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RESEARCH ARTICLE

Mineral-fungal interactions in response to biochar amendment: implications for carbon storage in saline-alkali soil

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Abstract
Background Biochar application has been widely acknowledged as an environment-friendly practice to promote soil organic carbon (SOC) stabilization and sequestration in agroecosystems. However, the interaction between fungal and minerals on organic carbon storage and stabilization with biochar application still remains unclear in saline-alkaline soil.
Methods In the present research, this interaction has been studied by following 6 years treatments at an experimental farm: i) CK, without any fertilization; ii) NPK, only mineral fertilizer; iii) BC, 8.0 Mg ha^{-1} biochar-based NPK and iv) FeBC, 8.0 Mg ha^{-1} Fe modified biochar-based NPK, respectively.
Results The results show that the relative content of illite in BC and FeBC treatments was 4.8%-5.1% higher than that in NPK treatment. Moreover, more stable OC fractions and functional groups, including particulate organic carbon (POC) and aromatic-C, were found in BC and FeBC treatments. Meanwhile, a positive relationship between illite and aromatic-C was found. The two of which might form organic-mineral complexes to decrease specific C mineralization rate. Besides, biochar application increased the diversity of soil fungal community and composition at the phylum level, such as *Ascomycota*. Redundancy analysis revealed that the content of soil POC and SOC was the major property affecting fungal diversity. Furthermore, the relative abundance of *Ascomycota* and *Basidiomycota* was positively correlated with SOC storage.
Conclusion Effects of biochar, especially Fe-modified biochar last up to six years to improve the stability and storage of SOC in saline-alkali paddy soils, which may be a better agro-management practice.

Keywords Biochar · Carbon · Clay minerals · Fungus · Paddy soils

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Introduction

The soil, as the largest carbon (C) reservoir within the terrestrial ecosystem, exchanges approximately 600 million tons of C with the atmosphere every year and absorbs about 20% of anthropogenic C emissions (Yang et al. 2021). Soil organic carbon (SOC) is not only a critical indicator to evaluate soil quality (Liu et al. 2023b; Samson et al. 2020), but also maintains the long-term sustainability of agroecosystems and global C storage. Small variations in SOC storage could have large impact on global warming (Huang et al. 2021). Previous studies have found that exogenous C addition could improve soil physicochemical properties, microbial activity, and interactions with minerals to promote SOC storage (Han et al. 2020; Hao et al. 2022; Rakhsh et al. 2017; Song et al. 2024). Therefore, further understanding of the mechanism of mineral-microbial interaction in SOC dynamics under the input of organic materials is of great significance for the mitigation of global climate change and sustainable agricultural development (Zeng et al. 2021).

Biochar is a C-rich residue produced by the thermal decomposition of organic matter in an oxygen-limited environment (Fernandez-Ugalde et al. 2017). Biochar is considered as a soil amendment that enhances SOC storage due to increasing the aromatic C content (Bi et al. 2021; Chen et al. 2018). After the application of biochar, SOC storage could be increased by promoting the combination of clay minerals with SOC (Xu et al. 2017). The adsorption of OC through the interactions between biochar and minerals will be accelerated by electrostatic interactions, hydrogen bonding, cation bridging, and reactions involving ligand exchange. Schweizer et al. (2019) found that mineral particles could affect the storage of OC in soil. Previous studies have confirmed that, compared with 1:1 clay minerals (such as kaolinite), 2:1 clay minerals (such as illite and vermiculite) could retain more OC (Chen et al. 2023c; Six et al. 2002). However, Bruun et al. (2010) assessed tropical soil respiration through different mineral chemical properties, and found that the OC stability of montmorillonite containing soil was lower than that of kaolinite containing soil. In addition, the input of exogenous C could promote the stability of soil aggregates, which provided physical protection for SOC (Kelly et al. 2017; Zhang et al. 2025). Numerous studies have found that Fe modified biochar enhances soil aggregates stability by forming complex OC-Fe associations (Han et al. 2020). However, until now, the role of Fe modified biochar on the mechanisms of SOC sequestration by changing the composition of clay minerals remains unclear.

Fungi play key role in soil biochemistry as their composition and activity are strongly related with SOC mineralization (Bello et al. 2021; Ullah et al. 2019). Generally, microbial necromass contributed greatly to OC stabilization. Liang et al. (2019) found that fungal necromass accounted for about 40% of SOC storage, whereas bacterial necromass accounted for about 20%. Besides that, fungal hyphae could provide transportation highways for soil bacteria and organic C by forming large mycelial networks (Emilia Hannula and Morriën 2022; Rudnick et al. 2015). Moreover, large amounts of fungal hyphae encapsulate soil particles and particulate organic matter (POM), which is contributing towards enhancing aggregate stability. Meanwhile, microbes could affect the formation and transformation of clay minerals (Chen et al. 2024a; Naĭmark et al. 2009), including the processes of crystallization, biomineralization and decomposition (Li et al. 2019). Nevertheless, it remains unclear how fungi drive the changes of clay mineral composition, which is crucial for C storage.

