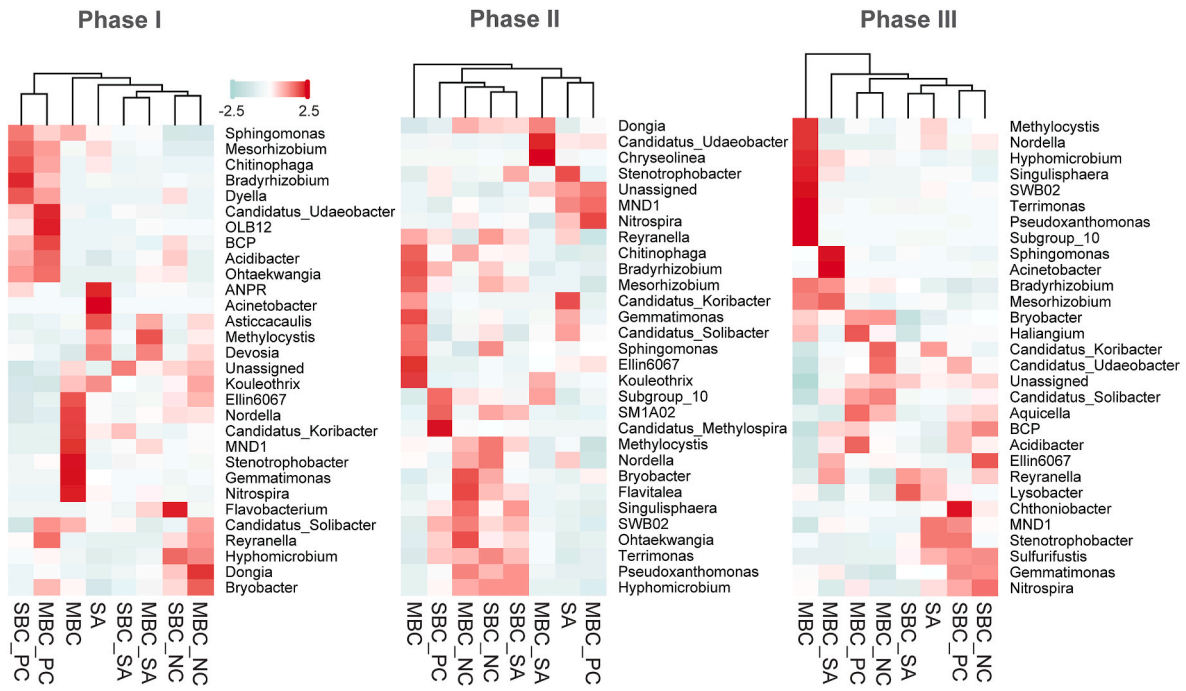

Fig. 6. Microbial cluster analysis for RS using the top 30 abundant genera in the three phases. ANPR is *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and BCP is *Burkholderia-Caballeronia-Paraburkholderia*.

dominant factor in reshaping community composition in later stages. Thus, biochar likely plays a more critical role in recovering rhizosphere methanotrophic activities in Phase III. In contrast to RS, no specific clustering pattern was observed for NS (Fig. S3).

Particularly for SA-planted soil, MBC treatment induced a more distinct microbial composition than SBC, as evidenced by comparisons among MBC_SA/RS, SBC_SA/RS, and SA/RS. This distinction was not observed in other vegetated soils, suggesting that MBC significantly altered microbial communities and activities in later stages compared to SBC.

4.3. Plant selection for rhizosphere communities in early stages

In Phase I, microbial community composition was primarily influenced by plant species rather than biochar type, supporting the notion that plants are the main drivers of core microbial communities in the rhizosphere. Plants exhibit selective recruitment in shaping rhizosphere microbial composition (Sasse et al., 2018; Hu et al., 2018), a process characterized by non-random phylogenetic conservation (Yu et al., 2021). Rhizosphere metabolites, derived from plant root exudates and microbial secretions, play a critical role in shaping microbial composition and activity (Baker et al., 2024; Zhuang et al., 2024). According to variance partitioning analysis (VPA) results in Fig. S4, plant and biochar factors explained 46.60 % and 20.90 % of the variation in rhizosphere metabolite composition, respectively, with no overlap in their explanatory power. This confirms that plants play a more decisive role than biochar in determining rhizosphere metabolite composition and early microbial community establishment.

