



Changes in bacterial community structure and carbon metabolism in sandy soil under the long-term application of chitin-rich organic material and attapulgite

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ABSTRACT

Continuous expansion of sandy soil deeply threatens crop security and has become one of the main challenges facing modern society. Chitin-rich organic material and attapulgite have been considered efficient materials to improve degraded soil based on their rich nutrient contents and unique structures. However, their potential effects on microbial community and functions in sandy soil are poorly understood. Therefore, we conducted a 4-year field experiment featuring amendment with chitin-rich organic material and attapulgite and investigated the effect of amendments on microbial community diversity, structure and function using amplicons (16S and ITS) and metagenomic sequencing. The four treatments were as follows: (1) CK: no soil amendment; (2) SA: CK + attapulgite amendment; (3) SC: CK + chitin-rich organic material; and (4) SCA: CK + chitin-rich organic material + attapulgite amendment. The results indicated that the concentrations of soil organic carbon (SOC), total nitrogen, available phosphate (AP), available potassium, and enzyme activities increased while pH decreased in the treatments with chitin-rich organic material application (SC and SCA). Microorganisms have different responses to different soil amendments. Bacteria are the main components of microorganisms and their community structure was altered under chitin-rich organic material treatments (SC and SCA). Furthermore, 14 OTUs belonging to five phyla (*Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Gemmatimonadota*, and *Patescibacteria*) related to the decomposition of organic matter were enriched in SC, while 22 OTUs belonging to six phyla (*Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Gemmatimonadota*, *Patescibacteria*, and *Cyanobacteria*) were enriched significantly in SCA, suggesting that the combination of the two amendments had the potential to further alter the bacterial community compared with a single amendment. The metagenomic analysis revealed a decrease in the relative abundance of genes involved in carbon metabolism in SCA, with soil pH, AP, enzyme activity, SOC, and bacterial community structure identified as primary influencing factors. In conclusion, we conducted a comprehensive analysis of the effects of chitin-rich organic material and attapulgite on microorganisms in sandy soil and found a robust link between soil properties, bacterial community structure, and microbial carbon metabolism function.

1. Introduction

The global area of sandy soil is approximately 5 billion ha worldwide, representing approximately 31 % of the total land area (Huang and Hartemink, 2020). China, the world's largest country in terms of food production and demand, is facing the challenges of continuous expansion of croplands across sandy soil. This deeply influences crop

security because sandy soils have low nutrient levels and poor water storage capacity. Traditionally, frequent fertilization has been a routine method of continuing crop production in sandy soils (Wu et al., 2022). However, excessive or unreasonable application of fertilizers leads to more serious soil degradation (Zhang et al., 2022) and ultimately inhibits crop growth.

Applying organic materials has been recommended for ameliorating

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sandy soil due to their capability to enhance the concentrations of soil organic carbon (SOC) and total nitrogen (TN) (Luan et al., 2019; Amdou et al., 2021) and thereby increase crop yields (Bonanomi et al., 2020). Chitin, a natural polysaccharide purified from the waste of shrimp heads and crab shells, has been widely used in agriculture (Rkhaila et al., 2021). Chitin-rich organic material has a lower carbon-to-nitrogen ratio (approximately 6–7) than conventional soil amendments (Niaz et al., 2022; Soria et al., 2023), and hence, it can be rapidly utilized by plants (Xue et al., 2018; Shamshina et al., 2020), promoting their growth (Aklog et al., 2016; Winkler et al., 2017) and increasing their yields (Sharp, 2013). Additionally, chitin-rich material application has been shown to directly regulate the soil nutrient content and thus improve soil fertility. For example, Zhan et al. (2021) reported that chitin fertilizer combined with Selenium addition increased the content of alkali-hydrolyzable nitrogen in red acid soil and thus enhanced soil nitrogen supply capacity. Similarly, studies conducted in saline-alkali soil in China have confirmed that chitin-rich organic material application effectively increased the content of SOC and the carbon pool management index (Ji et al., 2019). However, low clay content in sandy soil prevents SOC accumulation and nutrient retention. Thus, whether chitin-rich organic material has exceptional performance in sandy soil for SOC and nutrient improvement is still unknown.

Water holding capacity, which is low in sandy soil, affects not only nutrient movement, but also microbial capability to transform organic material into stable organic matter. Attapulgite, as a mineral colloid, has a large specific surface area and a high density of pores (Rafiq et al., 2017). Previous research has claimed that the application of attapulgite to sandy soil can improve the soil structure, promote the formation of macroaggregates (>0.25 mm), and increase soil water holding capacity (Yang et al., 2020). Yang et al. (2022) demonstrated that attapulgite addition greatly improves soil water holding capacity and the available water content. Jiang et al. (2015) noted that remediating fertilizer containing attapulgite effectively improved soil water retaining capacity due to the hydrogen bonds between the fertilizer and water.

Soil microorganisms are of crucial importance in the agricultural ecosystem, and they are very sensitive to changes in soil quality. Contradictory findings have been observed in previous research concerning changes in soil microbial diversity under chitin material application. Randall et al. (2020) indicated that the utilization of chitin amendment to sandy clay loam considerably increased the Shannon diversity indices for bacterial and fungal taxa. In contrast, as reported by Andreo-Jimenez (2021), chitin-rich amendment application in two types of sandy soil (pH 7.2 and 5.5) resulted in a decrease and an inversion of Shannon and Simpson's indexes. Possible reasons for the contradictory findings may involve the differences in soil physicochemical properties such as the C/N ratio, which presumably allowed different types of microbes to proliferate. Moreover, chitin-based amendment could enhance the growth of specific microbe populations. Zhan et al. (2021) found that co-incorporation of chitin fertilizer and Se to red soil resulted in the change of microbial community and its activities. Cretoiu et al. (2014) showed that *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes* underwent large and significant increases in chitin-amended sandy soil. Similarly, some studies have demonstrated that chitin amendment led to increases in *β-Proteobacteria*, *γ-Proteobacteria*, *Planctomycetes*, *Acidobacteria*, *Verrucomicrobia*, *Actinobacteria*, and *Bacteroidetes*, which are involved in plant growth and the nutrient cycle (Wieczorek et al., 2014; Debode et al., 2016). Therefore, chitin-rich material application may increase the growth of beneficial microorganisms and improve soil fertility. Close to the application of chitin-rich material, the application of attapulgite has also resulted in different findings concerning the changes in the microbial community. As shown in Liang et al. (2020), the addition of attapulgite altered microbial diversity and richness as well as the abundance of bacteria such as *Clostridiales*, *Syntrophobacteriales* and *Methanomicrobiales*. Rafiq et al. (2017) found that attapulgite biochar could strengthen the abundance of beneficial microorganisms, reduce nutrient leaching, and make better nutrient cycling. However, other

studies suggested that the composition of bacterial phyla and species diversity were unchanged (Cao et al., 2022). Overall, research tend to show that chitin-rich material and attapulgite application alone may alter the microbial community and enhance specific species of beneficial microorganisms. However, because of differences in soil types and material properties, it is still unknown whether both amendments can achieve similar results in sandy soil when applied alone or in combination.

Meanwhile, shifts in the microbial community may influence microbial function accordingly under amendment application (Fierer et al., 2013). Based on metagenomics, previous studies have reported that the addition of organic material can induce significant shifts in carbon metabolism such as C degradation and fixation (Hu et al., 2022). Similar to organic materials, the application of chitin-rich material can regulate soil function, especially related to nutrient cycling. For instance, De Tender et al. (2019) found that chitin addition increased enzyme activities and bacterial ammonium oxidation and influenced the N cycle. Tang et al. (2022) also reported that genes involved in phosphorus transport were enhanced by the application of chitin fertilizer, which means chitin fertilizer can immobilize more phosphorus and regulate the phosphorus cycle in agroecosystems. Furthermore, attapulgite can improve soil structure and enhance the water-holding capacity of the soil. These changes are beneficial for microbial activity and nutrient transport, and they may have an impact on microbial function. However, it is unclear on how microbial function, responds to attapulgite application, either alone or in combination with chitin-rich organic material in sandy soil.

Hence, we selected the sandy soil of the Huang-Huai-Hai plain as a representative system, and a 4-year field experiment was conducted in this area, where chitin-rich organic material and attapulgite were applied as amendments. The main objectives of the study were (i) to evaluate the long-term effects of chitin-rich organic material or/and attapulgite amendment application on microbial community diversity, structure and function, and (ii) to explore how chitin-rich organic material or/and attapulgite application regulates microbial community composition and function via shifting SOC and nutrients in sandy soil.

2. Materials and methods

2.1. Chitin-rich organic material and attapulgite

Chitin-rich organic material was produced in the laboratory. Chitin-rich organic material included shrimp heads and crab shells, peat, straw, and peanut shells with a weight ratio of 0.5:1.5:1:1, respectively. All organic materials mix thoroughly and was composted aerobically under high temperature. Attapulgite was produced from Hengyuan New Materials Co., Lt. The basic properties of the amendments are listed in Table 1.

2.2. Study site, field experiments, and soil sampling

A 4-year (2015–2019) field experiment was conducted in Langfang, Hebei Province, China (39.36 N, 116.36E; temperate continental monsoon climate). The annual mean temperature and precipitation in study site is 11.9 °C and 550 mm, respectively. The soil texture is sandy loam (5.26 % clay, 25.59 % silt, and 69.15 % sand), belongs to low yield soil. Before the start of study, the topsoil (0–20 cm) organic C, total N, available P, available K and pH were: 7.51 g kg⁻¹, 0.81 g kg⁻¹, 10.67 mg kg⁻¹, 153 mg kg⁻¹ and 8.84, respectively. Crop rotation comprises winter wheat (variety 'Langyan 43') and summer maize (variety 'Zhengdan 958').

Twelve trial plots (15 m² each) were laid out in a completely randomized block design, including four treatments with three replicates: (1) CK: no amendment; (2) SA: attapulgite amendment 2.25 t ha⁻¹; (3) SC: chitin-rich organic material 15 t ha⁻¹; and (4) SCA: the combination of chitin-rich organic material 15 t ha⁻¹ and attapulgite amendment

Table 1
Basic properties of the two amendments.

	pH	Total nitrogen (g kg ⁻¹)	Total phosphorus (g kg ⁻¹)	Total potassium (g kg ⁻¹)	Organic carbon (g kg ⁻¹)	Specific surface area (m ² g ⁻¹)	CEC (mol 100 g ⁻¹)
Chitin-rich organic material	7.75	14.7	21.05	22.88	73.74	–	–
Attapulгите	8.40	0.069	0.082	0.011	–	369	21.96

2.25 t ha⁻¹. Chemical fertilizers (N, P, and K input levels were 150, 52.39, and 46.68 kg ha⁻¹, respectively) were applied equally to each plot before wheat and maize sowing every year. After amendments and fertilizers were applied on the surface, they were immediately plowed into the arable layer. In October 2019, soil samples were collected at 0–20 cm depth after the maize harvested. Each treatment was randomly sampled in five points (0–20 cm), and then, the soil samples were thoroughly mixed and were divided into three subsamples for DNA extraction (stored at –80 °C), fresh sample analyses (stored at 4 °C) and chemical analysis.