The Yellow River Delta is formed by large amounts of sediment deposits, including vast areas characterized by saline-alkali soils in which typically both soil C stability and microbial activity are limited (Chen et al. 2021). Due to soil salinization, a large amount of SOC is lost by leaching, respiration (Chen et al. 2022). Therefore, it is particularly important to evaluate the relationships between clay mineral, fungi communities and SOC storage under different fertilization managements. In present study, a 6-year field experiment was conducted to investigate the interaction between fungal and minerals on SOC storage and stabilization with biochar application. The biochar was produced by rice straw, which was added to the soil with the same amount of carbon as the local straw incorporation (about 3.6 Mg C ha^{-1}) (Liu et al. 2023a). The study aimed to: (i) assess the changing characteristics of clay mineral composition with biochar application; (ii) investigate the role of clay minerals on SOC storage and aggregate stability; (iii) reveal the mechanisms of soil mineral-fungal interactions driving SOC storage in saline alkaline paddy soil. We hypothesized that (i) biochar, especially Fe-modified biochar, can increase the C sequestration potential of soil by increasing the 2:1 clay minerals; (ii) biochar can provide C source for fungi, promote mineral-fungal interactions and then enhance the stability and sequestration of SOC in saline-alkaline paddy soil.

Materials and methods

Site description

The Yellow River Delta, one of the largest saline-alkali wetlands in China, is characterized by low SOC content with poor stability (Chen et al. 2021). The improvement of saline-alkali soil in this region is of great significance for food security and climate change regulation. Therefore, the present research has been conducted at an experimental farm located on the Yellow River Delta (Kenli county) (118°32′35″E longitude and 37°31′28″N latitude). The area is flat with an average elevation of 16.8 m. The mean annual air temperature and precipitation are 12.8℃ and 556 mm, respectively, which belongs to temperate monsoon climate. According to the FAO system, the type of soil is the Aquic Inceptisol. Before the experiment began, the land was fallow. After two years of farming, the experiment was carried out. The original soil (0–20 cm) contained 17.2% of clay, 61.7% of silt and 21.1% of silt and has an pH of 8.1, a cation exchange capacity (CEC) of 5.9 cmol kg^{-1}, and bulk density of 1.57 g cm^{-3}, a water-holding capacity of 25.6%; a total nitrogen (TN) content of 1.1 g kg^{-1}, a total phosphorus (TP) content of 0.34 g kg^{-1}, a soil organic matter content of 8.4 g kg^{-1} and an electrical conductivity (EC) of 0.41 mS cm^{-1}.

Experimental design and biochar preparation

Four treatments have been established since June 2016, i.e. 1) CK, without any fertilization; 2) NPK, chemical fertilizer with application rates if 255 kg N ha^{-1}, 128 kg P ha^{-1} and 229 kg K ha^{-1} 3) BC, NPK plus 8 Mg ha^{-1} biochar; 4) FeBC, NPK plus 8 Mg ha^{-1} Fe modified biochar. The amount of chemical fertilizer applied is the traditional amount used in the local area. All biochar was applied only once in 2016 as basic fertilizer. Biochar (BC) was produced from straw of locally harvested rice crops, which was pyrolyzed at 600 °C in a muffle furnace for 4 h. Fe modified biochar (FeBC) was also made from rice straw, which was pre-impregnated with 0.1 M $FeCl_3$ for 24 h. Subsequently, this pretreated rice straw was further pyrolyzed at 600 °C, similar to the preparation of biochar. The characteristics of BC and FeBC has been described in Table S1. Each year, urea was applied four times, in which 20% was of the total amount was applied before planting, 40% during the early tillering stage 30% during a later phase of this tillering stage, and the remaining amount during the flowering period. All the superphosphate was used as basal fertilizer. In addition, 7.5 kg ha^{-1} zinc sulfate was foliar sprayed to all treatments during the tillering stage. The area of each plot was 11.05 m^2 (4.25 m × 2.6 m). The experiment was conducted following a completely randomized design with three replicates.

Soil sampling and soil aggregate sieving

After rice harvest in October 2021, soil (0–20 cm) sampling was carried out with an auger (5 cm diameter). 5 samples per plot were combined to form one composite sample. At the laboratory the composite samples were divided into three parts: one part was stored at −80 °C for fungi analysis; one part was stored at 4 °C to determine the content of dissolved organic carbon (DOC) and microbial biomass carbon (MBC); and the remaining part was air-dried for the soil properties determination.

As described by Six et al. (2000), the wet sieving method was used to obtain the different fractions of soil aggregates. Briefly, fresh soils (200 g) were slightly separated along the natural breakpoints and placed on a set of sieves, and shook up and down in the water for 2 min (50 times). Subsequently, the four fractions of water-stable aggregates were obtained, i.e. > 2 mm, 0.25–2 mm, 0.053–0.25 mm and < 0.053 mm, respectively.

The aggregate stability expressed by mean weight diameter (MWD) was calculated based on the formula given in equation (Chen et al. 2023b):

$$MWD = \sum_{i=1}^{n} X_i Y_i$$

where X_i is the mean diameter of the four aggregate size fractions; Y_i is the mass ratio of the remaining aggregate size fractions on each sieve. Y is the total mass of all size fractions of the aggregate.

i = 1, 2, 3, 4, represent the aggregate size > 2.0 mm, 0.25—2.0 mm, 0.053—0.25 mm, and < 0.053 mm, respectively.

