Stover and biochar can improve soil microbial necromass carbon, and enzymatic transformation at the genetic level

Yulan Zhang1,2,3 | CaiXia Sun4 | ShuQiang Wang1,5 | Hongtu Xie1 | Nan Jiang1 | Zhenhua Chen1 | Kai Wei6 | Xuelian Bao1 | Xueying Song7 | Zhen Bai1

1Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China
2Key laboratory of Conservation Tillage and Ecological Agriculture, Liaoning Province, Shenyang, China
3National Field Observation and Research Station of Shenyang Agro-Ecosystems, Shenyang, China
4Northeastern University, Shenyang, China
5University of Chinese Academy of Sciences, Beijing, China
6Key Laboratory of Mountain Surface Processes and Ecological Regulation, Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, Chengdu, China
7Key laboratory of Regional Environment & EcoRemediation of Ministry of Education, Shenyang, China

Correspondence
CaiXia Sun, Northeastern University, Shenyang, Liaoning Province 110819, China. Email: caixiasun@mail.neu.edu.cn
Hongtu Xie, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, Liaoning Province 110016, China. Email: htxie@iae.ac.cn

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Abstract
To understand how stover incorporation affects the levels of soil carbon and its biogeochemistry process, we examined soil carbon and amino sugar carbon (AS-C). Activity of N-acetylglucosaminidase (NAGase) and the abundance (real-time quantitative polymerase chain reaction, RT-qPCR) and communities of the bacterial chitinase-encoding gene (chiA) were monitored. We implemented a maize cropping system, which included four treatments, i.e., chopped maize stover returned directly (SD), compost produced by maize stover (SC), biochar produced by maize stover (BC), and stover removal (CK). The results showed that BC and SD enhanced soil organic carbon (SOC), microbial biomass carbon:SOC, total AS-C, glucosamine carbon, galactosamine carbon, NAGase activity, and chiA gene abundance significantly. There were significant positive correlations between the abundance of the soil chiA gene and NAGase activity. Biochar and stover treatments increased the number of total operational taxonomic units (OTUs), unique OTUs, and the chao1 and ACE richness indices, but decreased the Shannon and Simpson diversity indices. The dominant chiA-harboring bacterial phyla were Actinobacteria, Proteobacteria, and Bacteroidetes. Some detectable genera in Actinobacteria had high relative abundance, including Streptomyces, Actinoplanes, and Amycolatopsis. PLS-DA and CCoA revealed that the chiA community in the BC or SD plots was significantly different from that in the CK plots, and differences between BC and SD were not significant. Similar soil properties, diversity, and the abundance of chiA were observed between SC and CK. Greater abundance and diversity of the chiA gene with BC incorporation were associated with higher pH, SOC, and TN concentration. Soil pH was related to the composition and abundances of the chiA-harboring microbial communities (CCA).

KEYWORDS
abundance, diversity, gene community, soil amino sugar carbon, soil enzyme
1 | INTRODUCTION

Soil organic carbon (SOC) is fundamental for soil quality and productivity, crop growth and production, and plant–environment interactions. Owing to multiple nutrients in crop stover, stover return in various forms to the field is increasingly used to improve SOC and quality (Mackie et al., 2015; Sun et al., 2020). Much attention has been paid to the effect of stover on the accumulation and transformation of SOC. Soil amino sugar (AS) and microbial necromass C represent approximately 2%–7% of SOC and are considered to be relatively slowly cycling SOC fractions (Kallenbach et al., 2016; Wang, Qu, et al., 2021). The majority of AS (e.g., glucosamine, galactosamine) originate from the turnover of the soil microbial community and represent a major component of the cell walls of microorganisms (including bacteria and fungi, e.g., chitin, chitosan; Ding et al., 2013). AS turns over faster than SOC and is considered to be more sensitive to soil environmental disturbances (Wang, Liang, et al., 2021; Wang, Qu, et al., 2021). AS is the degradation product of chitin, which is the second most abundant polysaccharide in nature. The degradation of chitin to AS must be completed with the participation soil enzymes. There are few reports on the effect of stover incorporation on soil amino sugar carbon (AS-C) and its enzymatic mechanism including enzyme activity and the abundance and diversity of gene communities.