Soil pH was measured with a pH meter (water/soil ratio is 2.5:1). SOC and total nitrogen (TN) were determined using the potassium dichromate oxidation method and Kjeldahl method, respectively. Mineral N was measured using a continuous flow analytical system (AA3, SEAL, Germany) after being extracted with 2 M KCl. Available potassium (AK) extracted with 1 M ammonium acetate, while AP extracted with 0.5 M NaHCO₃. The AK was measured with a flame photometer, and AP was measured using the molybdenum blue method (Zheng et al., 2021a). Soil microbial biomass C (MBC) and microbial biomass N (MBN) were measured according to Vance et al. (1987). Firstly, soil samples were fumigated with CHCl₃, and then incubated in dark conditions at 25 °C for 24 h. Secondly, soil samples were extracted with 0.5 M K₂SO₄, and the filtrate were determined by C/N analyzer (Multi N/C3100, Analytik Jena AG, Germany).

2.3. Analysis of enzyme activity

β-glucosidase (BG), β-cellobiosidase (CBH), L-leucine aminopeptidase (LAP), N-Acetyl-glucosaminidase (NAG) were assayed using standard fluorescent method (Saiya-Cork et al., 2002). Briefly, fresh soil (1 g) was mixed with 100 mL of 50 mmol·L⁻¹ sodium acetate buffer solution (pH 7.8) and blended for 1 min with a magnetic agitator to produce a soil suspension. Then, 200 μL of soil suspension, 200 of μL buffer solution, 50 μL of standard buffer (LAP using 7-amino-4-methyl coumarin (AMC) as a standard substance and others using 4-methyl hydroxycoumarin (MUB) as a standard substance), and 50 μL of the corresponding substrate were transferred into a 96-well microplate. The microplates were incubated in the dark at 25 °C for 4 h, and were quantified with 365 nm excitation and 460 nm emission using a microplate fluorometer (Synergy, BioTek, USA).

2.4. Soil DNA extraction, Illumina Miseqsequencing, and metagenomic sequencing

Fresh soil (0.5 g) DNA was extracted using an E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) and then was checked on 1 % agarose gel. The bacterial 16S rRNA gene (V3-V4 region) and fungal ITS1 region were amplified using primers 338F and 806R, and ITS1F and ITS2R, respectively. The amplicons were paired-end sequenced (2 × 300) on the Illumina MiSeq platform (Illumina, San Diego, CA, USA). The sequencing reads were quality-filtered and merged using Trimmomatic and FLASH, respectively. The operational taxonomic units (OTU) were generated at a 97 % similarity cutoff using UPARSE (v7.1), and chimeric sequences were removed. The taxonomy of each OTU representative sequence was determined by using RDP Classifier against the SILVA database (v138) and UNITE database (v8.0), with a confidence threshold of 0.7. The raw sequence data generated in this study have been deposited in the Sequence Read Archive (SRA) with Accession No.

PRJNA977106.

Soil DNA samples of CK and SCA treatment were selected for metagenomic sequencing based on the amplicon sequencing data. In detail, six DNA samples being sequenced using Illumina NovaSeq 6000 (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology (Shanghai, China). For each DNA sample, clean data were obtained after removing low-quality reads (length < 50 bp or with a quality value < 20 or having N bases), and these reads were assembled high-quality sequences into contigs by using splicing software MEGAHIT (v1.1.2). And then, contigs were used for predicting open reading frames using MetaGene and translated into amino acid sequences. The sequences were further clustered using CD-HIT (v4.6.1) to construct a non-redundant gene catalog. Finally, the enrichment of related genes in each sample was calculated using SOAPaligner software. The raw sequence data generated have been deposited in the Sequence Read Archive (SRA) (No. PRJNA977730). Functional annotation results were performed using Diamond against the Kyoto Encyclopedia of Genes and Genomes database (KEGG v94.2), and the eggNOG database.

2.5. Statistical analysis

Differences between soil physiochemical properties and enzyme activities were tested using ANOVA (Duncan's test) in IBM SPSS Statistics 22. The R software (v4.1.1) was utilized to conduct the following analyses. The Richness and Shannon indices were calculated under different amendment treatments with the “vegan” package. The beta diversity of both bacterial and fungal communities was assessed via a principal coordinates analysis (PCoA) based on the Bray-Curtis distance matrices. Additionally, differences in community structure were analyzed under various amendment treatments using permutational multivariate analysis of variance (PERMANOVA), which was performed using the “vegan” package. To further analyze the differences in microbiome abundance between amendment and no amendment treatments, DESeq2 (the “DESeq2” package of R) was used (Donhauser et al., 2021). Redundancy analysis (RDA) was performed to reveal the relationship between soil properties and different bacteria using the “vegan” package. In order to explore how amendments affected bacterial community structure and microbial function, structural equation modeling (SEM) was implemented. The chi-square test ($P > 0.05$) and the root MSE of approximation (RMSEA; RMSEA < 0.05 and $P > 0.05$) were assessed to evaluate the fitness of the model (Chen et al., 2022). All the SEM-related analyses were conducted using AMOS 22.0.

3. Results

3.1. Effects of different amendments on maize yield and soil properties

After 4-year amendment application, the maize yield was ordered as follows: SCA > SC > SA ≈ CK. Compared with CK, crop yield was increased by 24.26 % under the SCA treatment (Table 2; $P < 0.05$). Compared with CK, the SC and SCA treatments significantly increased soil TN and SOC concentrations by 8.99–20.22 % and 16.97–27.97 %, respectively ($P < 0.05$). Soil AK, AP, and NO₃-N concentrations were also increased significantly in the SC and SCA treatments ($P < 0.05$). In addition, soil pH was lower under the SCA treatment than under other treatments and was decreased by 5.59 % ($P < 0.05$) compared with CK. MBC increased by 33.65 % under SCA compared with CK ($P < 0.05$). All soil properties mentioned above under SA did not change significantly

Table 2
Soil properties and crop yield after 4-year amendment application.

Items	CK	SA	SC	SCA
Organic carbon (g kg ⁻¹)	8.90 ± 0.18c	9.48 ± 0.89 bc	10.41 ± 0.86 ab	11.39 ± 0.56 a
Total nitrogen (g kg ⁻¹)	0.89 ± 0.01 c	0.90 ± 0.03 bc	0.97 ± 0.08 b	1.07 ± 0.02 a
Available phosphorus (mg kg ⁻¹)	12.93 ± 5.79 b	13.74 ± 0.72 b	55.13 ± 7.71 a	59.26 ± 15.42a
Available potassium (mg kg ⁻¹)	248.67 ± 20.10 b	255.67 ± 1.06 b	335.00 ± 21.93 a	364.67 ± 7.51 a
pH	7.87 ± 0.07 ab	7.94 ± 0.04 a	7.75 ± 0.04 b	7.43 ± 0.13 c
NH ₄ ⁺ -N (mg kg ⁻¹)	7.68 ± 1.26 b	7.10 ± 0.90 b	10.52 ± 0.83 a	6.53 ± 1.32 b
NO ₃ ⁻ -N (mg kg ⁻¹)	11.05 ± 1.14 b	8.95 ± 1.09 b	29.83 ± 5.99 a	29.32 ± 8.01 a
MBC (mg kg ⁻¹)	197.70 ± 6.07 b	209.46 ± 1.95 b	208.56 ± 25.68 b	264.24 ± 11.46 a
MBN (mg kg ⁻¹)	38.83 ± 2.97 a	40.24 ± 3.70 a	39.60 ± 4.94 a	48.18 ± 7.24 a
Yield (kg hm ⁻²)	6523 ± 591 b	6734 ± 561 ab	7586 ± 891 ab	8106 ± 961 a

Note: CK, no amendment; SA, soil amended with attapulgite amendment; SC, soil amended with chitin-rich organic material; and SCA, soil amended with the chitin-rich organic material and attapulgite amendment. Values are means ± standard errors (n = 3). Lowercase letters indicate significant differences under different treatments (P < 0.05, one-way ANOVA, Duncan's HSD test).

compared with CK (P > 0.05).

3.2. Effect of different amendments on enzyme activities

In general, enzyme activities were higher under SC and SCA compared to CK treatment. As shown in Fig. 1a, b, and c, BG, CBH, and NAG, enzymes that are crucial in C cycling, showed the highest activities in the SCA treatment, followed by the SC treatment. Compared with CK, the activities of BG, CBH, and NAG in the SC treatment were increased by 120 %, 529 %, and 281 %, respectively; the same activities increased by 148 %, 636 %, and 307 %, respectively, under the SCA treatment

(Fig. 1a, b, d). Furthermore, similar increases (78 % for SC and 99 % for SCA) were detected in the activity of LAP, an N-related enzyme (Fig. 1c). The BG, CBH, LAP, and NAG activities also increased in SA compared with CK. These findings indicated that amendments could enhance the activities of C- and N-related enzymes and accelerate the cycling of relevant nutrients.