Soil analyses

Soil EC and pH were determined using a glass electrode and pH meter (soil: distilled water = 1:5). SOC was extracted using H_2SO_4-$K_2Cr_2O_7$. TP was digested with H_2SO_4-$HClO_4$. Soil available P (AP) was extract with 0.5 M $NaHCO_3$ (Olsen 1954). DOC was determined using TOC analyzer (TOC-Vario, Elementar, Langenselbold, Germany). Particulate organic carbon (POC) was measured based on the revised method of Yuan et al. (2020): soil sample (10 g) was dispersed with hexametaphosphate solution (5 g L^{-1}) by shaking for 16 h (100 r min^{-1}), and sieved at 0.053 mm. The POC content was then determined using the same methodology as the SOC. The method of chloroform fumigation incubation was used to quantify MBC (Vance et al. 1987).

Preparation of soil colloids and clay minerals analyses

Soil colloids were extracted with the natural sedimentation and centrifugation methods (Gimbert et al. 2005; Zhang et al. 2021). Briefly, we used 0.2 M hydrochloric acid to remove carbonates from soil colloids, and then used 10% H_2O_2 to remove organic matter. And the dithionite-citrate-bicarbonate method was used to remove iron oxides. The clay fraction was extracted using a siphon tube and dried under an infrared light at 50℃ for further analysis.

Mg-glycerol and K were used to saturate soil colloids. The preparation method of Mg-glycerol saturated sample was as follows: dissolved the sample twice in a 0.5 M $MgCl_2$ solution, then added a 5% glycerol solution, centrifuged, and discarded the supernatant. The samples were stored in a desiccator containing saturated Ca $(NO_3)_2$ at room temperature. The soil colloids sample was stirred three times with KCl (1.0 M) solution, and the K-saturated oriented flakes were heated at 25℃ (K-25), 300℃ (K-300) and 550℃ (K-550) for 2 h, respectively.

To identify clay minerals, X-ray diffraction (XRD, D8ADVANCE, Bruker, Germany) was utilized. JADE 6.5 software was used to analyze the XRD results. The proportion (%) of clay minerals was calculated through the relative intensity of their diagnostic diffraction peaks (Zhou et al. 2018).

Fourier-transform infrared spectra analyses

As described by Bernier et al. (2013a), fourier-transform infrared spectra (FTIR) was used to analyze OC functional groups in soil samples with a Nicolet 6700 FTIR spectrometer (Thermo Scientific, Pittsburg, PA, USA). Before analysis, the soil samples were finely ground and dried at 105℃ to minimize the interference of moisture on FTIR spectrum, to subsequently being ground with KBr powder in an agate mortar. The spectrum records were scanned 32 times in average. The wavelength range was 400–4000 cm^{-1} considering a 4 cm^{-1} resolution. The semi-quantitative analysis of functional groups was achieved according to Zhu et al. (2016) and Szymański (2017). The data of FTIR absorption region was processed using OMSNIC 8.0 spectrometer software.

CO_2 emission experiment

Soil incubation experiment (60 days) was carried out to quantify CO_2 emission from the soil. Fresh soil (20 g dry weight equivalent) was adjusted to 60% moisture content and placed in a 500 ml jar. Each sample was replicated 4 times, and non-soil treatment was taken as control. Before the incubation test, soil microbial activity was restored by pre-incubation at 25℃ for 7 days. Briefly, 20 ml NaOH (0.5 M) trap was precipitated with $BaCl_2$ solution and then titrated with 0.1 M HCl to calculate CO_2 emissions. The CO_2 measurements were caried-out on days 1, 3, 5, 7, 9, 11, 13, 15, 20, 25, 30, 40 and 60 of the experiment (Huang et al. 2016).

The SOC storage (0–20 cm) was calculated by the method described by Chen et al. (2022):

SOC storage (Mg C ha^{-1}) = SOC (g kg^{-1}) × BD (g cm^{-3}) × D (0.2 m) × 10.

The C mineralization rate (CMR) and the specific C mineralization rate (SCMR) were obtained using the following equation (Chen et al. 2022):

$$\mathrm{CMR} = \frac{\text{total amount of CO2}}{\text{time}}$$

$$\mathrm{SCMR} = \frac{\mathrm{CMR}}{\text{SOC content}}$$

Fungal community characterization

The Powersoil® DNA Isolation Kit was used to extract DNA from 0.5 g soil samples. The fungal internal transcribed spacer (ITS) was sequenced by specific ITS 1F/2R primer set (White et al. 1990). Generally, PCR contained 1 μL of each primer (5 μM), 0.25 μL of TransStart Fastpfu DNA Polymerase, 2 μL of dNTPs (2.5 mM), 4 μL of 5 × Reaction Buffer and 10 ng DNA Template. PCR initial denaturation was performed at 98℃ for 2 min, at 98℃ for 15 s, at 50℃ for 30 s, at 72℃ for 30 s, and at 72℃ for 5 min. Illumina high throughput sequencing platform of Majorbio (China) was used to sequence.

Date analysis

Before analyzing the data, we checked the normality and homogeneity of variance of the data. A one-way ANOVA was used to evaluate the difference in soil properties, organic C functional groups, clay mineral composition and soil fungus community. Significance among treatments were tested by least significant difference (LSD) at $p < 0.05$. Redundancy analysis (RDA) was carried out using Canoco 5 in order to evaluate the relationship between soil physicochemical properties and fungus community. All figures were performed using Origin 2021 software.