Soil AS formation and accumulation process are controlled by soil extracellular chitinase (1,4-β-N-acetylglucosaminidase, NAGase, EC.3.2.1.52), which is the dominant factor involved in the production of soil AS from soil chitin decomposition (Terahara et al., 2009). The abundance of soil microbial functional genes controls the mode and degree of the enzyme-catalyzed transformation and degradation of soil organic matter. NAGases are the most widely studied chitin-degrading enzymes; chitinases (EC 3.2.1.14) are categorized as glycoside hydrolases produced by plants and bacteria (Metcalfe et al., 2002). Chitinases originate mainly from bacteria (especially Streptomyces) and plants. Bacterial chitinase is subdivided into groups A, B, and C; group A accounts for 70%–80% of soil chitin degradation and is more abundant in soil than groups B or C (Terahara et al., 2009). Many researchers aimed to determine the relationships between chiA gene diversity and the degradation of soil organic nitrogen. More attention should be paid to the association between chiA gene diversity and NAG enzymatic hydrolysate and enzymatic products, i.e., AS. Soil NAGase activities and chiA gene diversity are related to soil properties such as the pH and organic matter content. The addition of organic materials (such as chitin) to soil stimulated the growth of bacteria with chitinolytic action and changed the bacterial community (Vionis et al., 1996). Ekenler and Tabatabai (2002) reported that NAGase activity was sensitive to the variation in soil pH and was positively correlated with the soil pH. The activity of NAGase was the highest in double-mulched soils but the lowest in residue-abandoned soils (Ekenler & Tabatabai, 2002). Metcalfe et al. (2002) explored the bacterial community diversity of the soil chiA gene with the incorporation of lime and activated sludge. Ekenler and Tabatabai (2003) reported that the addition of lime significantly increased 1,4-β-N-acetyl-glucosamine (NAG) activity. The results showed that Actinobacteria played a major role in chitin degradation, and sludge incorporation enhanced chitinase activity but decreased its diversity. However, the effects of the incorporation of chopped stover, compost, and biochar into soil on AS, related enzyme gene community diversity and the exact mechanisms underlying this phenomenon remain unknown despite their potential importance.

With iterative community turnover under stover return, how microbes contribute to the formation and accumulation of AS is a fundamental and much-debated question related to soil carbon dynamics (Ma et al., 2018). Monitoring and exploring the soil microbial communities related to AS can lead to a better understanding of the soil stable carbon pool (Guo et al., 2016). In our previous work, we found that the SOC, nitrogen, and phosphorous contents were influenced to a remarkable extent by the addition of maize stover biochar (Wang et al., 2017). Biochar has unique properties compared with stover or compost and has different effects on the microbiome (Liu et al., 2014; Luo et al., 2017; Yu et al., 2018). The contents of microbial biomass carbon (MBC) with biochar addition were higher than those after stover treatments on an equal carbon basis, while extracellular enzymes and the stoichiometric ratios of soil microorganisms were significantly lower with biochar incorporation than those stover incorporation into the field (Sun et al., 2021).

The present study speculated that 3 years of stover-return treatments (stover, biochar, and compost from stover) will alter the chiA: (1) community structure and diversity (via NGS) and (2) gene abundance (based on qPCR). We hypothesized that chitinolytic enzyme (NAG) activity and the accumulation of soil microbial residue carbon (AS) would increase with stover incorporation. We also predicted that biochar treatment (BC) would have different impacts on soil compared with treatments with stover compost (SC) and where stover was returned directly (SD).
2 | MATERIALS AND METHODS

2.1 | Site description and set-up of the field experiment

One field experiment with maize stover biochar, compost, and chopped stover was conducted in the spring of 2015. Information on the experiment is described in our previous paper (Li et al., 2020). The experiment was conducted at the National Field Observation and Research Station of Shenyang Agro-ecosystems in a typical temperate agricultural region of the Liaohe Plain, northeastern China (N41°31′, E123°24′). The weather at the site is typical of a temperate, humid, continental monsoon climate. The average temperature ranges between 7.0 and 8.1°C, and the nonfrost period is approximately 153–180 day. The annual average precipitation ranges between 575 and 684 mm, with most rainfall occurring during July and August. The soil type in the experimental field is classified as an Alfisol (meadow brown soil). No plots were treated with fertilizer to ensure uniform soil fertility from 2010 to 2014, and the experiment was implemented in 2015. Before 2010, conventional tillage was practiced for more than 30 years, and all of the above ground maize biomass (above 10 cm) was removed after the annual harvest in late September.