3.3. Shifts in soil microbial community diversity, composition, and structure

In total, 854,266 bacterial 16S rDNA and 726,467 fungal ITS high-

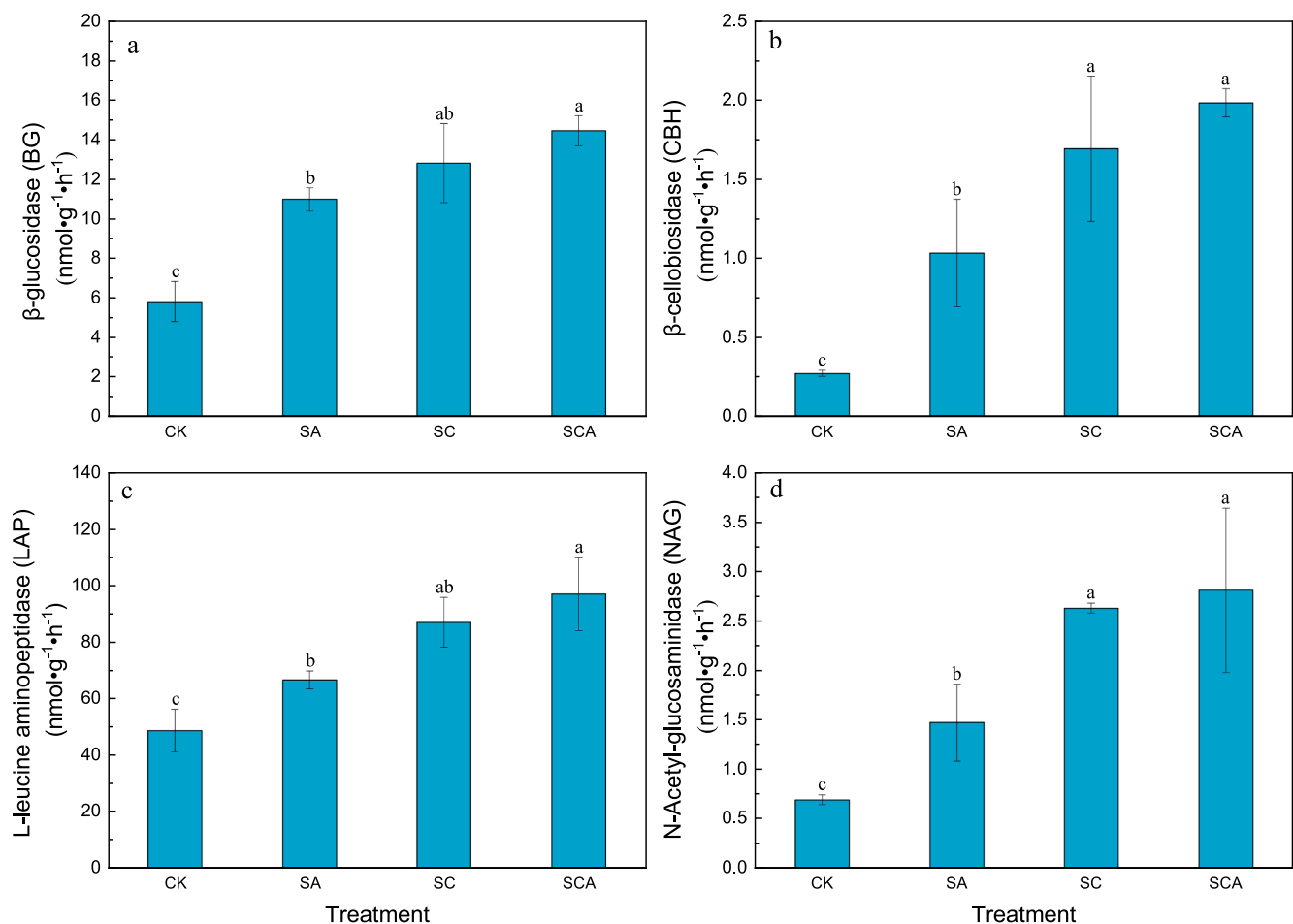


Fig. 1. Extracellular enzyme activities in different treatments. CK, no amendment; SA, soil amended with attapulgite; SC, soil amended with chitin-rich organic material; and SCA, soil amended with chitin-rich organic material and attapulgite. Values are means ± standard errors (n = 3). Lowercase letters indicate significant differences under different treatments (P < 0.05, one-way ANOVA, Duncan's HSD test).

quality sequences were obtained from 12 samples. These sequences were classified as 4760 bacteria OTUs and 1313 fungal OTUs. No significant changes were found in the bacterial or fungal α diversity under SA, SC, or SCA treatments compared with CK (Fig. 2a and b). PCoA and PERMANOVA analysis revealed that bacterial β diversity was notably different between different treatments (Fig. 2c; PERMANOVA, $R^2 = 0.388$, $P = 0.008$). Along the PC1 axis, the bacterial β diversity separated chitin-rich organic material treatments (SC and SCA) from other amendment treatments (CK and SA) at the bacterial genus level. In contrast, the fungal community structure was unchanged under different amendments (Fig. 2d; PERMANOVA, $R^2 = 0.348$, $P = 0.076$).

Acidobacteriota (ranging from 26.37 % to 28.64 %), *Proteobacteria* (ranging from 19.94 % to 22.90 %), and *Actinobacteriota* (ranging from 13.76 % to 15.20 %) were the predominant bacterial phyla across all samples (Fig. 2e). Further differential analysis revealed that almost no taxa difference existed in SA (except for OTU3753) compared with CK treatment (Fig. S2). Contrary to SA treatment, SC and SCA had obvious taxa enrichment and depletion compared with CK treatment, and were more pronounced in SCA than in SC. Specifically, 14 and 22 OTUs were significantly enriched in SC and SCA, respectively, compared with CK (Fig. 3a and b; FDR adjusted $P < 0.05$). These enriched microbial taxa mostly belonged to six phyla, namely *Proteobacteria* (*Alphaproteobacteria* and *Gammaproteobacteria* at the class level), *Actinobacteriota* (*Actinobacteria*, *Acidimicrobiia*, and *Thermoleophilina* at the class level), *Cyanobacteria*, *Firmicutes*, *Gemmatimonadota*, and *Patescibacteria* (Table S1). Furthermore, 13 and 31 OTUs had been depleted in the SC and SCA treatments, respectively (Fig. 3a and b; FDR adjusted $P < 0.05$). These belonged to three phyla, namely *Actinobacteriota* (*Acidimicrobiia*, *Actinobacteria*, *MB-A2-108*, and *Thermoleophilina* at the class level), *Acidobacteriota*, and *Chloroflexi* (Table S2). Moreover, a notable overlap of OTUs in SC and SCA was observed. Twelve bacterial OTUs were enriched in both treatments (Fig. 3c), accounting for 54.55 % in the SCA treatment and 85.71 % in the SC treatment; 10 and two specific OTUs were enriched in the SCA and SC treatments, respectively. Only nine OTUs with significant reduction were found in both treatments (Fig. 3d), accounting for 29.03 % in the SCA treatment and 69.23 % in the SC treatment.

3.4. Changes of microbial carbon metabolism function

The microbiomes under the SCA treatment presented a considerable difference compared with CK than other amendment treatments. Thus, we selected the CK and SCA treatments for metagenomic sequencing. The KEGG and eggNOG annotation results suggested that carbon metabolism and amino acid transport and metabolism were the most active functions (Fig. S3). Moreover, the results confirmed that the functional composition (i.e., NMDS ordinations of KO and COG) of the SCA differed significantly from that of CK (stress < 0.05 ; Fig. 4a and b). To determine how the combined addition of chitin-rich organic material and attapulgite affected the microbiome's functional properties, we performed differential abundance analysis using the most dominant functional pathways (TOP10) on KEGG level 3 and COG level 2. Compared with CK, the enrichment of genes related to carbon metabolism, carbon fixation pathways in prokaryotes, the citrate cycle (TCA cycle) (KEGG; Fig. 4c), posttranslational modification, protein turnover, and chaperones (COG; Fig. 4d) was significantly lower in the SCA treatment than in CK.

3.5. Linkages between microbial communities and soil properties

RDA was conducted to explore the relationship between soil properties with dominant bacterial OTUs (TOP 100) and enriched OTUs in chitin-rich organic material treatments (Fig. 5). The first two axes together explained 79.55 % of the total variation in the dominant bacterial OTUs. Soil $\text{NH}_4^+\text{-N}$ and SOC were major factors and explained 21.34 % and 14.25 % of the variation, respectively. Moreover, the first

axis of the RDA explained 77.52 % of the variation in the enriched OTUs community, and the second axis explained 15.16 % of the variation. AK, pH, $\text{NO}_3^-\text{-N}$, and AP were the main factors that significantly altered bacterial OTUs under chitin-rich organic material application ($P < 0.01$).

SEM was used to investigate how soil properties affected the bacterial community and carbon metabolism (enrichment of ko00020 pathway functional genes) (Fig. 6a). The results showed that soil AP was the key factor, as it positively influenced the carbon metabolism via increasing enzyme activities (standardized total effect = 0.801). Furthermore, soil AP was significantly associated with bacterial community structure ($r = -0.825$, $P < 0.001$), but there was no significant relationship between bacterial community structure and carbon metabolism ($r = -0.276$, $P > 0.05$). SOC was also a key factor that was affected by soil AP ($r = 0.475$, $P < 0.05$) and that could directly regulate carbon metabolism, even though the effect was negative ($r = -0.677$, $P < 0.05$). Overall, this model met the significance criteria ($\chi^2 = 17.166$, $P = 0.443$, CFI = 0.998, $P = 0.029$, AIC = 71.166) and could explain 61.8 % of the variation in soil AP, 82.9 % of the variation in enzyme activities, and 66.5 % the variation in carbon metabolism.

4. Discussion

4.1. Effects of chitin-rich organic material and attapulgite on soil properties and crop yield

In the present study, chitin-rich organic material application (alone and in combination) significantly increased SOC (Table 2), while attapulgite application alone did not had a significant effect. This indicates that the input of organic substances can increase the SOC, similar to the results of manure or straw return experiments (Liu et al., 2022; Song et al., 2022). Compared with the single application of each amendment, the combined application was able to improve SOC more effectively. One possible reason is the physical protection of soil aggregate (Liu et al., 2021). In our previous research (Zhang et al., 2021b), compared with the CK treatment, SCA remarkably increased the proportion of soil macro-aggregate, thereby increasing the content of SOC. In addition, microbial growth results in the increase of microbial-derived C into the soil by biomass turnover and necromass accumulation, which could also influence the accumulation of SOC to a certain degree. In our research, the SCA treatment increased MBC by 33.66 % in comparison to the CK treatment (Table 2). This suggested that the ample nutrients provided by chitin-rich organic material and the wide spacing of attapulgite effectively promoted microbial growth and activity, thereby increasing the content of SOC. Besides, our results showed that TN and $\text{NO}_3^-\text{-N}$ increased under SC and SCA (Table 2). This could be the result of the low C/N ratio (6–7) of chitin-rich material that can rapidly release N and thereby result in higher N mineralization (Shamshina et al., 2020). These results may also be linked to functional genes associated with soil denitrification and nitrogen fixation under chitin fertilization (Zhan et al., 2021). Other nutrients such as AP and AK were also increased under chitin-rich organic material application (Table 2). These increases can be attributed to high P and K contents in the organic material (Table 1). In this study, activities of C- and N-cycling-related enzymes were increased significantly under chitin-rich organic material application (Fig. 1). This is consistent with the results of (Liu et al., 2010; Mangalassery et al., 2019; Kang et al., 2021; Wang et al., 2021). In general, when a certain nutrient is deficient in the soil, microorganisms will secrete more enzymes to meet the demand for the nutrient. However, Fierer et al. (2021) suggested that when the nutrient content is high, this will also increase the activity of the corresponding enzymes. Our results are more in accordance with the hypothesis of Fierer, the high C content of organic material provides sufficient substrate for microbial participation in nutrient cycling and transformation, thus increasing enzyme activity (Fierer et al., 2021).