Results

Physical and chemical properties of soil

Compared with NPK treatment, soil pH was higher than that in the two types of biochar application treatments. The content of SOC was 7.7 g C kg^{-1} in FeBC treatment, which was 26.2% higher than that in NPK treatments ($p < 0.05$). There was no significant difference between BC and FeBC treatment. Compared with NPK treatment, soil AP content was 28.1% higher in the FeBC treatment. Compared with NPK treatments, TN content was also higher with biochar addition. And compared with NPK, the content of POC and MBC was increased by 50.0% and 20.0% in FeBC treatments, respectively (Table 1).

Soil organic C functional groups

Four main functional groups of OC were found on the soil surface: polysaccharide-C, Aliphatic C, Aromatic-C, and Carboxyl or Amidogen-C (Table 2). The proportion of polysaccharide-C was 37.2%–41.1%. Compared with NPK treatment, the relative content of Aromatic-C was about 13.9% greater with biochar addition ($p < 0.05$). Moreover, the Aromatic-C content in FeBC treatment was 24.3% higher than that in NPK treatment. Aliphatic-C was also increased in FeBC treatment relative to NPK treatment. While the proportion of Carboxyl or Amidogen-C was reduced with the application of biochar.

Table 1 Effects of different fertilizer regimes on soil basic physicochemical properties

Soil properties	Treatments			
	CK	NPK	BC	FeBC
pH	8.74 ± 0.06a	8.61 ± 0.25a	8.47 ± 0.26a	8.49 ± 0.12a
EC (mS cm^{-1})	0.33 ± 0.02a	0.31 ± 0.03ab	0.28 ± 0.02b	0.28 ± 0.02b
SOC (g kg^{-1})	5.6 ± 0.5c	6.1 ± 0.5b	7.1 ± 1.0ab	7.7 ± 0.9a
AP (mg kg^{-1})	6.5 ± 0.7d	19.7 ± 1.9c	23.6 ± 2.5b	25.2 ± 2.3a
TP (g kg^{-1})	0.2 ± 0.03c	0.7 ± 0.03b	0.8 ± 0.02ab	0.8 ± 0.07a
TN (g kg^{-1})	0.6 ± 0.07b	0.7 ± 0.09ab	0.7 ± 0.04a	0.8 ± 0.05a
POC (g kg^{-1})	0.7 ± 0.0c	1.0 ± 0.1b	1.3 ± 0.1a	1.5 ± 0.1a
MBC (mg kg^{-1})	71.5 ± 2.7b	178.9 ± 6.5a	197.5 ± 13.5a	214.6 ± 14.1a

Values are means ± standard deviation ($n = 3$). Different lower-case letters show significant differences between treatments ($p < 0.05$). Abbreviations: *EC* Electrical conductivity; *SOC* Soil organic carbon; *TP* Total phosphorus; *AP* Available phosphorus; *POC* Particulate organic carbon; *MBC* Microbial biomass carbon; *Feo* Fe oxides

Table 2 Effects of different fertilizer regimes on soil organic carbon functional group content

Treatment	Organic C function group (%)			
	Polysaccharide-C	Aliphatic-C	Aromatic-C	Carboxyl or Amidogen-C
CK	37.2 ± 1.5c	32.4 ± 0.5b	10.3 ± 0.7d	20.1 ± 1.5a
NPK	38.6 ± 0.7bc	32.7 ± 0.8ab	11.5 ± 0.7c	17.2 ± 2.1b
BC	40.6 ± 0.8ab	33.3 ± 1.2ab	13.1 ± 0.7b	13.0 ± 1.3c
FeBC	41.1 ± 1.4a	34.1 ± 0.4a	14.3 ± 0.3a	10.5 ± 0.7c

Values are means ± standard deviation ($n = 3$). Different lower-case letters show significant differences between treatments ($p < 0.05$)

Analyses of dominant clay mineral in soil

Vermiculite, illite and kaolinite were found as the dominant minerals in soil (Fig. 1). Compared with NPK treatment, the proportion of illite was increased by 19.4% and 14.7% in FeBC and BC treatments, respectively. While no significant differences were observed in the proportion of vermiculite among those four treatments. In comparison to NPK treatment, the proportion of kaolinite in BC