Our field experiment was implemented using a complete randomized block design, which included four treatments with four replicates: CK, i.e., control, stover removal (cut to 10–15 cm) and incorporation of the stubble into the soil by tilling (this is the traditional residue management practice); BC, i.e., maize stover-produced biochar addition (approximately 2000 kg · ha⁻¹ · year⁻¹); SD, i.e., incorporation of chopped stover directly into the soil (approximately 6000 kg · ha⁻¹ · year⁻¹); and SC, i.e., maize stover-produced compost addition (approximately 2000 kg · ha⁻¹ · year⁻¹, made from stover in situ). Each plot consisted of nine rows (0.55 m wide and 30 m long) with a 1 m buffer zone between two adjacent treatments. The maize cultivar “Fuyou 9” was sown at a plant population density of 5.5 plants · m⁻². The experiment was conducted under natural rainfall conditions with conventional NPK application. The application rates were recommended based on initial soil tests and maize requirements. All of the basal fertilizers (90 kg · ha⁻¹ · N 75 kg · ha⁻¹ · P₂O₅; 75 kg · ha⁻¹ · K₂O) were applied annually before the crops were planted, and side-dressing (90 kg · ha⁻¹ · N) was applied 2 months after planting. According to local agronomic practices, intensive management of the maize fields was performed unless otherwise indicated. The tested biochar was prepared by carbonizing corn stover for 2–3 h under low oxygen at 350–500°C. The stover was composted through the addition of decomposing microbial inoculums and was fully decomposed at high temperature for 1–2 months. The basic physicochemical characteristics of the test soil, stover, biochar, and compost are listed in Table 1.

2.2 | Soil sample collection and preparation

During the harvest of the maize in October 2017, we collected a composite surface (0–20 cm) soil sample from each plot from 10 locations that were randomly sampled within a plot. All of the soil fauna, stones, and visible plant materials such as roots in each sample were carefully removed. Samples were mixed evenly and screened (<2 mm screen). A coning and quartering method was used to divide each fresh soil sample into four parts. Part of the sieved samples was rapidly frozen for chiA determination; part of the sieved samples was used for the determination of soil microbial biomass carbon (MBC) within 24 h of sampling; part of the sieved samples was stored at 4°C (for less than 14 day) for assays on enzyme activity; and part of the sieved samples was air-dried at room temperature for further analysis (e.g., pH). Then, parts of the air-dried soils were ground and sieved (<0.15 mm) for SOC, TN, and TP determination.

2.3 | Soil physical and chemical property assays

The soil pH was determined in a soil:water suspension (1:2.5) with a glass electrode. Soil bulk density (ρb) was determined from a core sample (two soil core samples were collected in each microplot), which was obtained by
driving a 100-cm³ stainless steel cylinder into the 0–10 cm soil layer. SOC and total nitrogen (TN) were determined for each air-dried soil sample using an elemental analyzer (Elementar Vario EL III) in the C-N operation mode. The available inorganic nitrogen (AN) was determined by continuous-flow analysis (CFA) after extraction with KCl (2 mol · L⁻¹; Keeney & Nelson, 1982). The soils were analyzed for total phosphorus, following digestion with H₂SO₄-HClO₄ (Koide et al., 2011). Within 24 h of sampling, four field-moist soil subsamples (12.5 g oven-dried equivalent) were prepared, and two portions were fumigated with ethanol-free chloroform at 25°C for 24 h. All of the samples were extracted (50 ml 0.5 mol · L⁻¹ K₂SO₄) after shaking (0.5 h at 200 rpm), the soil suspension was filtered using a Waterman membrane filter and the filtrate was analyzed using automatic TOC analyzer (Elementor). We used extractable OC from fumigated soil minus OC extracted from nonfumigated soil to obtain EOC, and then we calculated MBC (MBC = EOC/K, where K = 0.45).

Soil AS-C was obtained through conversion from the AS concentration, and the proportion of AS-C to SOC was used to reflect the relative accumulation of dead microbial residue carbon in the SOC. The present study measured three fractions of soil AS-C: glucosamine carbon (GluN-C), muramic acid carbon (MurA-C) and galactosamine carbon (GalN-C). (We did not measure mannosamine because of the low content of mannosamine, and the source was not obvious although it can also be separated and quantified). The determination of three individual AS, namely, glucosamine (GluN), galactosamine (GalN), and muramic acid (MurA), was performed by means of gas chromatography (GC) after their conversion to aldonitrile acetates according to published protocols (Liang et al., 2017; Zhang & Amelung, 1996).

2.4 | Soil enzyme activity assays

Substrates of test enzymes were purchased from Sigma-Aldrich, Inc., and J & K Chemical Ltd. Within 2 weeks of sampling, the soil enzyme activities were assayed using colorimetric methods of determination based on an increase or decrease in the concentration of a substrate (Tabatabai, 1994). The N-acetyl-β-D-glucosaminidase (NAGase) activity (EC 3.2.1.30) was estimated by using 4-nitrophenyl-β-D-glucosaminide (SIGMA) as a substrate, in 0.1 acetate buffer at pH 5.2, 50°C for 1 h, using 1 g d.w. soil. The soil activities of β-D-glucosidase (E.C.3.2.1.21, pH 6.0; β-Glu) were assayed using p-nitrophenyl-β-D-glucopyranoside (SIGMA) as a substrate at pH 6.0, 37°C for 1 h, using 1 g d.w. soil, and β-D-galactosidase (E.C.3.2.1.23, pH 6.0; β-Gal) was assayed with p-nitrophenyl-β-D-galactoside (SIGMA) as a substrate at 37°C for 1 h, using 1 g d.w. soil (Tabatabai, 1994; Zhang et al., 2009, 2010).