Numerous studies have demonstrated that SOC accumulation has

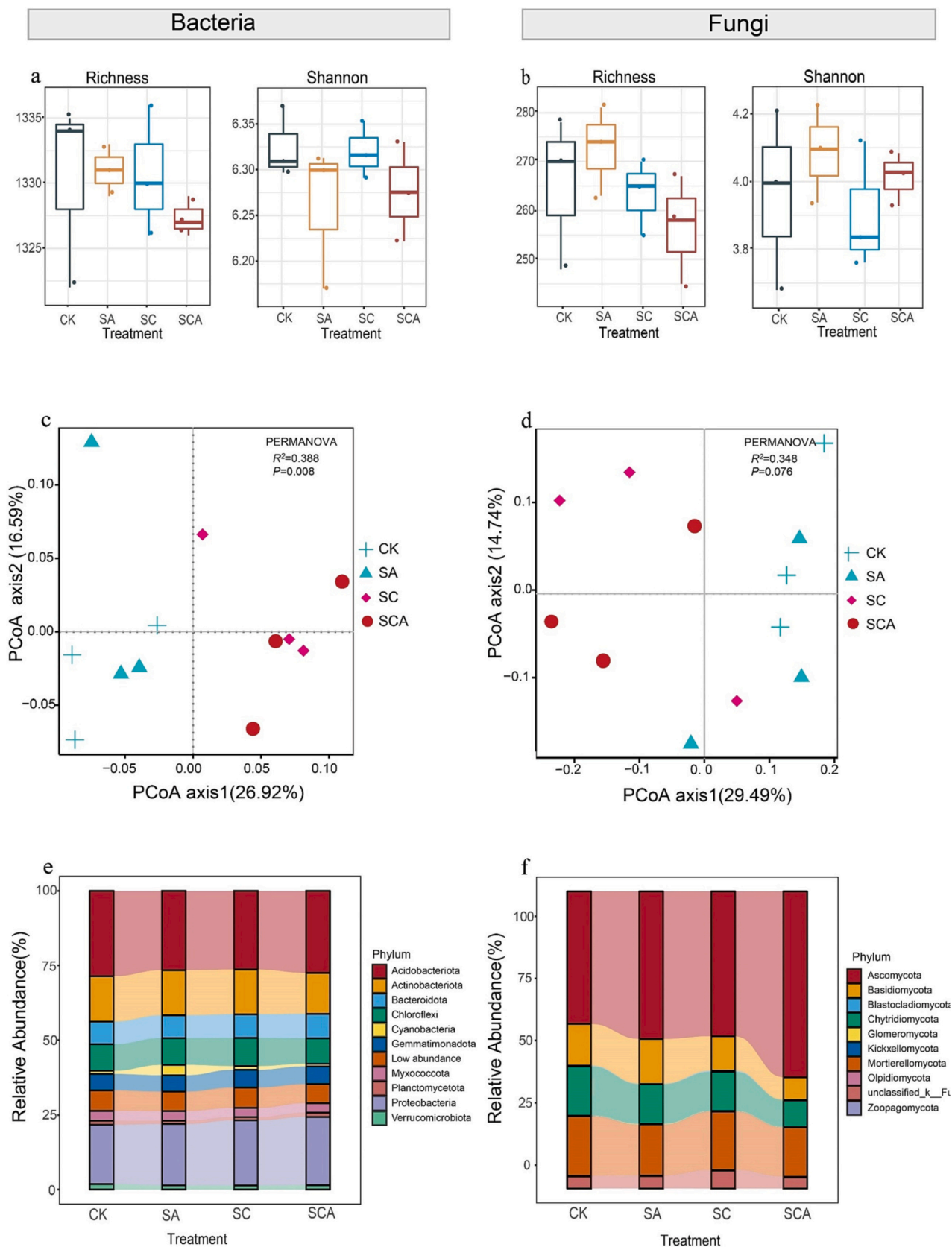


Fig. 2. Alpha and beta diversity of both bacterial and fungal communities in different treatments. Alpha diversity indices of bacterial (a) and fungal (b) communities (n = 3). Principal coordinate analysis of the bacterial community structure (c) and fungal community structure (d) based on the Bray-Curtis distance under different treatments. Relative abundances (%) of the bacterial (e) and fungal (f) phyla. CK, no amendment; SA, soil amended with attapulgite; SC, soil amended with chitin-rich organic material; and SCA, soil amended with chitin-rich organic material and attapulgite. R and P values were calculated via the Adonis test using vegan package.

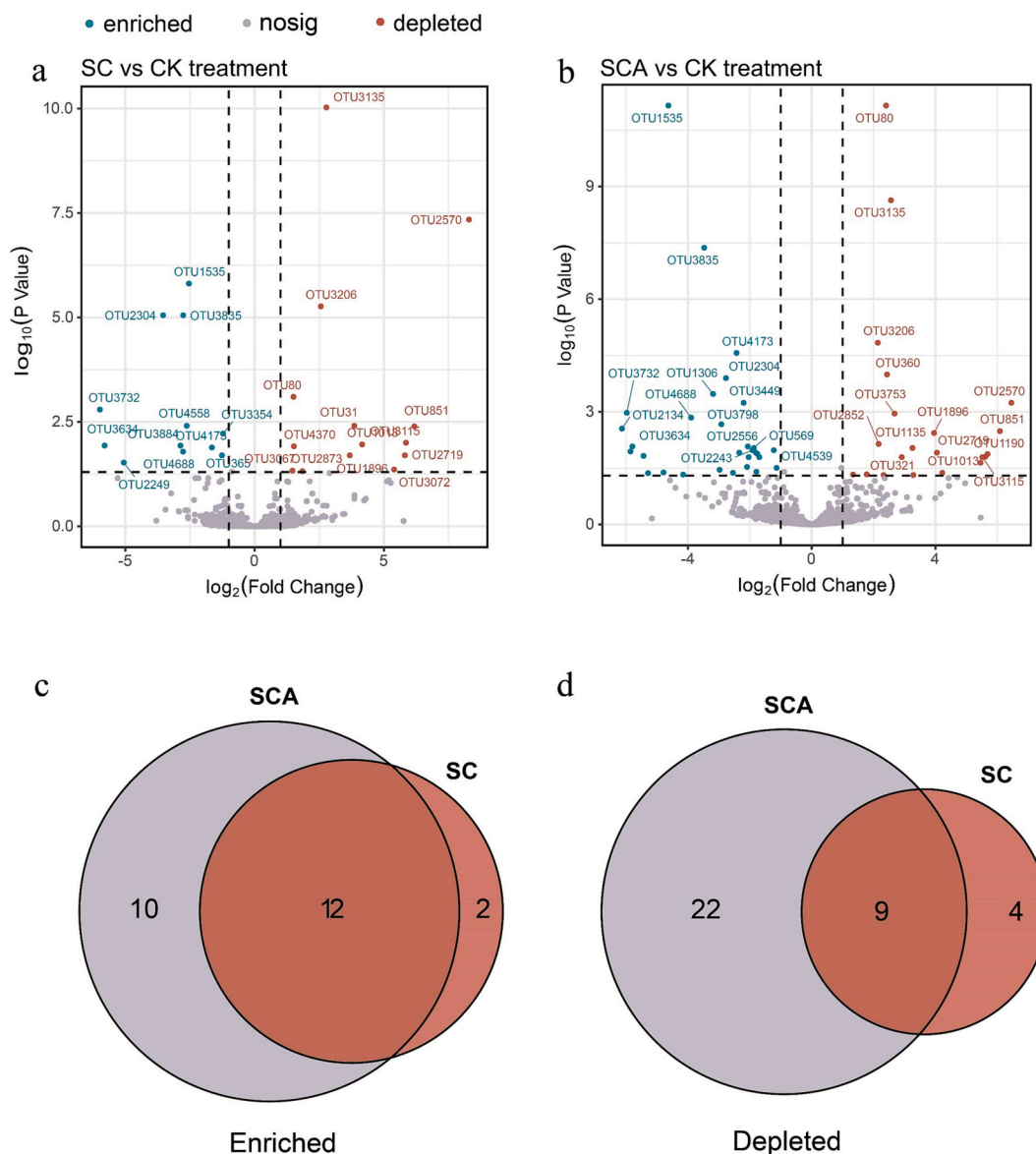


Fig. 3. Differences in bacterial taxonomic composition under amendment treatments. Volcano plots showing differential bacteria OTUs in chitin-rich organic material treatment (a) and co-incorporation of chitin-rich organic material and attapulgite amendment (b). Overlapping OTUs enriched (c) and depleted (d) in chitin-rich organic material and chitin-rich organic material and attapulgite amendment treatments. SC, soil amended with chitin-rich organic material; SCA, soil amended with chitin-rich organic material and attapulgite.

beneficial effects on crop yield (Cai et al., 2019; Li et al., 2019). However, the beneficial effects depend on the type of organic amendment, the management of application, and the soil properties. For example, Li et al. (2018) reported that crop yield increased by 9.9, 13.2, and 17.4 %, under different organic amendments (pig manure, straw, and pig manure + straw, respectively). By contrast, Wu et al. (2022) suggested that 100 % organic manure application had no significant impact on yield. Our results showed that the crop yield increased by 24.26 % under SCA, better than those under SC and SA. This may have been due to the high content of polysaccharides in chitin that can quickly provide nitrogen and energy for plants and microorganisms (Shamshina et al., 2020). Moreover, chitin-rich organic material contains functional hydroxyl and amino groups that can be used as sustainable alternatives to synthetic chelation agents (such as EDTA) to deliver soil nutrients and improve crop growth (Sharp, 2013). Also, the attapulgite amendment can increase water retention. Thus, the combination of these two materials is more favorable to maintaining the balance of the soil nutrient system and achieving increases in crop yield.