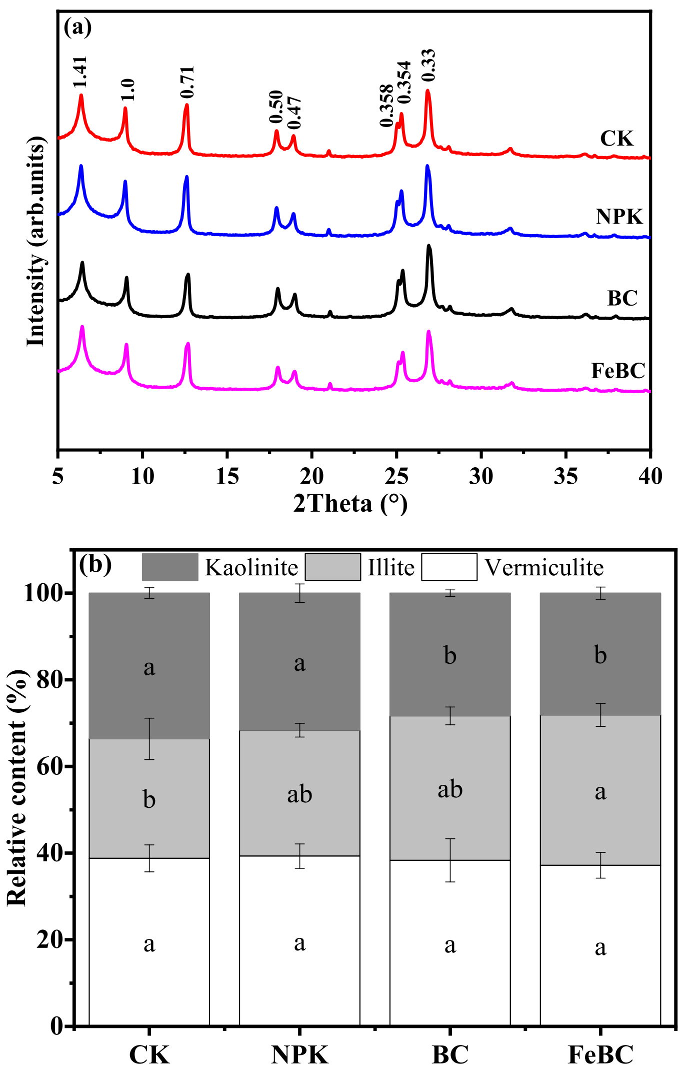

Fig. 1 The clay minerals analysis results using XRD in four fertilization treatments. Note: Values are means ± standard deviation ($n = 3$). Different lower-case letters show significant differences between treatments ($p < 0.05$). Abbreviations: CK, no fertilization; NPK, application of mineral fertilizer; BC, mineral fertilizer application combined with biochar; FeBC, mineral fertilizer application combined with biochar

and FeBC treatment was significantly decreased by 10.4% and 11.1%, respectively ($p < 0.05$).

SCMR and SOC storage

The application of biochar and Fe modified biochar could significantly reduce SCMR, which was decreased by more than 51.1% with biochar addition, compared with NPK treatment (Fig. 2a). SOC storage was increased with biochar application, in which it was 15.7% higher in FeBC treatment, compared with NPK treatment, and the order of SOC storage was CK < NPK < BC < FeBC (Fig. 2b).

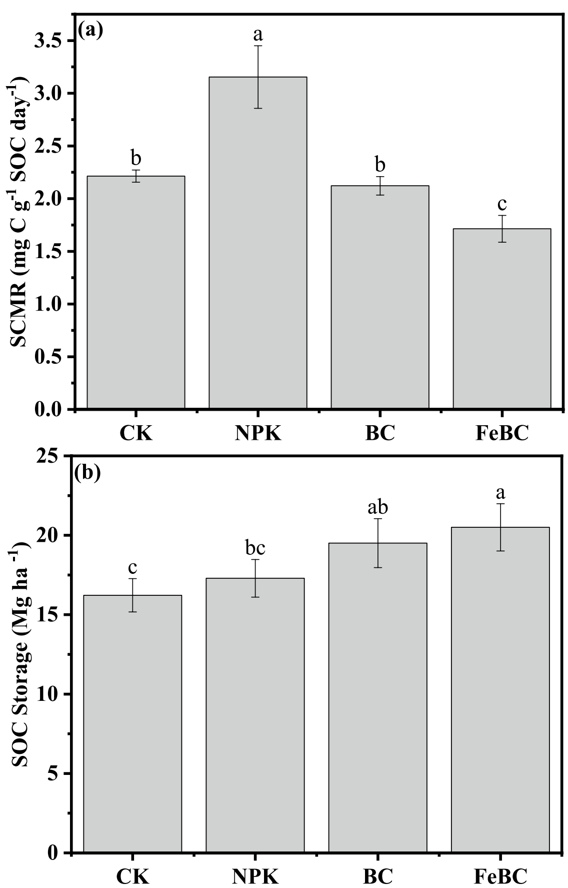

Fig. 2 The SCMR (**a**) during the 60-day incubation period and SOC storage (**b**) as affected by different fertilizer treatments. Note: Values are means ± standard deviation ($n=3$). Different lower-case letters show significant differences between treatments ($p < 0.05$)

Fungus community

Compared with NPK, the Chao1 (Fig. 3a), Evenness (Fig. 3b), Shannon (Fig. 3c), and Simpson index of soil fungus communities were increased in FeBC treatment. *Ascomycota* was the dominant phyla of fungi, the relative abundance of which was improved by more than 6.3% in the two treatments with biochar amendments. Furthermore, the content of *Sordariomycetes* was increased with biochar addition, which was increased by more than 4%, relative to NPK (Fig. 3f). However, compared with NPK treatment, the relative abundance of *Basidiomycota*, *Chytridiomycota* and *Glomeromycota* was reduced across the three different fertilization treatments (Fig. 3e).

Correlation analysis

SOC stock was found to be positively correlated with MWD, illite, Aliphatic-C and Aromatic-C (Table S2). Besides that, illite was also positively correlated with SOC stock ($p < 0.05$). While the proportion of Carboxyl or Amidogen-C showed a negative relationship with SOC stock, and the same relationship was found between kaolinite and SOC stock ($r = -0.779$, $p < 0.05$).

RDA results showed that the composition of soil fungal community was positively correlated with POC ($F = 6.3$, $p = 0.002$) and SOC contents ($F = 4.9$, $p = 0.002$) (Fig. 5). The results revealed that POC explained 38.5% of the total variable. The Linear and Pearson correlation analysis showed that SOC and POC were positively related to *Ascomycota* (Table S3). The composition of soil fungal community, especially *Ascomycota*, showed a strong positive correlation with MWD (Figs. 5 and S3), whereas soil pH and EC were negatively correlated with soil fungal community composition.