2.5 | Soil DNA extraction, real-time quantitative PCR analysis, Illumina MiSeq sequencing and data processing

We collected four replicates of soil samples from each treatment to extract soil DNA via a soil DNA extraction Kit, according to the manufacturer’s protocol. The NDA quality was checked on 0.8% agarose gel, and its quantity was assayed using a fluorometer.

Chitinase chiA genes were amplified from DNA and then cloned and sequenced. Real-time quantitative PCR and analyses were conducted on the number of chitinase chiA gene copies (Quant-IT PicoGreen dsDNA Assay Kit, and Microplate reader BioTe, FLx800). The forward primer chi2 (GACGGATCGACATCGATTGG) and reverse primer chir (CSGTCCAGCGCGSCCRTA) were used (Xiao et al., 2005). PCR amplicons were purified with Agencourt AMPure Beads (Beckman Coulter) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen) 0.11.

After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2 × 300 bp sequencing was performed using the Illumina MiSeq platform with MiSeq Reagent Kit v3. Sequence data analyses were mainly performed using QIIME (v1.9.1) and R packages (v3.2.0). Quality filtering of joined sequences was performed, and sequences that did not satisfy the following criteria were discarded: sequence length <200 bp, ambiguous bases, and mean quality score ≥ 20. The effective sequences were grouped into operational taxonomic units (OTUs) using the clustering program VSEARCH (1.9.6) against the Silva 132 database and were pre-clustered at 85% sequence identity (Kielak et al., 2013).

2.6 | Statistical analysis

All of the determinations were performed in quadruplicate, and the values reported are means based on oven-dried soil (105°C). The treatment of the data and the statistical analyses were performed using the SPSS 10.0 statistical software package (SPSS Inc.). For each variable, the data were analyzed with one-way ANOVA, and the means were separated using the least significant difference (LSD) method at p = 0.05. p values lower than 0.05 were considered to be statistically significant. The correlation of the soil parameters was based on Pearson correlation coefficients.
The chiA gene community richness and diversity indices were calculated using Mothur software (version 1.21.1). Principal components analysis was completed using community OTU data. Heatmap analysis was performed to visualize these associations using the R package (version 3.1.1). The differences in the chiA gene community richness and diversity indices among the stover input treatments were determined using one-way ANOVA. The differences in the relative abundance of the chiA gene were compared using Metastats (http://metastats.cbcb.umd.edu/). To identify the significant variations between different treatments, a t-test was performed. Spearman’s correlation coefficient was calculated to explore the associations between members of chiA and enzyme activity using SPSS version 18.0 for Windows (SPSS Inc.). PLS-DA (partial least squares discriminant analysis) was introduced using the R package as a supervised model to reveal the microbiota variation among groups.

3 | RESULTS

3.1 | Effects of Stover and biochar incorporation on SOC, microbial viable carbon, and necromass carbon

The biochar treatment BC had a significant positive effect on SOC, Glu-C, MurA-C, and total AS-C but markedly decreased the water-soluble organic carbon in soil (WSOC):SOC ratio (Figure 1). SD treatments markedly enhanced soil Gal-C and total AS-C. There were no observable differences between the BC or the SD treatment and CK for soil MBC, and also no significantly differences between the SC treatment and CK for all of the tested indices related to soil C.

The incorporation of biochar and chopped stover increased the concentration of total AS-C and the accumulation of its fractions; however, MurA-C in the chopped stover treatment varied little.

3.2 | Effects of stover and biochar incorporation on soil N-acetylglucosaminidase activity, chiA gene abundance, and diversity (richness, community diversity, and structure)

Stover incorporation significantly increased both soil N-acetylglucosaminidase activity (NAGase) and chiA gene copy number (Figure 2). The influence of the stover treatments on NAGase followed the order SD > BC ≈ SC (Figure 2a). The abundance of the chiA gene in the SD, BC, and SC treatments was 2.7-, 1.8-, and 1.5-fold, respectively, of that in CK (Figure 2b). The average copy numbers ranged from 5.46 to 14.6 × 10^7 per g dry soil. Pearson’s correlation analysis showed that the chiA gene abundance was positively correlated with soil SOC, C:N, MBC, AS-C, Gal-C, and NAGase activities (Table 2).