4.2. Chitin-rich organic material application altered the bacterial community structure and taxonomic composition

In general, organic material application increases soil energy (organic carbon and other nutrients) and thus sustains a greater soil microbial diversity according to the species-energy hypothesis (Clarke and Gaston, 2006). According to a meta-analysis, bacterial alpha diversity increases under organic amendments compared with mineral fertilizer (Shu et al., 2022). However, our results indicated that chitin-rich organic material did not have any significant impact on the alpha diversity of bacterial or fungal communities (Fig. 2a, b). Specifically, the richness indices were lower under organic material treatments compared with CK for both bacteria and fungi, in agreement with studies by Ji et al. (2022) and Zhang et al., 2021a. The reason for the contradicting results may be the chemical heterogeneity of organic materials. Bacteria usually utilize labile organic carbon (Xun et al., 2018), while chitin-rich organic material increases stable organic carbon more than active organic carbon (Zhou et al., 2020), and this may restrict bacterial

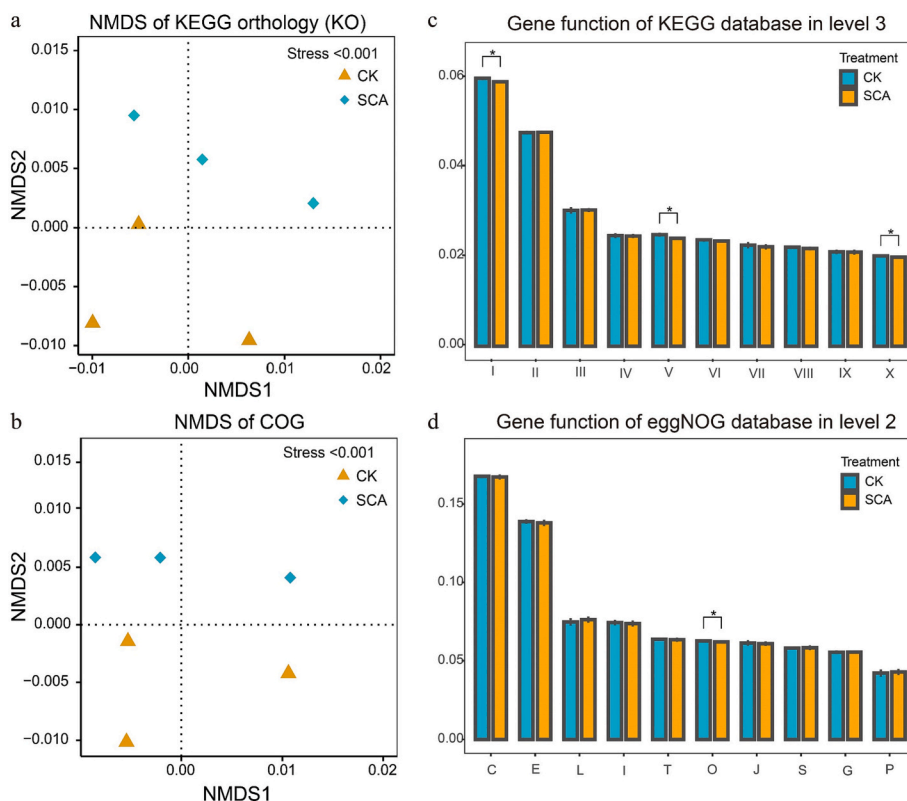


Fig. 4. Non-metric multidimensional scaling analysis between no amendment and co-incorporation of chitin-rich organic material and attapulgite amendment based on the KEGG database (a) and the eggNOG database (b). Differential function based on the KEGG database at level 3 (c). Differential function based on the eggNOG database at level 2 (d). KEGG function: I, Carbon metabolism; II, Biosynthesis of amino acids; III, Purine metabolism; IV, Pyrimidine metabolism; V, Carbon fixation pathways in prokaryotes; VI, Pyruvate metabolism; VII, ABC transporters; VIII, Glyoxylate and dicarboxylate metabolism; IX, Oxidative phosphorylation; and X, Citrate cycle (TCA cycle). COG function: C, Energy production and conversion; E, Amino acid transport and metabolism; L, Replication, recombination, and repair; I, Lipid transport and metabolism; T, Signal transduction mechanisms; O, Posttranslational modification, protein; J, Translation, ribosomal structure, and biogenesis; S, Function unknown; G, Carbohydrate transport and metabolism; and P, Inorganic ion transport and metabolism. CK, no amendment; SCA, soil amended with chitin-rich organic material and attapulgite. Significant differences were detected using the *t*-test ($P < 0.05$), means + standard error ($n = 3$).

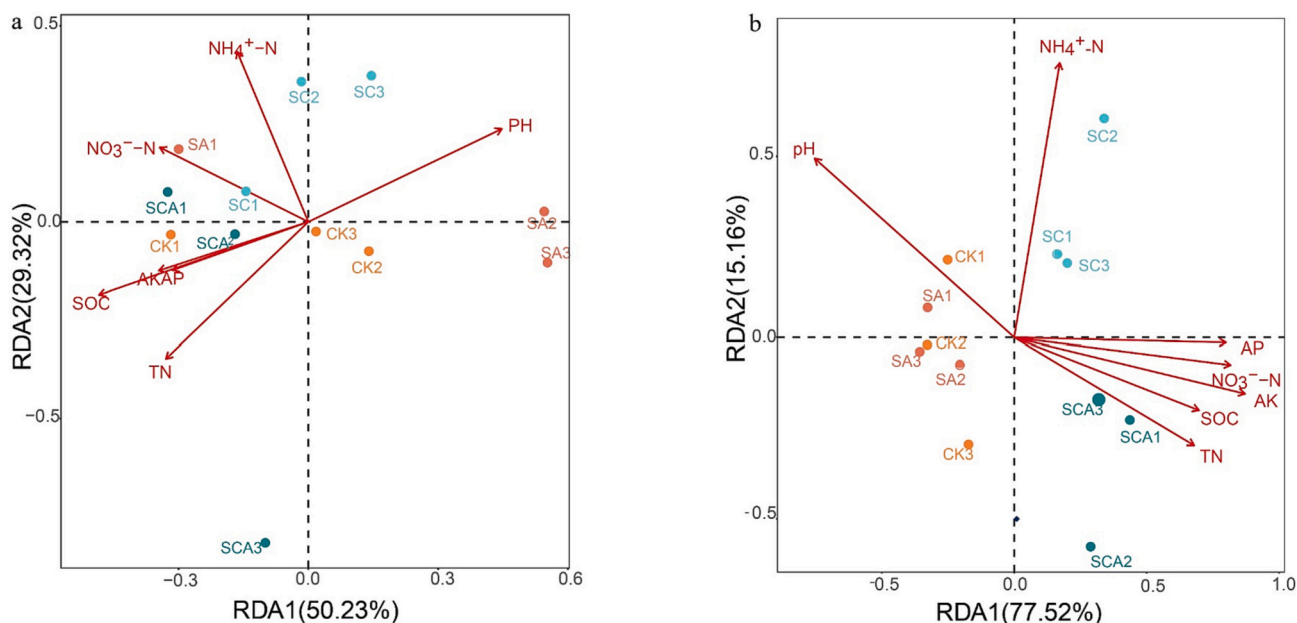


Fig. 5. Redundancy analysis between dominant bacterial OTUs (TOP 100) (a) and enriched OTUs in chitin-rich organic material treatments (b) and soil properties. SOC, soil organic carbon; TN, total nitrogen; AP, available phosphorus; AK, available potassium; NO_3^- -N, nitrate nitrogen; and NH_4^+ -N, ammonium nitrogen.

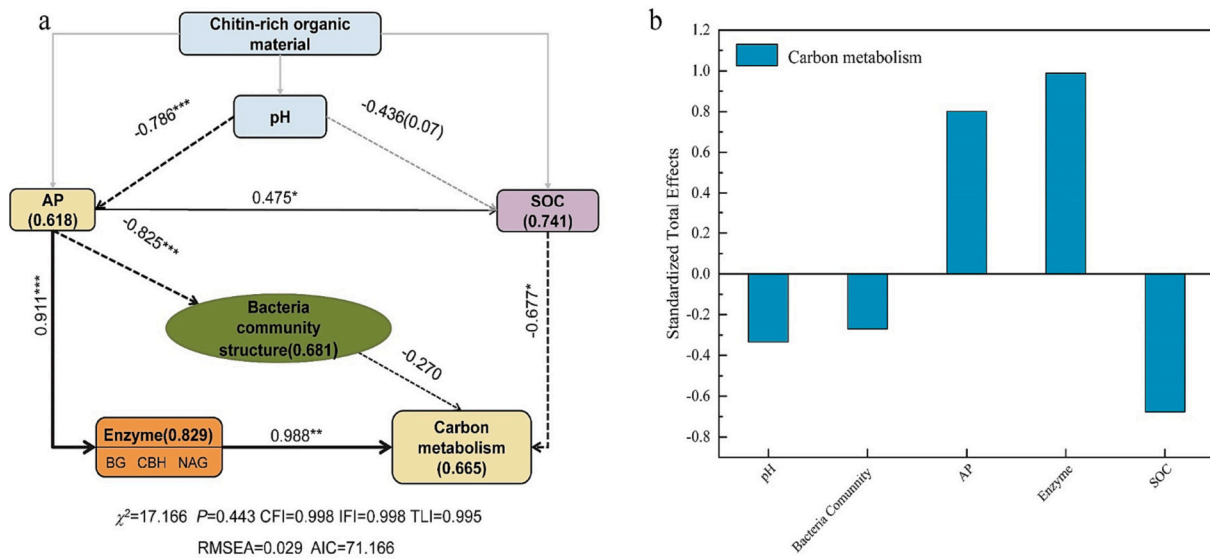


Fig. 6. Structural equation model (SEM) showing the effects of soil properties, enzyme activities, and the bacterial community structure on carbon metabolism. Solid and dashed arrows indicate significant positive and negative relationships, respectively. The width of the arrow indicates the strength of the effect; the number next to the arrow is the standardized path coefficient; and the significance level is as follows: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

C utilization and reduce diversity. At the same time, the addition of organic material may lead to competition among fungal community members because the materials supply narrow-spectrum nutrients (Sun et al., 2020). This compresses the living niche for certain fungal species and ultimately reduces diversity. In contrast to the alpha diversity, bacterial beta diversity was significantly shifted under SC and SCA (Fig. 2c), like it was also observed by Cheng et al. (2022). This can be partially explained by the variation in soil physicochemical properties under SC and SCA (Table 2). In contrast to bacteria, the current research showed that fungal beta diversity changed little with different treatments (Fig. 2d). This finding was also shown by Han et al. (2021). The reason may be due to the different responses of fungi to soil pH. Bacteria exhibit relatively narrow growth tolerance for soil pH, while fungi have a wide pH optimum (covering 5–9 pH units) (Hu et al., 2017).