Discussion

The changes of clay mineral composition with biochar application

Clay minerals are widely distributed across soil colloids, which are the key factors affecting soil physicochemical properties (Chen et al. 2024b; Zhang et al. 2018). Based on XRD analysis, vermiculite, illite and

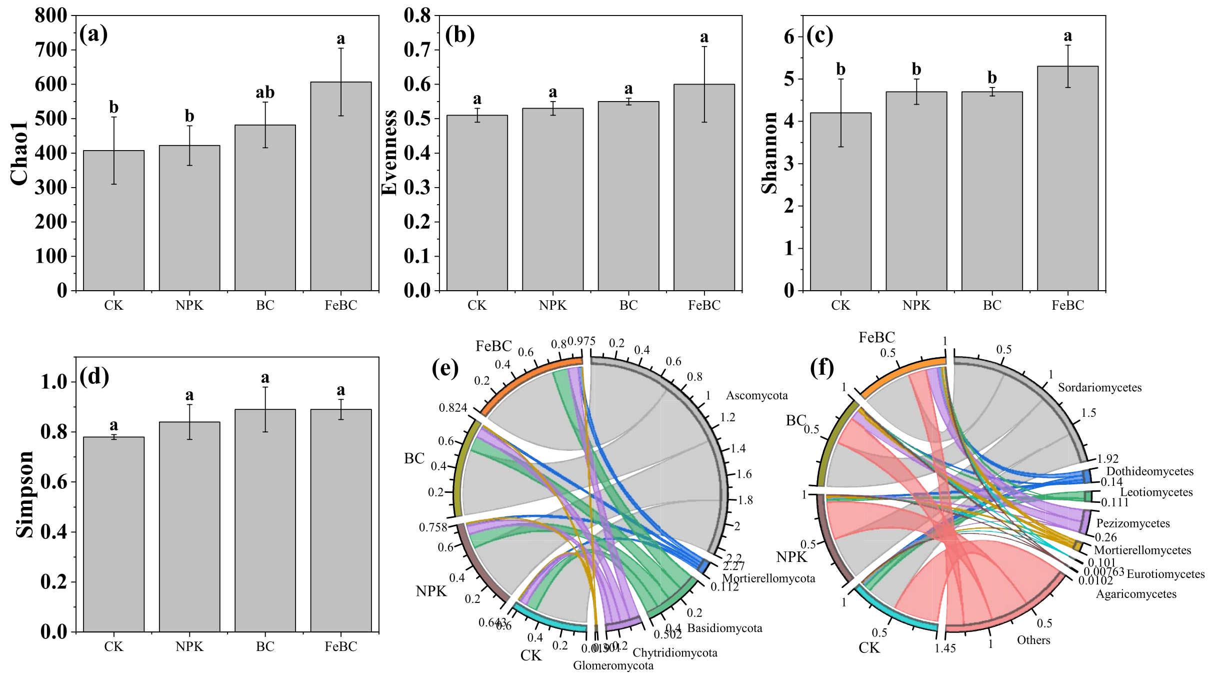

Fig. 3 The fungus community Chao1 (**a**), Evenness (**b**), Shannon (**c**), Simpson (**d**) index, and the chord diagram shown the relative abundance of fungal community at the phylum (**e**) and class level (**f**) in the four treatments. Note: Values are means ± standard deviation ($n = 3$). Different lower-case letters show significant differences between treatments ($p < 0.05$)

kaolinite were the dominant clay minerals (Fig. 1), which was consistent with previous studies who reported that vermiculite, kaolinite and illite were the main clay minerals in north China (Chen et al. 2023b; Zhang et al. 2016). This was determined by soil parent material and climate conditions. Meanwhile, Jing et al. (2022) demonstrated that biochar addition could promote the change of clay mineral composition. As such, also in present research, the relative content of illite and vermiculite in biochar applied treatments did increase, while the opposite trend was found for kaolinite (Fig. 1). This might be the consequence of increased K^+ with biochar application which could increase the 2:1 type of clay minerals in saline-alkali soil (Andrade et al. 2020; Chen et al. 2023b).

In addition, the proportion of clay minerals is frequently affected by the redox condition in paddy soils (Jia et al. 2014), such as the oxidation and reduction processes of Fe (Andrade et al. 2014). In our study, the content of illite was increased with Fe modified biochar addition (Fig. 1), which might be due to the fact that: (1) biochar could act as an electron acceptor to promote Fe reduction in paddy soil; (2) more Fe (III) in Fe modified biochar which could also enhance the reduction of Fe, and hence, increasing the layer charge and promoting the transformation of clay mineral.

Response of SOC stability to clay mineral transformation

Clay particles, as an abiotic factor, affect the microbial decomposition rate of OC (Kleber et al. 2015). Compared with kaolinite, illite and vermiculite have larger specific surface area and CEC, which leads to the stronger interaction between SOC and clay, thereby promoting the stabilization of SOC (Rakhsh et al. 2017). The present research found that compared to NPK treatment, the SCMR in BC and FeBC treatments was reduced (Fig. 2a), which was negatively correlated with illite (Fig. 4a). This indicates that the legacy effect of biochar addition after 6 years could increase the stability of SOC in saline-alkali soil by increasing the proportion of illite.