3.3 | Effect of stover and biochar incorporation on the richness, community diversity, and structure of the soil chiA gene diversity

Operational taxonomic units were classified at 85% nucleotide similarity cut off (Kielak et al., 2013). The number of OTUs based on the rarefaction of 1200 randomly sampled sequences was used to represent the diversity. The number of OTUs varied depending on samples (Figure 3a). The number of total OTUs was greatest in BC (3472), followed by SD (3415) and then CK (3167) and SC (2914), i.e., SD > BC > SC ≈ CK. Significant variations were observed in the total OTU number among areas with BC and where SD was returned (p < 0.01), but no significant difference was found between the SC and CK treatments. The number of unique OTUs in the BC, SD, CK, and SC treatments was 992, 986, 917, and 694, respectively. Shared OTUs (1005) accounted for 31.7%, 28.9%, 34.5%, and 29.4% of the total number in the respective CK, BC, SC, and SD samples.

The treatments showed similar chitinase-secreting microorganism phyla composition (Figure 3b). The dominant chiA-harbouring bacterial phyla in all of the treatments were Actinobacteria, Proteobacteria, and Bacteroidetes. Four other typical phyla were Acidobacteria, Firmicutes, Planctomycetes, and Chordata. The relative abundance differed among the SD, BC, and SC treatments. Single factor analysis of variance showed that the Proteobacteria abundance of BC was the highest, and that in the SC treatment was the lowest (p < 0.05; Figure 3c). SC significantly decreased the abundance of Proteobacteria and Bacteroidetes.

The most abundant chiA-harbouring bacterial genera were Streptomyces, Actinoplanes, Amycolatopsis, Burkholderia, and Lysobacter (Figure 4a). Stover incorporation affected 12 genera significantly (Figure 4b). The genera Streptomyces and Actinoplanes produce Actinomycin, and a number of other antibiotics. SD and BC incorporation had a slight effect on the most abundant genera, Streptomyces, and Amycolatopsis, while SC increased these genera. Stover incorporation did not affect the second most abundant genus Actinoplanes (Actinobacteria). BC incorporation enhanced the abundance of the third most abundant genus and the fourth most abundant...
genus *Burkholderia* (Proteobacteria) and *Cellulomonas*, respectively. Stover treatments decreased the abundance of *Aeromonas* (Proteobacteria), *Janthinobacterium* (Proteobacteria), and *Candidatus Symbiobacter*, and SC decreased more genera, including *Kitasatospora*, *Rhodothermus*, *Saccharopolyspora*, *Saccharopolyspora*, *Serratia*, and *Sporangium*.

The 20 most abundant groups at different levels (phylum to genus) were shown using a classification-level tree graph, and the samples showed similar taxa compositions for the relative abundance at the phylum and genus levels. From the tree diagram of the total sample classification generated using GraPhlAn (Figure 5), we learned that Actinobacteria (*Streptomyces*, *Actinoplanes*, and *Amycolatopsis*), Bacteroidetes, and Betaproteobacteria (*Burkholderiaceae*) were dominant populations. Linear discriminant analysis (Lefse; Figure 5) also showed that the CK treatment had dominant *Janthinobacterium* and *Oxalobacteraceas*. *Burkholderiaceae* had a higher LDA score in BC treatment than *Enterobacterales* had in SD treatment.
3.4 Similarity/difference analysis of the community structure between the stover and biochar treatments

Similar to the patterns of the OTU density, the Chao 1 or ACE index of BC and SD (2793 and 2549) was higher than that of SC and CK (2382 and 2243; Figure 6a). The Shannon and Simpson indices showed the opposite trend to Chao and ACE, and BC and SD slightly decreased the bacterial chiA community diversity.

A comparison of the β-diversity (partial least squares discriminant analysis, PLS-DA) revealed that samples belonged to four groups (Figure 6b), i.e., the chiA community structure differed among the four treatments. CK, BC, and

**TABLE 2** Pearson’s correlation between total chiA gene copy numbers and various soil variables

|               | pH  | pb  | SOC  | TN  | TP  | C:N  | WSOC | MBC  | AS-C | GluN-C | GalN-C | MurA-C | NAGase |
|---------------|-----|-----|------|-----|-----|------|------|------|------|--------|--------|--------|--------|
| chiA Gene     | 0.465 | −0.398 | 0.543* | 0.074 | −0.211 | 0.578* | −0.128 | 0.544* | 0.508* | 0.448  | 0.569* | 0.004  | 0.952**|
| copies        |     |     |      |     |     |      |      |      |      |        |        |        |        |

Different lowercase letters above the columns indicate significant differences among organic matter treatments at \( p < 0.05 \). *\( p < 0.01 \); *\( p < 0.05 \).