In the current study, SA had no influence on bacterial community (Fig. S2). This result was attributed to the content of nutrient input being very low (Fig. S1), which may not significantly affect microbial growth and activity. In contrast to the SA treatment, we found that chitin-rich organic material (SC and SCA) altered the bacterial composition (Fig. 3). For example, the two dominant phyla *Proteobacteria* and *Actinobacteriota* were significantly enriched in chitin-rich organic material treatments. These phyla have the potential for decomposing organic material and promoting plant growth (He et al., 2020). At the order level, some OTUs belonging to *Xanthomonadales*, *Burkholderiales*, *Sphingomonadales*, and *Rhizobiales* are positively related with the organic material; *Xanthomonadales* and *Sphingomonadales* are positively correlated to complex organic compound decomposition (Dominguez et al., 2019; Wu et al., 2021; Qi et al., 2022), and *Burkholderiales* and *Rhizobiales* are important plant growth-promoting bacteria (Garrido-Oter et al., 2018; Xiong et al., 2021). These results imply that chitin-rich organic material application alters microbial function. Soil factors such as AK, SOC, AP, TN, and $\text{NO}_3\text{-N}$ positively affected bacterial communities, especially in those groups enriched under organic material application (Fig. 5). These results were similar to those of previous studies (Ali et al., 2022; Gupta et al., 2023) and suggested that nutrient input may have significant effects on the bacterial community. Soil pH showed an unfavorable relationship with other factors (Fig. 5). This result was different from those of Xiao et al. (2021) and Zhao et al. (2020) who found that soil pH was positively correlated with some taxa and with the overall bacterial community. The varied results may be due to the soil type. The present study was conducted in a slightly alkaline

sandy soil, and the decrease in soil pH made the soil environment closer to a neutral environment that was more suitable for bacterial growth (Lauber et al., 2008).

4.3. Microbial carbon metabolism was altered under the combined application of chitin-rich organic material and attapulgite

Carbon metabolism is the main metabolic pathway of microorganisms and comprises the most basic function of life (Pang et al., 2021). It encompasses the uptake, degradation and transformation of carbon sources. However, our metagenomic analyses indicated that the functional genes involved in carbon metabolism, carbon fixation pathways in prokaryotes, and the citrate cycle (TCA cycle) decreased significantly under SCA. This result was in opposition to that of Chen et al. (2019), who found that the genes participating in carbon metabolism were enriched under long-term organic fertilization. The reason may be due to negative priming effect: (i) microorganisms preferentially utilize easily available carbon from chitin-rich organic material and hence inhibit the decomposition of native SOC based on 'preferential substrate utilization' theory (Qiu et al., 2022); (ii) a high density of pores from attapulgite most likely results in more native SOC to enter, which reduce the decomposition of native SOC (Zheng et al., 2021b). Furthermore, application of chitin-rich organic material and attapulgite had obvious increasing effects on soil aggregate formation, especially macroaggregate (>0.25 mm), which protect more SOC from microbial decomposition.

Several studies have suggested that changes in carbon metabolism are closely related to the activities of bacteria. For example, activities of phyla *Gemmatimonadetes*, *Actinobacteria*, *Proteobacteria*, and *Chloroflexi*, which are the main contributors to carbon metabolism, increased under manure treatment (Khodadad et al., 2011; Tao et al., 2023). However, Yu et al. (2020) found that *Bacteroidetes* and *Gemmatimonadetes* have negative effect on the decomposition of SOC. These opposite results may indicate that carbon metabolism was also related with community structure (Fig. 6) not just special taxonomic composition. This is in line with Wang et al. (2020). They found that bacterial community structure was negatively associated with carbon metabolism, especially in carbohydrate utilization related to glucose and cellobiose. The above result due to less bacterial functional redundancy in the late stages, which lead to inadequate functional complementary for recalcitrant SOC decomposition (Yu et al., 2020).

In addition, soil factors such as AP, SOC, and pH play important roles in influencing microbial carbon metabolism. Soil AP was the most effective factor for microbial carbon metabolism, as confirmed by Hu et al. (2022). Possible reasons may link to the higher P availability being important for microbial growth. Higher P availability also enhances microbial demand for other nutrients, such as C, and thus drives the community to switch investment (Yao et al., 2018). By contrast, SOC significantly inhibited microbial carbon metabolism (Fig. 6). This may be related to the characteristics of organic carbon. In the current study, chitin-rich organic material introduced a large amount of organic carbon from different materials such as peat, straw, and peanut shells, and this enhanced the content of stable organic carbon in the soil (Zhou et al., 2020). Therefore, the microbes invested more energy into motility and enzyme production to consume the complex organic C (Allison et al., 2010), resulting in a lower growth rate and lower C use efficiency (Morrissey et al., 2023). Altogether, our results revealed that microbial carbon metabolism was affected by different amendments through changing soil properties and soil enzyme activities, but further research is needed to assess specific carbon metabolism pathways and verify the correlation relationships between soil amendment, soil physicochemical properties, and microbial function.

5. Conclusions

Collectively, we show a mechanistic link between soil properties, bacterial community structure and carbon metabolism function. Combined application of chitin-rich organic material and attapulgite shifted sandy soil nutrition retention (i.e., SOC, AP and pH), led to notable variation of bacterial community structure and increase of beneficial species, and inhibited microbial carbon metabolism function. These results suggest that chitin-rich organic material and attapulgite addition is an effective way to build SOC and nutrient stocks, improving soil fertility and keeping sandy soil sustainable. But further research is needed to determine more specific pathways related to carbon metabolism.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2023.105161>.

References

- Aklog, Y., Egusa, M., Kaminaka, H., Izawa, H., Morimoto, M., Saimoto, H., Ifuku, S., 2016. Protein/caco3/chitin nanofiber complex prepared from crab shells by simple mechanical treatment and its effect on plant growth. *Int. J. Mol. Sci.* 17, 1600. <https://doi.org/10.3390/ijm7101600>.
- Ali, I., Yuan, P., Ullah, S., Iqbal, A., Zhao, Q., Liang, H., Khan, A., Imran Zhang, H., Wu, X., Wei, S., Gu, M., Jiang, L., 2022. Biochar amendment and nitrogen fertilizer

- contribute to the changes in soil properties and microbial communities in a paddy field. *Front. Microbiol.* 13, 834751 <https://doi.org/10.3389/fmicb.2022.834751>.
- Allison, S.D., Weintraub, M.N., Gartner, T.B., Waldrop, M.P., 2010. Evolutionary-Economic Principles as Regulators of Soil Enzyme Production and Ecosystem Function. In: Shukla, G., Varma, A. (Eds) *Soil Enzymology. Soil Biology*, Vol 22. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-14225-3_12.
- Amadou, A., Song, X., Huang, S., Song, A., Tang, Z., Dong, W., Zhao, S., Zhang, B., Yi, K., Fan, F., 2021. Effects of long-term organic amendment on the fertility of soil, nodulation, yield, and seed quality of soybean in a soybean-wheat rotation system. *J. Soil. Sediment.* 21, 1385–1394. <https://doi.org/10.1007/s11368-021-02887-1>.
- Andreo-Jimenez, B.S.M.N.E., 2021. Chitin- and keratin-rich soil amendments suppress rhizoctonia solani disease via changes to the soil microbial community. *Appl. Environ. Microbiol.* 87, e00318–e00321. <https://doi.org/10.1128/AEM.00318-21>.
- Bonanomi, G., De Filippis, F., Zotti, M., Idbella, M., Cesarano, G., Al-Rowaily, S., Abd-ElGawad, A., 2020. Repeated applications of organic amendments promote beneficial microbiota, improve soil fertility and increase crop yield. *Appl. Soil Ecol.* 156, 103714 <https://doi.org/10.1016/j.apsoil.2020.103714>.
- Cai, A., Xu, M., Wang, B., Zhang, W., Liang, G., Hou, E., Luo, Y., 2019. Manure acts as a better fertilizer for increasing crop yields than synthetic fertilizer does by improving soil fertility. *Soil Tillage Res.* 189, 168–175. <https://doi.org/10.1016/j.still.2018.12.022>.
- Cao, Q., You, B., Liu, W., Xie, L., Jiang, W., Cheng, C., 2022. Using soil amendments to reduce microcystin-LR bioaccumulation in lettuce. *Environ. Pollut.* 292, 118354 <https://doi.org/10.1016/j.envpol.2021.118354>.
- Chen, D., Wang, X., Carrion, V.J., Yin, S., Yue, Z., Liao, Y., Dong, Y., Li, X., 2022. Acidic amelioration of soil amendments improves soil health by impacting rhizosphere microbial assemblies. *Soil Biol. Biochem.* 167, 108599 <https://doi.org/10.1016/j.soilbio.2022.108599>.
- Chen, L., Redmile-Gordon, M., Li, J., Zhang, J., Xin, X., Zhang, C., Ma, D., Zhou, Y., 2019. Linking cropland ecosystem services to microbiome taxonomic composition and functional composition in a sandy loam soil with 28-year organic and inorganic fertilizer regimes. *Appl. Soil Ecol.* 139, 1–9. <https://doi.org/10.1016/j.apsoil.2019.03.011>.
- Cheng, Z., Shi, J., He, Y., Wu, L., Xu, J., 2022. Assembly of root-associated bacterial community in cadmium contaminated soil following five-year consecutive application of soil amendments: evidences for improved soil health. *J. Hazard. Mater.* 426, 128095 <https://doi.org/10.1016/j.jhazmat.2021.128095>.
- Clarke, A., Gaston, K.J., 2006. Climate, energy and diversity. *Proc. R. Soc. B* 273, 2257–2266. <https://doi.org/10.1098/rspb.2006.3545>.
- Cretoiu, M.S., Kielak, A.M., Schluter, A., van Elsas, J.D., 2014. Bacterial communities in chitin-amended soil as revealed by 16S rRNA gene based pyrosequencing. *Soil Biol. Biochem.* 76, 5–11. <https://doi.org/10.1016/j.soilbio.2014.04.027>.
- De Tender, C., Mesuere, B., Van der Jeugt, F., Haegeman, A., Ruttink, T., Vandecasteele, B., Dawyndt, P., Debode, J., Kuramae, E.E., 2019. Peat substrate amended with chitin modulates the N-cycle, siderophore and chitinase responses in the lettuce rhizobiome. *Sci. Rep.* 9 <https://doi.org/10.1038/s41598-019-46106-x>.
- Debode, J., De Tender, C., Soltaninejad, S., Van Malderghem, C., Haegeman, A., Van der Linden, I., Cottyn, B., Heyndrickx, M., Maes, M., 2016. Chitin mixed in potting soil alters lettuce growth, the survival of zoonotic bacteria on the leaves and associated rhizosphere microbiology. *Front. Microbiol.* 7 <https://doi.org/10.3389/fmicb.2016.00565>.
- Dominguez, J., Bacosa, H.P., Chien, M.F., Inoue, C., 2019. Enhanced degradation of polycyclic aromatic hydrocarbons (PAHs) in the rhizosphere of sudangrass (sorghum x drummondii). *Chemosphere.* 234, 789–795. <https://doi.org/10.1016/j.chemosphere.2019.05.290>.
- Donhauser, J., Qi, W., Bergk Pinto, B., Frey, B., 2021. High temperatures enhance the microbial genetic potential to recycle C and N from necromass in high-mountain soils. *Glob. Change Biol.* 27, 1365–1386. <https://doi.org/10.1111/gcb.15492>.
- Fierer, N., Ladau, J., Clemente, J.C., Luff, J.W., Owens, S.M., Pollard, K.S., Knight, R., Gilbert, J.A., McCulley, R.L., 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. *Science.* 342, 621–624. <https://doi.org/10.1126/science.1243768>.
- Fierer, N., Wood, S.A., Bueno De Mesquita, C.P., 2021. How microbes can, and cannot, be used to assess soil health. *Soil Biol. Biochem.* 153, 108111 <https://doi.org/10.1016/j.soilbio.2020.108111>.
- Garrido-Oter, R., Nakano, R.T., Dombrowski, N., Ma, K., McHardy, A.C., Schulze-Lefert, P., 2018. Modular traits of the Rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. *Cell Host Microbe* 24, 155–167. <https://doi.org/10.1016/j.chom.2018.06.006>.
- Gupta, S., Graham, D.W., Sreekrishnan, T.R., Ahammad, S.Z., 2023. Exploring the impacts of physicochemical characteristics and heavy metals fractions on bacterial communities in four rivers. *J. Environ. Manage.* 325, 116453 <https://doi.org/10.1016/j.jenvman.2022.116453>.
- Han, J., Dong, Y., Zhang, M., 2021. Chemical fertilizer reduction with organic fertilizer effectively improve soil fertility and microbial community from newly cultivated land in the loess plateau of China. *Appl. Soil Ecol.* 165, 103966 <https://doi.org/10.1016/j.apsoil.2021.103966>.
- He, Y., Lu, L., Peng, C., Li, H., Zhang, J., Li, R., Zhou, C., 2020. High-yield grass Pennisetum sinense Roxb plantation and organic manure alter bacterial and fungal communities structure in an ecological agriculture farm. *AMB Express.* 10 <https://doi.org/10.1186/s13568-020-01018-2>.
- Hu, X., Liu, J., Wei, D., Zhu, P., Cui, X.A., Zhou, B., Chen, X., Jin, J., Liu, X., Wang, G., 2017. Effects of over 30-year of different fertilization regimes on fungal community compositions in the black soils of Northeast China. *Agric. Ecosyst. Environ.* 248, 113–122. <https://doi.org/10.1016/j.agee.2017.07.031>.