The Fe^{3+} provided by Fe modified biochar acts as a bridge to neutralize the negative charges and organic

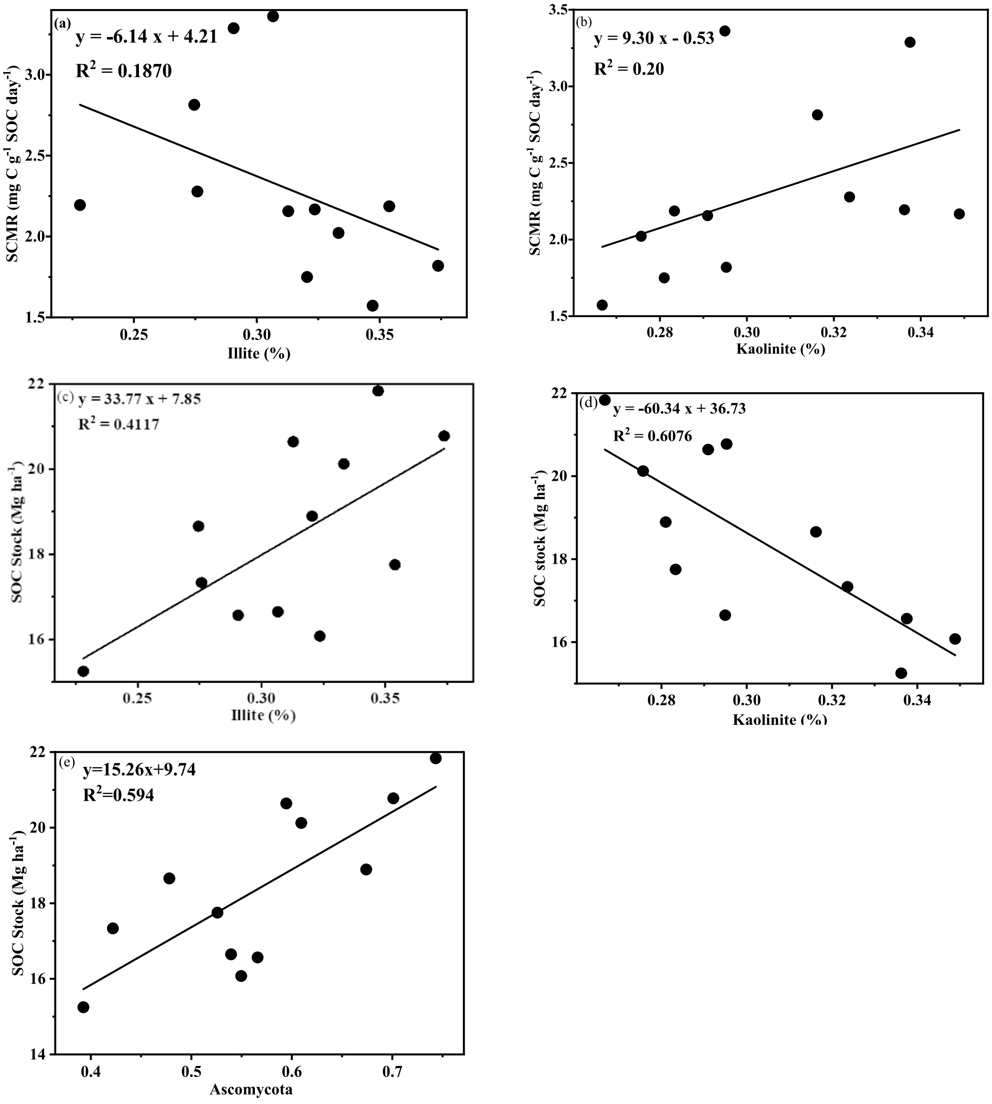

Fig. 4 Correlation analysis between illite and SCMR (**a**), kaolinite and SCMR (**b**), illite and SOC stoock (**c**), kaolinite and SOC stock (**d**) Asomycota and SOC Stock in soil

anions on the clay surface, and links organic biopolymers (such as aromatic molecules) to the clay surface (Kaiser et al. 2012). Illite was positively correlated with aromatic-C (Table S2), which was the main reason why clay minerals preferentially combined with aromatics compounds to form organic-mineral complexes reducing SCMR (Lanson et al. 2015; Xue et al. 2022). Meanwhile, SOC could be protected by soil aggregates (Chen et al. 2021). Chen et al. (2022) found that clay minerals could promote the formation of water stable aggregates by increasing the physical retention of SOC. In the present study, MWD was increased in FeBC treatments (Fig. S1b). This might be because illite, as the main binder, promoted the stability of aggregates (Chen et al. 2023b; Hu et al. 2021).

The role of microbial communities on SOC storage

Biochar has the physical characteristics of high porosity and large surface area, which could improve the aeration and water retention of soil, which on its