Abbreviations: AS-C, amino sugar carbon; GalN-C, galactosamine carbon; GluN-C, glucosamine carbon; MBC, microbial biomass carbon; MurA-C, muramic acid carbon; NAGase, N-acetylglucosaminidase; pb, soil bulk density; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus.
SD overlapped with SC, meaning that SC had few differences with the other treatments. The samples belonging to the BC or SD groups were further apart from the CK groups than SC, indicating that BC and SD had a more evident shift than SC did in comparison with CK. The BC and SD groups overlapped in a larger area, i.e., when compared with CK, SD induced a shift of bacterial $\text{chiA}$ gene communities that was similar to that induced by BC.

Samples were clustered according to the similarity of their composition with each other and were then arranged horizontally according to the clustering results. Classification units were also clustered according to the
similarity of their distribution in different samples and were arranged vertically according to the clustering results (Figure 7). Heatmap analysis showed that treatments could be divided into two groups, one for CK and SC and the other for the BC and SD treatments. Tested genera were divided into three clusters. In cluster 1, SC had a similar influence on all genera as CK, whereas the SD treatment had a low abundance of 15 genera. The genus abundance of the first cluster in BC was low except for Lysobacter and Stenotrophomonas. In the second cluster, almost all genera had low abundance in SC; nearly half of the genera had high abundance in SD or BC, although the biome at the genus level was different. In the third cluster, the stover incorporation treatments weakened the bacterial communities with respect to the chiA gene.

3.5 | Relationship between the soil chiA gene community compositions and soil properties

The top 30% of the genera was selected for plotting to analyze the relationship between the soil chiA community structure compositions and the tested soil physicochemical properties (Figure 7). The community in the BC or SD plots was significantly different from that in the CK plots, and the main influential factors may be pH and TP. In the CK treatment, soil AN and the AN:TN ratio had a closer correlation with the chiA community structure. Soil pH and TN were more important in shaping the chiA community structure in the BC soil. The soil C, N, and C:N ratio affected the chiA community structure in the SC plots. The relative distribution of the chiA community in the SD plots indicated that all of these physical and chemical factors were correlated with the chiA community structure. Soil pH and TN were more important in shaping the chiA community structure in the BC soil. The soil C, N, and C:N ratio affected the chiA community structure in the SC plots. The relative distribution of the chiA community in the SD plots indicated that all of these physical and chemical factors were correlated with the chiA community structure in the SD treatment. The possible reason may be the regeneration of microorganisms in SD soils. Lysobacter and Burkholderia were significantly correlated with soil pH (Figure 7b). Nonomura was significantly correlated with soil pH and TP. Myxococo was highly correlated with TP.

4 | DISCUSSION

4.1 | Enhancement of the SOC fractions and physicochemical properties

In general, the SOC content is affected by soil type, plants, the amount and nature of organic amendments and other factors. In line with our initial hypothesis, biochar had significantly positive effects on SOC, Glu-C, MurA-C, and total AS-C, similar to previous studies on soil chemical and physical properties (Liu et al., 2021;
The content of SOC under biochar incorporation was higher than that in the chopped stover or compost treatment, possibly because it's unique properties include high carbon content, the ability to absorb and retain carbon and resistance to decomposition (Wang et al., 2015). On the other hand, the mineralization of biochar or compost was lower than that of the chopped stover treatment (Siedt et al., 2021; Zavalloni et al., 2011). The biochar and the chopped stover plots preserved more microbial biomass carbon and microbial residue carbon than the compost plots. Such differences may be because the compost-added soil had fewer available nutrients than the stover-added soil, and compost degraded faster than the same weight of biochar (Sun et al., 2020). The chopped stover incorporation created a nutrient-rich micro-ecological soil environment and provided abundant available carbon, nitrogen, and other nutrient sources for soil microorganisms. The growth and reproduction of more microorganisms were promoted and thus the flora and quantity of the soil microbial changed. Stover incorporation improved the soil biological activity (Six et al., 2002; West & Six, 2007).

Soil MBC accounted for only a small portion of SOC in our results, which meant that soil microbial biomass was low. Both biochar and chopped stover incorporation enhanced soil MBC lightly, i.e. there were no distinct differences for MBC between CK and the biochar plots or the chopped stover plots. Both the insignificant decrease in WSOC and the significant increase in SOC in the BC treatment markedly decreased the WSOC:SOC ratio. We speculated that BC contained less available C.