- Hu, X., Gu, H., Liu, J., Wei, D., Zhu, P., Cui, X., Zhou, B., Chen, X., Jin, J., Liu, X., Wang, G., 2022. Metagenomics reveals divergent functional profiles of soil carbon and nitrogen cycling under long-term addition of chemical and organic fertilizers in the black soil region. *Geoderma* 418, 115846 <https://doi.org/10.1016/j.geoderma.2022.115846>.
- Huang, J., Hartemink, A.E., 2020. Soil and environmental issues in sandy soils. *Earth Sci. Rev.* 208, 103295 <https://doi.org/10.1016/j.earscirev.2020.103295>.
- Ji, C., Ye, R., Yin, Y., Sun, X., Ma, H., Gao, R., 2022. Reductive soil disinfection with biochar amendment modified microbial community composition in soils under plastic greenhouse vegetable production. *Soil Tillage Res.* 218, 105323 <https://doi.org/10.1016/j.still.2022.105323>.
- Ji, Z., Zhou, J., Zhang, H., Li, G., Zhang, J., 2019. Effect of soil conditioners on the soil chemical properties and organic carbon pool of saline-sodic soil. *J. Agro-Environ. Sci.* 2019 (8), 1759–1767. <https://doi.org/10.11654/jaes.2019-0426>. (in Chinese).
- Jiang, N., Cai, D., He, L., Zhong, N., Wen, H., Zhang, X., Wu, Z., 2015. A facile approach to remediate the microenvironment of saline-alkali soil. *ACS Sustain. Chem. Eng.* 3, 374–380. <https://doi.org/10.1021/sc500785e>.
- Kang, Y., An, X., Ma, Y., Zeng, S., Jiang, S., Wu, W., Xie, C., Wang, Z., Dong, C., Xu, Y., Shen, Q., 2021. Organic amendments alleviate early defoliation and increase fruit yield by altering assembly patterns and of microbial communities and enzymatic activities in sandy pear (*Pyrus pyrifolia*). *AMB Express* 11. <https://doi.org/10.1186/s13568-021-01322-5>.
- Khodadad, C.L.M., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. *Soil Biol. Biochem.* 43, 385–392. <https://doi.org/10.1016/j.soilbio.2010.11.005>.
- Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol. Biochem.* 40, 2407–2415. <https://doi.org/10.1016/j.soilbio.2008.05.021>.
- Li, C., Ma, S., Shao, Y., Ma, S., Zhang, L., 2018. Effects of long-term organic fertilization on soil microbiological characteristics, yield and sustainable production of winter wheat. *J. Integr. Agric.* 17, 210–219. [https://doi.org/10.1016/S2095-3119\(17\)61740-4](https://doi.org/10.1016/S2095-3119(17)61740-4).
- Li, T., Gao, J., Bai, L., Wang, Y., Huang, J., Kumar, M., Zeng, X., 2019. Influence of green manure and rice straw management on soil organic carbon, enzyme activities, and rice yield in red paddy soil. *Soil Tillage Res.* 195, 104428 <https://doi.org/10.1016/j.still.2019.104428>.
- Liang, Y., Xu, L., Bao, J., Firmin, K.A., Zong, W., 2020. Attapulgite enhances methane production from anaerobic digestion of pig slurry by changing enzyme activities and microbial community. *Renew. Energy* 145, 222–232. <https://doi.org/10.1016/j.renene.2019.06.037>.
- Liu, E., Yan, C., Mei, X., He, W., Bing, S.H., Ding, L., Liu, Q., Liu, S., Fan, T., 2010. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in Northwest China. *Geoderma* 158, 173–180. <https://doi.org/10.1016/j.geoderma.2010.04.029>.
- Liu, X., Wu, X., Liang, G., Zheng, F., Zhang, M., Li, S., 2021. A global meta-analysis of the impacts of no-tillage on soil aggregation and aggregate-associated organic carbon. *Land Degrad. Dev.* 32, 5292–5305. <https://doi.org/10.1002/ldr.4109>.
- Liu, X., Yang, J., Tao, J., Yao, R., 2022. Integrated application of inorganic fertilizer with fulvic acid for improving soil nutrient supply and nutrient use efficiency of winter wheat in a salt-affected soil. *Appl. Soil Ecol.* 170, 104255 <https://doi.org/10.1016/j.apsoil.2021.104255>.
- Luan, H., Gao, W., Huang, S., Tang, J., Li, M., Zhang, H., Chen, X., 2019. Partial substitution of chemical fertilizer with organic amendments affects soil organic carbon composition and stability in a greenhouse vegetable production system. *Soil Tillage Res.* 191, 185–196. <https://doi.org/10.1016/j.still.2019.04.009>.
- Mangalassery, S., Kalaivanan, D., Philip, P.S., 2019. Effect of inorganic fertilisers and organic amendments on soil aggregation and biochemical characteristics in a weathered tropical soil. *Soil Tillage Res.* 187, 144–151. <https://doi.org/10.1016/j.still.2018.12.008>.
- Morrissey, E.M., Kane, J., Tripathi, B.M., Rion, M.S.I., Hungate, B.A., Franklin, R., Walter, C., Sulman, B., Brzostek, E., 2023. Carbon acquisition ecological strategies to connect soil microbial biodiversity and carbon cycling. *Soil Biol. Biochem.* 177, 108893 <https://doi.org/10.1016/j.soilbio.2022.108893>.
- Niaz, S., Wehr, J.B., Dalal, R.C., Kopittke, P.M., Menzies, N.W., 2022. Organic amendments and gypsum reduce dispersion and increase aggregation of two sodic Vertisols. *Geoderma* 425, 116047 <https://doi.org/10.1016/j.geoderma.2022.116047>.
- Pang, Z., Dong, F., Liu, Q., Lin, W., Hu, C., Yuan, Z., 2021. Soil metagenomics reveals effects of continuous sugarcane cropping on the structure and functional pathway of Rhizospheric microbial community. *Front. Microbiol.* 12 <https://doi.org/10.3389/fmicb.2021.627569>.
- Qi, Y., Liu, H., Zhang, B., Geng, M., Cai, X., Wang, J., Wang, Y., 2022. Investigating the effect of microbial inoculants Frankia F1 on growth-promotion, rhizosphere soil physicochemical properties, and bacterial community of ginseng. *Appl. Soil Ecol.* 172, 104369 <https://doi.org/10.1016/j.apsoil.2021.104369>.
- Qiu, Q., Wang, H., Zhang, Q., Said Mgelwa, A., Zhu, B., Hu, Y., 2022. Negative priming effect from tree leaf and root residues with contrasting chemical composition. *Geoderma* 427, 116118 <https://doi.org/10.1016/j.geoderma.2022.116118>.
- Rafiq, M.K., Joseph, S.D., Li, F., Bai, Y., Shang, Z., Rawal, A., Hook, J.M., Munroe, P.R., Donne, S., Taherymoosavi, S., Mitchell, D.R.G., Pace, B., Mohammed, M., Horvat, J., Marjo, C.E., Wagner, A., Wang, Y., Ye, J., Long, R., 2017. Pyrolysis of attapulgite clay blended with yak dung enhances pasture growth and soil health: characterization and initial field trials. *Sci. Total Environ.* 607–608, 184–194. <https://doi.org/10.1016/j.scitotenv.2017.06.186>.
- Randall, T.E., Fernandez-Bayo, J.D., Harrold, D.R., Achmon, Y., Hestmark, K.V., Gordon, T.R., Stapleton, J.J., Simmons, C.W., VanderGheynst, J.S., 2020. Changes of fusarium oxysporum f.sp. lactucae levels and soil microbial community during soil biosolarization using chitin as soil amendment. *PLoS One* 15, e232662. <https://doi.org/10.1371/journal.pone.0232662>.
- Rkhalila, A., Chtouki, T., Erguig, H., El Haloui, N., Ounine, K., 2021. Chemical properties of biopolymers (chitin/chitosan) and their synergic effects with endophytic *Bacillus* species: unlimited applications in agriculture. *Molecules* 26, 1117. <https://doi.org/10.3390/molecules26041117>.
- Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biol. Biochem.* 34, 1309–1315. [https://doi.org/10.1016/S0038-0717\(02\)00074-3](https://doi.org/10.1016/S0038-0717(02)00074-3).
- Shamshina, J.L., Kelly, A., Oldham, T., Rogers, R.D., 2020. Agricultural uses of chitin polymers. *Environ. Chem. Lett.* 18, 53–60. <https://doi.org/10.1007/s10311-019-00934-5>.
- Sharp, R., 2013. A review of the applications of chitin and its derivatives in agriculture to modify plant-microbial interactions and improve crop yields. *Agronomy* 3, 757–793. <https://doi.org/10.3390/agronomy3040757>.
- Shu, X., He, J., Zhou, Z., Xia, L., Hu, Y., Zhang, Y., Zhang, Y., Luo, Y., Chu, H., Liu, W., Yuan, S., Gao, X., Wang, C., 2022. Organic amendments enhance soil microbial diversity, microbial functionality and crop yields: a meta-analysis. *Sci. Total Environ.* 829, 154627 <https://doi.org/10.1016/j.scitotenv.2022.154627>.
- Song, D., Dai, X., Guo, T., Cui, J., Zhou, W., Huang, S., Shen, J., Liang, G., He, P., Wang, X., Zhang, S., 2022. Organic amendment regulates soil microbial biomass and activity in wheat-maize and wheat-soybean rotation systems. *Agric. Ecosyst. Environ.* 333, 107974 <https://doi.org/10.1016/j.agee.2022.107974>.
- Soria, R., Rodríguez-Berbel, N., Sánchez-Cañete, E.P., Villafuerte, A.B., Ortega, R., Miralles, I., 2023. Organic amendments from recycled waste promote short-term carbon sequestration of restored soils in drylands. *J. Environ. Manage.* 327, 116873 <https://doi.org/10.1016/j.jenvman.2022.116873>.
- Sun, R., Chen, Y., Han, W., Dong, W., Zhang, Y., Hu, C., Liu, B., Wang, F., 2020. Different contribution of species sorting and exogenous species immigration from manure to soil fungal diversity and community assemblage under long-term fertilization. *Soil Biol. Biochem.* 151, 108049 <https://doi.org/10.1016/j.soilbio.2020.108049>.
- Tang, Y., Zhan, T., Fan, G., Huang, J., Zhou, Y., Yuan, X., Li, K., Chen, S., Zhao, X., Hu, C., 2022. Selenium combined with chitin reduced phosphorus leaching in soil with pomelo by driving soil phosphorus cycle via microbial community. *J. Environ. Chem. Eng.* 10, 107060 <https://doi.org/10.1016/j.jece.2021.107060>.
- Tao, Y., Shen, L., Han, S., Li, Z., Cui, Y., Lin, Y., Qu, J., Zhang, Y., 2023. Metagenomic study of carbon metabolism in black soil microbial communities under lead-lanthanum stress. *J. Hazard. Mater.* 446, 130666 <https://doi.org/10.1016/j.jhazmat.2022.130666>.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707. [https://doi.org/10.1016/0038-0717\(87\)90052-6](https://doi.org/10.1016/0038-0717(87)90052-6).
- Wang, H., Zhang, W., Chen, L., Xu, Q., Jiang, Y., Sun, B., 2020. Biochar induced negative priming effect on soil organic carbon mineralisation by changing the microbial community structure across plant growth stages. *J. Soil. Sediment.* 20, 3340–3350. <https://doi.org/10.1007/s11368-020-02662-8>.
- Wang, J., Fu, X., Ghimire, R., Sainju, U.M., Jia, Y., Zhao, F., 2021. Responses of soil bacterial community and enzyme activity to organic matter components under long-term fertilization on the loess plateau of China. *Appl. Soil Ecol.* 166, 103992 <https://doi.org/10.1016/j.apsoil.2021.103992>.
- Wieczorek, A.S., Hetz, S.A., Kolb, S., 2014. Microbial responses to chitin and chitosan in oxic and anoxic agricultural soil slurries. *Biogeosciences* 11, 3339–3352. <https://doi.org/10.5194/bg-11-3339-2014>.
- Winkler, A., Dominguez-Núñez, J., Aranaz, I., Poza-Carrión, C., Ramonell, K., Somerville, S., Berrocal-Lobo, M., 2017. Short-chain chitin oligomers: promoters of plant growth. *Mar. Drugs* 15, 40. <https://doi.org/10.3390/md15020040>.
- Wu, F., Jiao, S., Hu, J., Wu, X., Wang, B., Shen, G., Yang, Y., Tao, S., Wang, X., 2021. Stronger impacts of long-term relative to short-term exposure to carbon nanomaterials on soil bacterial communities. *J. Hazard. Mater.* 410, 124550 <https://doi.org/10.1016/j.jhazmat.2020.124550>.
- Wu, W., Lin, Z., Zhu, X., Li, G., Zhang, W., Chen, Y., Ren, L., Luo, S., Lin, H., Zhou, H., Huang, Y., Yang, R., Xie, Y., Wang, X., Zhen, Z., Zhang, D., 2022. Improved tomato yield and quality by altering soil physicochemical properties and nitrification processes in the combined use of organic-inorganic fertilizers. *Eur. J. Soil Biol.* 109, 103384 <https://doi.org/10.1016/j.ejsobi.2022.103384>.
- Xiao, E., Ning, Z., Xiao, T., Sun, W., Jiang, S., 2021. Soil bacterial community functions and distribution after mining disturbance. *Soil Biol. Biochem.* 157, 108232 <https://doi.org/10.1016/j.soilbio.2021.108232>.
- Xiong, C., Singh, B.K., He, J., Han, Y., Li, P., Wan, L., Meng, G., Liu, S., Wang, J., Wu, C., Ge, A., Zhang, L., 2021. Plant developmental stage drives the differentiation in ecological role of the maize microbiome. *Microbiome* 9 <https://doi.org/10.1186/s40168-021-01118-6>.
- Xue, W., Han, Y., Tan, J., Wang, Y., Wang, G., Wang, H., 2018. Effects of Nanochitin on the enhancement of the grain yield and quality of winter wheat. *J. Agric. Food Chem.* 66, 6637–6645. <https://doi.org/10.1021/acs.jafc.7b00641>.
- Xun, W., Yan, R., Ren, Y., Jin, D., Xiong, W., Zhang, G., Cui, Z., Xin, X., Zhang, R., 2018. Grazing-induced microbiome alterations drive soil organic carbon turnover and productivity in meadow steppe. *Microbiome* 6 <https://doi.org/10.1186/s40168-018-0544-y>.
- Yang, S., Li, C., Xu, C., Wu, D., Wang, J., Zhang, Y., Ai, Y., Li, H., 2020. Effects of adding straw and Attapulgite on soil structure and carbon and nitrogen contents of old Yellow River course. *Bull. Soil Water Conserv.* 2, 199–224. <https://doi.org/10.13961/j.cnki.stbctb.2020.02.029>. (in Chinese).