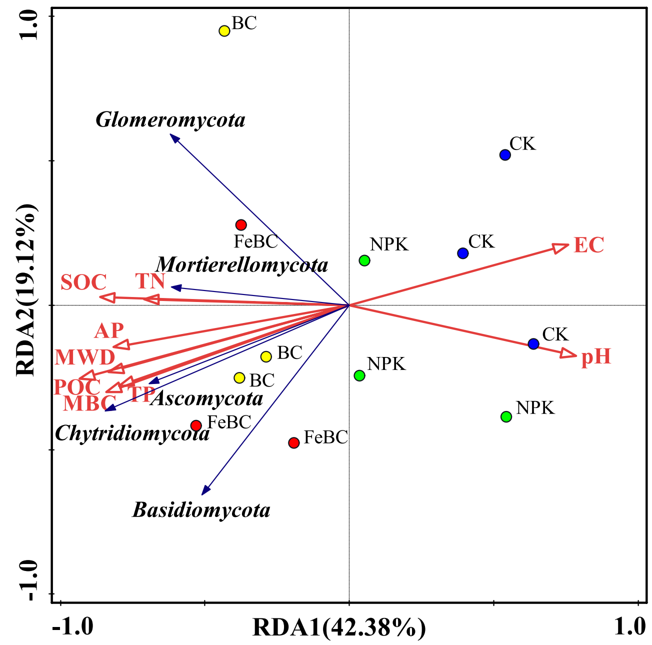

Fig. 5 Redundancy analysis (RDA) between soil basic physicochemical properties and soil fungus community compositions in phylum level

turn could provide better habitat for soil fungi (Jones et al. 2012; Thies and Rilling 2009). In this study, the application of biochar increased the diversity of soil fungi (Fig. 3), which was consistent with Yao et al. (2017) who found that biochar addition could promote fungal growth. This could be the consequence of a fungal hyphae colonization in biochar pores, increasing fungal diversity (Bernier et al. 2013b; Yan et al. 2021). Furthermore, SOC was the main determinant of fungal community composition (Zhalnina et al. 2015), which was increased with biochar addition, suggesting that biochar addition could increase fungal diversity by providing available C sources. In this respect it is important to note that biochar is rich in cellulose, and that the decomposition rate of cellulose determines the increase or decrease of SOC (Chen et al. 2023a; Wu et al. 2021). It was found that *Ascomycota* was the most dominant phylum with biochar addition (Fig. 3), which was positively related with SOC storage from RDA results (Figs. 5 and S3). This might be the consequence of *Ascomycota* was rich in hemicellulolytic and cellulolytic fungal communities, which could lead to the decomposition of recalcitrant lignocellulose in biochar, thereby increasing SOC stock (Couturier et al. 2016; Ge et al. 2021).

The mineralization and sequestration of SOC were affected by fungal diversity, composition and activity (Emilia Hannula and Morriën 2022). Fungi could promote the stability of aggregates through mycelial entanglement, which provided physical protection for OC (Rosenzweig et al. 2018). In our results, in comparison to NPK, the relative abundance of *Chytridiomycota* did increase with biochar addition (Fig. 3), which could produce rhizoid, and hence, provide more POC to the soil (Couturier et al. 2016). POC and MWD were positively correlated with *Chytridiomycota* (Fig. 5), indicating that fungi might transport organic matter to form soil aggregates, where organic matter bond with minerals, thereby improving the stability of SOC (Guhra et al. 2022). In addition, clay minerals are beneficial for microbial growth (Uroz et al. 2015), and protect microorganism from adverse environment conditions. On the other hand, due to the filamentous growth habit of fungi and the secretion of organic acids and protons (Rudnick et al. 2015), it was highly suitable as a major bioconversion agent (Fomina et al. 2005). Burford et al. (2018) found that the oxalicacid-producing fungi could promote the formation of 2:1 clay mineral, which was consistent with the finding from our study illustrating that illite was positively correlated with fungi (Table S3). Moreover, the illite was increased with biochar application (Fig. 1), which was also positively correlated with SOC storage (Fig. 4). Hence, biochar, especially Fe modified biochar, could change the composition of clay minerals by providing a C source for fungal growth, thereby increasing SOC sequestration in saline-alkali paddy soil.

Although we have explored the mineral-fungal mechanism, there are still some limitations. For example, we have only explored fungi in terms of microorganisms, and have not explored bacteria, archaea, etc. And, we still lack direct evidence to identify the mechanism of mineral-fungal interaction on carbon sequestration in saline-alkali soils. These limitations may be the focus of future research. In addition, the indexes of OC fractions (POC, MBC) and functional groups showed no significant difference between BC and FeBC treatments. However, the relatively stable aromatic-C was increased significantly in FeBC compared with BC treatment, and we noticed that SCMR was decreased significantly in FeBC. Therefore, biochar, especially modified biochar, might be a better agro-management practice to improve the stabilization and storage of SOC in saline-alkaline paddy soils.

Conclusion

After six-year of biochar application, the relative content of kaolinite did decrease, while the relative content of illite did increase, which indicated that the composition of clay minerals was changed by biochar application in saline-alkaline soil. Meanwhile, the proportion of aromatic-C was also increased with biochar addition, which could form organic-mineral complexes with illite. As such soil organic carbon (SOC) tended to stabilize with a lower specific C mineralization rate. In addition, Fe modified biochar addition increased fungal diversity, which was mainly affected by SOC. Compared with NPK treatment, the relative abundance of *Ascomycota* and *Basidiomycota* was increased with biochar addition, which was also positively correlated with SOC storage. Consequently, biochar, especially Fe modified biochar, could change the composition of clay minerals by providing a C source for fungal growth, thereby improving the stabilization and sequestration of SOC in saline-alkali paddy soil. Overall, our study found that the effect of modified biochar on saline-alkali soil improvement could last for 6 years, which is of great significance for mitigating global climate change and guiding saline-alkali soil improvement.

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Data availability Data will be made available on request.

Declarations

Competing interests There have no competing financial interests or personal relationships in this study.

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