The exudation of bioactive metabolites varies significantly among plant species (Hu et al., 2018), which likely contributes to the observed differences in rhizosphere metabolite composition. Heatmap analysis in Fig. S5 reveals that methanotrophs were negatively correlated with most soil metabolites, including the dominant amines (2-Ethylbutan-1-amine, 2-Methylbutylamine, and 1-Heptanamine). In contrast, only a few metabolites, such as cis-9-palmitoleic acid (a long-chain organic acid) and 5-(6-Methyl-7-oxooctyl)furan-2(5H)-one (a polyketide), were positively correlated with methanotrophs, though it was not significant ($P > 0.05$). Long-chain organic acids, including palmitoleic acid, exhibit significant rhizosphere recruitment capabilities and can induce disease-suppressive responses (Cao et al., 2024). Polyketides, a class of secondary metabolites, indirectly enhance plant adaptation by modifying soil nutrients and rhizosphere microflora (Zhuang et al., 2024). Both MBC_SA/RS and SBC_SA/RS exhibited relatively higher concentrations of these metabolites. Notably, biochar could significantly reduce amine concentrations (Fig. 5(c)). Given the higher CH_4 removal efficiencies of MBC_SA and SBC_SA in Phase I, the combination of biochar and SA (particularly MBC_SA) fostered a superior rhizosphere metabolite composition for supporting methanotrophic activities compared to other plant-biochar combinations in the early stage. Besides, Fig. 5(c) reveals that NS of SA planted groups presented much less MOB-favored metabolites (notably cis-9-palmitoleic acid) but more MOB-non-favored amines than RS, regardless of biochar type. The result demonstrates RS's superior metabolite profile for methanotrophic activity.

Rhizosphere metabolites also serve as accessible substrates for microbes (Pei et al., 2020), mitigating environmental stress during CH_4-C starvation in Phase II. The transport and utilization of these metabolites vary among rhizosphere microbiomes. Based on 16S functional predictions (Fig. S6), the RNA polymerase sigma-70 factor (ECF subfamily, k03088) was more abundant in all RS compared to controls. Sigma factors are core elements of RNA polymerase that direct promoter specificity and regulate transcription (Pátek et al., 2023). Additionally, ABC transporters (k01990, K01992, K02004), which are primarily involved in nutrient import and waste excretion (Seeger and van Veen, 2009; Dong et al., 2024), were consistently more abundant in biochar-amended RS than in non-amended soils. Among biochar-amended plant treatments, these transporters were most abundant in SA/RS, indicating more efficient substrate transport and utilization. However, both MBC and SBC significantly reduced rhizosphere metabolite concentrations in SA/RS (Fig. 5(b)), likely due to physical adsorption, chemisorption, and redox reactions with root exudates (Fan et al., 2023).

Biochar also influences the metabolic potential of the rhizosphere microbiome. For instance, biochar-amended soils are associated with higher bacterial diversity and enhanced utilization rates of

carbohydrates and phenolic compounds, coupled with the stimulation of bacteria capable of degrading phenolic compounds (Kolton et al., 2017). Therefore, during CH_4-C deficiency, biochar's interaction with rhizosphere metabolites reduces substrate availability to microbes. Given the larger surface area, pore volume, and ash (mineral) content of MBC (Table 1), its greater interaction with rhizosphere metabolites compared to SBC can be anticipated. This explains the lower resistance of MBC-planted groups to CH_4-C starvation in Phase II (Fig. 2). In contrast, ABC transporters were particularly abundant in PC/NS (Fig. S6), aligning with the highest resistance in CH_4 removal capacity observed for SBC_PC in Phase II. These findings collectively highlight the advantages of reduced biochar-rhizosphere metabolite interactions and efficient substrate transport and utilization in buffering the stress of CH_4-C starvation on methanotrophic activities.

4.4. Biochar dominance in shaping microbial composition and methanotrophic activities in later stages

The influence of plants on soil microbial communities began to diminish in Phase II, while the role of biochar became more pronounced in Phase III. In addition to modulating rhizosphere metabolites, biochar also shapes microbial community structure by altering soil physicochemical properties. As shown in the db-RDA results in Fig. 7(a), soil properties significantly influenced microbial community composition ($P < 0.05$). The primary constraint axes, CAP1 and CAP2, accounted for 42.84 % and 28.60 % of the constraint variance, respectively. NH_4^+ and MC were the major drivers for CAP1, while NH_4^+ and total organic carbon (TOC) were the primary drivers for CAP2. Consequently, as biochar's impact intensified in Phase III, variations in soil NH_4^+, TOC, and MC likely drove the differences in CH_4 removal efficiencies among the experimental groups.

Biochar has been widely reported to enhance soil water retention (Wei et al., 2023). In this study, SBC resulted in slightly higher MC in SA_RS and PC_RS compared to MBC, particularly in Phases I and II. This aligns with the findings of Louthan and Morris (2021), who noted that biochar derived from animal-based feedstock is less effective at improving soil water retention than biochar derived from plant-based feedstock. The optimal MC range for methanotrophic activity typically falls between 10 % and 20 % (Scheutz et al., 2009). Excessive MC can reduce soil porosity and gas transport rates, thereby inhibiting methanotrophic activity. Given that the average MC in Phase III (27 %) was significantly higher than in Phase I (19 %), the decreased mass transfer efficiency may explain the reduced CH_4 removal efficiencies observed after the resumption of continuous CH_4 supply.