- Yang, T., Xing, X., Gao, Y., Ma, X., 2022. An environmentally friendly soil amendment for enhancing soil water availability in drought-prone soils. *Agronomy*. 12, 133. <https://doi.org/10.3390/agronomy12010133>.
- Yao, Q., Li, Z., Song, Y., Wright, S.J., Guo, X., Tringe, S.G., Tfaily, M.M., Paša-Tolić, L., Hazen, T.C., Turner, B.L., Mayes, M.A., Pan, C., 2018. Community proteogenomics reveals the systemic impact of phosphorus availability on microbial functions in tropical soil. *Nat. Ecol. Evol.* 2, 499–509. <https://doi.org/10.1038/s41559-017-0463-5>.
- Yu, Z., Ling, L., Singh, B.P., Luo, Y., Xu, J., 2020. Gain in carbon: deciphering the abiotic and biotic mechanisms of biochar-induced negative priming effects in contrasting soils. *Sci. Total Environ.* 746, 141057 <https://doi.org/10.1016/j.scitotenv.2020.141057>.
- Zhan, T., Hu, C., Kong, Q., Shi, G., Tang, Y., Zhou, Y., Guo, Z., Zhai, H., Xiao, X., Zhao, X., 2021. Chitin combined with selenium reduced nitrogen loss in soil and improved nitrogen uptake efficiency in Guanxi pomelo orchard. *Sci. Total Environ.* 799, 149414 <https://doi.org/10.1016/j.scitotenv.2021.149414>.
- Zhang, H., Wang, S., Zhang, J., Tian, C., Luo, S., 2021a. Biochar application enhances microbial interactions in mega-aggregates of farmland black soil. *Soil Tillage Res.* 213, 105145 <https://doi.org/10.1016/j.still.2021.105145>.
- Zhang, H., Yang, J., Zhou, J., Li, G., Zhang, J., 2021b. Effects of organic and inorganic amendments on aggregation and crop yields in sandy fluvo-aquic soil. *Plant Nutr. Fert. Sci.* 27, 791–801. <https://doi.org/10.11674/zwyf.20576>. (in Chinese).
- Zhang, Y., Ye, C., Su, Y., Peng, W., Lu, R., Liu, Y., Huang, H., He, X., Yang, M., Zhu, S., 2022. Soil acidification caused by excessive application of nitrogen fertilizer aggravates soil-borne diseases: evidence from literature review and field trials. *Agric. Ecosyst. Environ.* 340, 108176 <https://doi.org/10.1016/j.agee.2022.108176>.
- Zhao, Z., He, J., Quan, Z., Wu, C., Sheng, R., Zhang, L., Geisen, S., 2020. Fertilization changes soil microbiome functioning, especially phagotrophic protists. *Soil Biol. Biochem.* 148, 107863 <https://doi.org/10.1016/j.soilbio.2020.107863>.
- Zheng, J., Zhang, J., Gao, L., Wang, R., Gao, J., Dai, Y., Li, W., Shen, G., Kong, F., Zhang, J., 2021a. Effect of straw biochar amendment on tobacco growth, soil properties, and rhizosphere bacterial communities. *Sci. Rep.* 11 <https://doi.org/10.1038/s41598-021-00168-y>.
- Zheng, T., Zhang, J., Tang, C., Liao, K., Guo, L., 2021b. Positive and negative priming effects in an ultisol in relation to aggregate size class and biochar level. *Soil Tillage Res.* 208, 104874 <https://doi.org/10.1016/j.still.2020.104874>.
- Zhou, J., Zhang, H., Yang, J., Li, G., Zhang, J., 2020. Effects of continuous application of soil amendments on fluvo aquic soil fertility and active organic carbon components. *Sci. Agric. Sin.* 53, 3307–3318. <https://doi.org/10.3864/j.issn.0578-1752.2020.16.009>. (in Chinese).