The content of GluN-C was dominant in microbial necromass C, which proved that fungi dominated in microbial residues and contributed more C to the necromass carbon pool than bacteria. BC and SD incorporation enhanced not only the concentration of total AS-C, but also the accumulation of its fractions, with
the exception of MurA-C in SD, which had few variations. The BC and SD treatments enhanced the net accumulation of soil AS-C, which can be accumulated to supplement the carbon pool with a sufficient carbon source (Liang, Zhang, & Balser, 2007; Liang, Zhang, Rubert, et al., 2007). Liang, Zhang, and Balser (2007) and Liang, Zhang, Rubert, et al. (2007) speculated that greater microbial synthesis was the reason, and the nutrient amount was the primary attribute. The results showed that the SD and BC treatments increased the soil pH, and soil pH have positive impacts on SOC increase (Table S1). Increases in SOC, TN, TP, C:N, and C:P in BC also benefited soil C accumulation. Liang, Zhang, and Balser (2007) and Liang, Zhang, Rubert, et al. (2007) pointed out that organic material with a higher C:N ratio appeared to sustain the net AS production for a longer period. We inferred that the quantity of organic materials added was not sufficient and the addition time was not long enough to enhance bulk density distinctly, and we plan to test this conclusion in further research. The results for the SC treatment were contrary to our expectations. The SC treatment had little impact on the soil C indexes, TN accumulation, and pH. These results for the SC treatment may be due to its lower pH and fewer amounts input organic materials compared with the SD and BC treatments. BC or SD incorporation altered the soil temperature and moisture, oxygen supply, water potential within microbial cells and other environmental factors (Obia et al., 2020).

4.2 | Soil NAGase activity, chiA gene abundance, and community diversity

Chitinases in the form of different isoenzymes secreted by organisms are usually expressed at low levels under farmland ecosystem, and various chitinases can be induced by oligomers degraded from chitin. Organic material incorporation improved the soil biological activity related to soil carbon transformation, including not only MBC, but also the activity of the NAG enzyme and the abundance of chiA. The SD treatment provided a higher nutrient content and had the greatest enhancement effect on NAGase activity and the chiA gene ($p < 0.01$). Stover had more total and available nutrients than biochar and compost, and provides more nutrients during its composting process in soil. Thereby, stover increased the bacterial abundance and extracellular enzyme activities. As previously reported, the presence of chitin enhanced the production of chitinases (Nawani & Kapadnis, 2005). The soil nutritional status (e.g., TOC, TN, and TP) positively influenced the bacterial chiA abundance, only soil pH was the predominant factor for the bacterial chiA abundance and structure. The absolute function of the chitin-C cycle increased greatly, which can increase the content of soil available C. Soil MBC and the response of soil NAGase activity and chiA gene abundance to SD are determined by the nutrient-rich micro-ecological soil environment, abundant carbon, and nitrogen sources for soil microorganisms and the promotion of the growth and
reproduction of more microorganisms. Consequently, it is also reasonable that chiA gene abundance was significantly associated with NAGase activity in our study.

Stover incorporation shifted the genetic constitution (unique OTUs) and still sustained most similar microbiome (shared OTUs). Shared OTUs (1005) accounted for nearly 30% of the total number in all each treatment (BC-phylum exhibit disease-suppression activity, such as de-thesis, and their high abundance is vital for crop growth (unique OTUs) and still sustained most similar microbiome. The results suggested the significantly associated with NAGase activity in our study.

Three treatments significantly increased the abundance, richness, and diversity of the chiA gene. We distinguished the chiA gene community structure (i.e., β-diversity) between the CK and stover incorporation samples to some degree. The results suggested the chiA gene richness (i.e., α-diversity) of CK was preserved in soil in which BC, SC, and SD were incorporated. According to PLSDA and the heatmap, the chiA gene OTUs clustered into two groups. SD and BC were grouped together and coincided, meaning that they had a relatively large impact on the chiA community and had lower biodiversity; however, the BC and SD treatments increased the community richness. SC and CK were grouped together, and the distance between the SC treatment and CK treatment was relatively close, i.e., SC addition had little impact on the chiA community and had higher biodiversity compared with CK. Stover incorporation improved the colony structure related to the chitin carbon cycle, and the colonies gradually flourished. Sun et al. (2015) pointed out that higher bacterial diversity was observed with organic matter than with chemical fertilizer alone. We propose that higher bacterial diversity might be responsible for the increase in the chiA gene diversity, while our study about the response of soil bacterial richness and diversity in the rhizosphere was different with the chiA gene (Li et al., 2020). We should thoroughly study the relationship between the chitinase gene and bacteria in rhizosphere soil and bulk soil.