When comparing TOC levels among different groups, the effects of SA and MBC alone were limited, but their combination significantly increased TOC in RS of MBC_SA. A similar trend was observed for the SBC and PC combination. The increase in TOC can be attributed to several factors. First, enhanced CH_4-C assimilation likely contributed to the higher TOC content, as substantial CH_4 was consumed in the presence of biochar (Scheutz et al., 2009). Increased soil TOC following biochar amendment has been widely documented in previous studies (Huang et al., 2025). Second, root exudates, which contain abundant and diverse metabolites, contribute to the soil carbon pool (Panchal et al., 2022). Additionally, biochar can release carbon that is utilized by microbes, thereby promoting microbial growth and increasing microbial

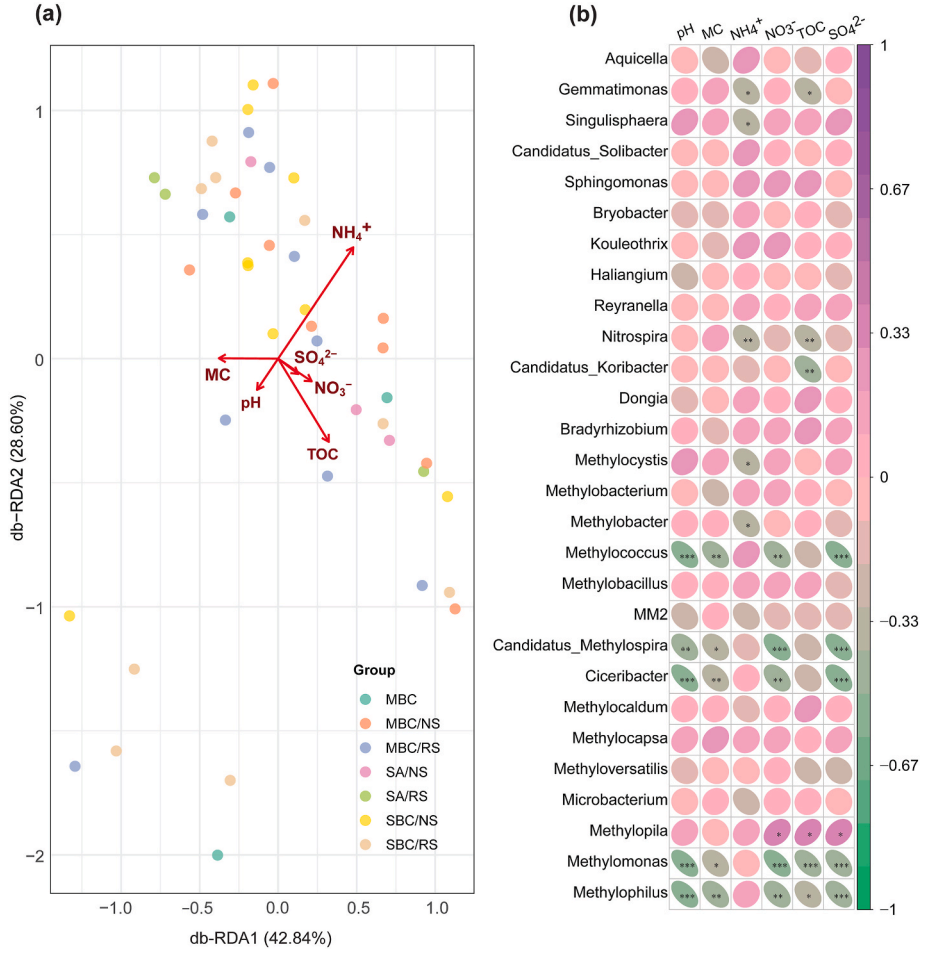

Fig. 7. Relationships between soil properties and microbial communities. (a) db-RDA results; (b) Correlation heatmap. *, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively.

biomass (Zhou et al., 2025). Although it is challenging to quantify the contributions of these different sources to TOC, MBC amendments consistently resulted in higher TOC levels than SBC amendments (Fig. S7). Elevated TOC levels support microbial activity by providing essential nutrients and energy (Fang et al., 2018). Furthermore, increased TOC content can drive microbial community assembly, facilitating functional restoration, such as methanotrophic activity (Dini-Andreote et al., 2015).

In the control MBC, NH_4^+ content exhibited a decreasing trend over the phases (Fig. S8). Biochar can reduce soluble NH_4^+ through adsorption while enhancing soil nitrification in soils with high soil organic matter (SOM) content (Gul and Whalen, 2016). In Phase I, the abundance of *Nitrospira*, a major nitrifying bacterium, was significantly higher in the upper soil of MBC/CK, confirming biochar's role in promoting nitrification during the early stages. However, the adsorption of NH_4^+ by biochar may have reduced its availability to *Nitrospira*, potentially explaining the decline in its abundance in later phases. In contrast, NH_4^+ levels increased in SA/RS in the absence of biochar. Microbes require inorganic nitrogen to assimilate labile carbon substrates in the rhizosphere. To enhance nitrogen availability, microbial activity is stimulated, leading to the production of extracellular enzymes that break down SOM and release mineral nitrogen (Ma et al., 2023).

While NO_3^- content remained similar across all groups due to inevitable plant-biochar interactions, NH_4^+ dynamics were significantly influenced by plant-biochar interactions. As shown in Table 2, MBC effectively reduced NH_4^+ levels in the RS of all plant species and NS of SA-planted soils. In contrast, SBC only reduced NH_4^+ levels in SA_RS. These results suggest that SBC's impact on NH_4^+ dynamics is more influenced by rhizosphere effects than MBC, with the least rhizosphere impact observed for MBC in SA-planted soils. According to the correlation heatmap in Fig. 7(b), the dominant methanotroph *Methylocystis* was negatively correlated with NH_4^+ content ($P < 0.05$). This inhibition is attributed to NH_4^+ competing for the methane monooxygenase enzyme site or the toxicity of intermediates (e.g., hydroxylamine or nitrite) produced during ammonia oxidation (He et al., 2019). Given its efficacy in reducing NH_4^+ levels in both RS and NS, MBC—particularly in combination with SA—is a favorable choice for engineering rhizospheres to optimize soil CH_4 oxidation capacities in the absence of CH_4-C starvation.

4.5. Implications and limitations

This study provides the first evidence of synergistic enhancement of CH_4 oxidation in LCS through biochar-vegetation co-application. Our findings establish the rhizosphere effect as the dominant driver of early-stage methanotrophic activity, while highlighting biochar's critical role in maintaining functional resilience during LFG fluctuations. Current limitations include partial soil metabolite profiling (Phase I only), leaving CH_4-C starvation (Phase II) and recovery (Phase III) dynamics uncharacterized. Future work should elucidate biochar's pathways for modulating soil metabolite composition and turnover rates, and employ isotope tracing to quantify relative contributions of plant-versus biochar-derived carbon to microbial communities. Such investigations would fully clarify the biochar-rhizosphere mechanisms shaping microbial structure and methanotrophic function.

5. Conclusions

Plants play a pivotal role in the early establishment of microbial communities, with SA-planted soils exhibiting a superior rhizosphere metabolite composition that more effectively supports CH_4 oxidation capacities compared to NC and PC-planted soils. Among the identified metabolites, palmitoleic acid and polyketides were positively correlated with methanotrophic activity, highlighting their significance in enhancing CH_4 oxidation. The resistance of microbial communities to environmental stress is primarily determined by plant species but is significantly modulated by biochar amendments. Microbial communities in both rhizosphere and non-rhizosphere soils with enhanced nutrient-importing capabilities demonstrated greater resilience to CH_4 fluctuations. However, strong interactions between biochar and root exudates can attenuate the benefits of the rhizosphere effect. Compared to MBC, SBC, which exhibits weaker interaction with root exudates, proved more advantageous in maintaining elevated CH_4 removal efficiencies under stress conditions. When environmental stress was alleviated, the recovery of methanotrophic activities was primarily driven by biochar's influence. MBC demonstrated greater efficacy than SBC in increasing TOC content and reducing NH_4^+ levels, facilitating the restoration of CH_4 removal capacities to pre-disruption levels. These findings underscore the synergistic potential of combining plants and biochar to enhance the resistance and resilience of soil microbial communities and methanotrophic activities. While soil amended with either plants or biochar alone appeared vulnerable to environmental changes, the integration of specific plant-biochar combinations (e.g., MBC_SA and SBC_PC) optimized soil microbial functions and performance. These results highlight the potential of tailored plant-biochar systems to improve the stability and efficiency of CH_4 biofiltration systems under dynamic environmental conditions.

CRediT authorship contribution statement

Rujie Zhang: Writing – original draft, Investigation, Data curation. **Jianfei Ye:** Writing – original draft, Formal analysis. **Jiahui Chen:** Visualization, Data curation. **Jiang Wu:** Methodology. **Jie Wang:** Validation. **Xinyue Bai:** Validation. **Huaihai Chen:** Data curation. **Qiyong Xu:** Writing – review & editing, Resources. **Dandan Huang:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.126348.

Data availability

Data will be made available on request.

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