The most dominant phyla that secreted the chiA gene were Actinobacteria and Proteobacteria. The members of Actinobacteria play major roles in the process of decomposing and utilizing a wide range of complex organic molecules (Bouskill et al., 2010; Singh et al., 2018). Members of Actinobacteria have been reported to facilitate the horizontal transfer of genes related to photosynthesis, and their high abundance is vital for crop growth (Ma et al., 2018). Furthermore, many taxa within this phylum exhibit disease-suppression activity, such as defending plant roots from fungal pathogen infection, which may improve the health of the plant and soil (Chen & Sinsabaugh, 2020). Stover incorporation did not distinctly affect the abundance of Actinobacteria with respect to chiA, meaning the above ability was not changed by stover incorporation. The results of the bacterial changes in response to stover incorporation are contradictory to those observed for the chiA gene (Banerjee et al., 2016; Zhao et al., 2019). Li et al. (2020) reported that Actinobacteria have a copiotrophic nature, and stover treatments increased the relative abundance of Actinobacteria in rhizosphere soil. Other researchers reported contradictory results regarding the abundance, reproduction rate, community composition, and diversity of soil bacteria in response to stover and biochar (Graber et al., 2010; Liu et al., 2017; Luo et al., 2017; Tian et al., 2016). The contradictory results may be due to the C:N ratio, residual stover texture and percentage, input and aging periods, soil type, and soil physiochemical properties (Li et al., 2018; Ma et al., 2016; Zhang et al., 2017).

The abundance of the third most dominant phylum (Bacteroidetes) that secreted the chiA gene in SC was significantly lower than that in CK, and SD and BC did not differ significantly from SC or CK. Members of Bacteroidetes have the ability to secrete a diverse array of carbohydrate-active enzymes (CAZymes), which target a wide variety of glycans in the soil. The difference in the Bacteroidetes community between SC and other plots may be due to the composition of compost and the fewer glycan substrate amounts. Acidobacteria was not the dominant population with respect to the chiA gene community. Our previous study found that Acidobacteria formed the second largest group of bacteria, and stover treatments decreased its abundance (Li et al., 2020). Banerjee et al. (2016) also pointed out that Acidobacteria were the keystone taxa during organic matter decomposition in arable soil. As oligotrophic bacteria, members of Acidobacteria are highly abundant in agricultural soils and their abundance was reported to be negatively correlated with soil nutrients; in addition, the Acidobacteria population increased with a decrease in the soil pH (Ma et al., 2018). However, the abundance of Acidobacteria varied little. The variation in Acidobacteria secretin chiA and the distribution of Acidobacteria among bacteria need to be explored.

Some detectable genera in Actinobacteria had high relative abundance, including Streptomyces, Actinoplanes, and Amycolatopsis. Those genera generate Actinomycin and a number of other antibiotics. Stover incorporation increased or slightly affected the Actinobacteria. The abundance of Aeromonas (Proteobacteria), Janthinobacterium (Proteobacteria), and Candidatus Symbiobacter (Firmicutes) decreased in the presence of stover, biochar, and compost. Biochar incorporation enhanced the abundance of Burkholderia (Proteobacteria) and Cellulomonas (Actinobacteria). Burkholderia fixes nitrogen, releases phosphorus and potassium, and inhibits pathogenic bacteria. The Cellulomonas genus secretes
enzymes to break down cellulose, glucose, arabinose, and others. Compost treatment decreased more genera, including Kitasatospora, Rhodothermus, Saccharopolyspora, Saccharopolyspora, Serratia, and Sporangium.

In sum, stover incorporation significantly affected the microbial community that secretes the chiA gene. The most important environmental factors were pH and TP, and we speculated that pH is an important environmental factor that affects the soil microbial community structure.

5 | CONCLUSIONS

In general, the SOC content was the most important factor regulating the pattern of microbial carbon utilization. Owing to the greater contents of available C, pH and nutrients, stover and biochar application resulted in the highest amount of SOC 9.7% and 13%, soil necromass carbon 10.5% and 5.8%, stover and biochar application also enhanced enzymatic activity, and enzymatic transformation of soil necromass carbon at the genetic level. The growth and propagation of some microbes that secrete chiA were improved, and the gene community abundance, richness, and diversity were reshaped. Test soil with stover incorporation did not result in the formation of oligotrophic habitats, as evidenced by the microbial flora Actinobacteria, which were the dominant chiA-harboring bacterial phyla. The dominant phyla in test samples were similar, while the relative phylum abundance was significantly different. The predominance or disappearance of some microbial populations resulted from interspecific competition under the addition of different types of organic matter. The enzyme-catalyzed transformation and degradation of organic matter in soil ecosystems are likely to be driven by microbial functional gene abundance as well as enzyme activity, which were evidenced by a positive correlation between the soil chiA gene abundance and NAGase activity. Linking the vast database of microbial functional genes to a variety of different enzyme activities remains a major challenge. Future studies should focus on the gene communities related to carbon transformation and the relationships between genes and soil properties.

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CONFLICT OF INTEREST

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

DATA AVAILABILITY STATEMENT

All data, models, or code generated or used during the study are available in a repository or online in accordance with funder data retention policies (http://doi.org/10.57760/sciencedb.01819).

ORCID

Yulan Zhang © https://orcid.org/0000-0002-4041-9645

CaiXia Sun © https://orcid.org/0000-0003-1390-9814

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