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Host-induced silencing of a nematode chitin synthase gene decreases abundance of rhizosphere fungal community while enhancing *Heterodera glycines* resistance of soybean

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ABSTRACT

Context. A transgenic variety of soybean (*Glycine max* (L.) Merr.), H57, has been developed from wild-type variety Jack, with host-induced gene silencing of a chitin synthase gene (*CHS*) in soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe), a devastating pathogen in soybean. H57 needs to be characterised for suitability to manage SCN, especially because rhizosphere microbial communities may be sensitive to genetically modified crops. **Aims.** We aimed to evaluate the SCN resistance of H57 at the T_7 generation, and analyse the impact on the rhizosphere microbial community of planting H57 into SCN-infected soil. **Methods.** Infection with SCN was assessed at 60 days after planting of H57 and Jack into SCN-infected soil by examining recovered cysts from rhizosphere soil and comparing with an infected bulk soil control. For analysis of rhizosphere microbial communities (bacterial and fungal), *16S* and ITS amplicons were identified by high-throughput sequencing, and bioinformatic analysis was used to define operational taxonomic units. Alpha diversity, using five indexes, and relative abundance were determined. **Key results.** Soybean H57 showed significantly enhanced and heritable resistance to SCN compared with Jack. The diversity and richness (abundance) of the bacterial community of H57 and Jack were significantly and similarly increased relative to the bulk soil. The fungal community of H57 had considerably lower abundance than both other treatments, and lower diversity than the bulk soil. The relative abundance of only two bacterial phyla (Acidobacteria and Actinobacteria) and one fungal phylum (Glomeromycota), and three bacterial genera (*Candidatus*_Solibacter, *Candidatus*_Udaeobacter and *Bryobacter*) and one fungal genus (*Aspergillus*), differed significantly between rhizosphere soils of H57 and Jack. **Conclusions.** Host-induced gene silencing of *SCN-CHS* substantially and heritably enhanced SCN resistance in soybean, did not significantly alter the rhizosphere bacterial community, but greatly suppressed the abundance of the rhizosphere fungal community, which was likely associated with boosted SCN resistance. **Implications.** This study established a basis for interaction research between soybean with *SCN-CHS* host-induced gene silencing and the rhizosphere microbial community, and for potentially planting soybean H57 to manage SCN.

Keywords: abundance, chitin synthase, diversity, host-induced silencing of gene, rhizosphere microbial community, SCN resistance, soybean, transgenic soybean H57.

Introduction

Soybean (*Glycine max* (L.) Merr.) is a major crop globally, as an important source of sustainable supply of protein and oil. A steady increase in soybean production is required to meet growing world food and energy demands. Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is one of the most economically damaging pathogens in soybean worldwide, significantly constraining soybean production and causing huge yield losses annually (Peng *et al.* 2021). SCN secretes a series of effectors

mainly through a spear-like feeding structure called a stylet, penetrating host root cell walls and inducing the formation of complex feeding sites (syncytia) near the vascular cylinder, from which SCN obtains nutrition. Aboveground symptoms are not always visible after infection by SCN, meaning that infection is usually recognised at later stages of infection when significant damage has already occurred; the nematode is difficult to eradicate, and damage is often devastating (Niblack *et al.* 2006). Applications of fertilisers and pesticides provide some mitigation, but the use of improved cultivars with underlying resistance to pathogens is the most attractive option for farmers in soybean production (Kim *et al.* 2016; Shaibu *et al.* 2020).

Host-induced gene silencing (HIGS) has shown potential for controlling various pathogens, through generation of transgenic plants carrying hairpin constructs against the target genes in the harmful organisms (Mao *et al.* 2007; Zhang *et al.* 2016). For instance, HIGS targeting of fungal genes is reported to be an efficient strategy for controlling the fungal diseases caused by *Blumeria graminis*, *Fusarium graminearum* and *Verticillium dahlia* (Nowara *et al.* 2010; Cheng *et al.* 2015; Zhang *et al.* 2016). A high level of accumulated small interference RNAs (siRNAs) played an important role in the defence of HIGS cotton plants against pathogenic *V. dahlia* (Zhang *et al.* 2016). However, there are only a few preliminary studies on the application of HIGS against plant parasitic nematodes. In eggplant, HIGS targeting three nematode effector genes (*Mi-msp-1*, *Mi-msp-18* and *Mi-msp-20*), whose transcripts were accumulated in subventral pharyngeal gland cells of *Meloidogyne incognita*, conferred resistance to the nematode (Shivakumara *et al.* 2017; Chaudhary *et al.* 2019). In hairy roots of soybean, HIGS targeting six reproduction or fitness-related genes (*Cpn-1*, *Y25*, *Prp-17*, *J15*, *J20* and *J23*), showed a significant suppression of SCN populations; however, HIGS was confined to transient composite soybeans, and not heritable (Li *et al.* 2010; Tian *et al.* 2016). To date, HIGS soybean lines with broad-spectrum and durable resistance to *H. glycines* have not been reported.

Chitin is a linear polymer of *b*-(1,4)-linked *N*-acetylglucosamine (GlcNAc) synthesised by chitin synthase (Chs) that is present in fungi and nematodes but absent in plants and vertebrate animals (Kong *et al.* 2012). Therefore, Chs is widely considered an important target for designing new fungicides and developing novel resistant crop varieties via HIGS (Kong *et al.* 2012; Cheng *et al.* 2015). Our previous studies demonstrated three *CHS* genes (*MoCHS1*, *MoCHS6* and *MoCHS7*) in a model phytopathogenic fungus *Magnaporthe oryzae* as potential targets for developing novel fungicides (Kong *et al.* 2012). HIGS wheat lines targeting a *CHS* gene (*Chs3b*) in *F. graminearum* showed durable resistance to Fusarium head blight and seedling blight, even the HIGS-T_5 generation showing strong resistance (Cheng *et al.* 2015).

Interactions between plants and rhizosphere microorganisms are vital in carbon sequestration, ecosystem functioning and nutrient cycling in natural ecosystems as well as agricultural and forest systems (Singh *et al.* 2004), and the rhizosphere microbial community is often influenced by plant genotype and environmental conditions (Filion 2008). Plants interact with soil microorganisms, thereby affecting rhizosphere microbial community dynamics (Wu *et al.* 2014). Diversity changes in rhizosphere microbial communities may influence bio- and geo-chemical processes in soil ecology (Chauhan *et al.* 2014; Kostov *et al.* 2014). Rhizosphere microbial communities may be very sensitive to genetically modified (transgenic) crops (Gao *et al.* 2015). Therefore, the structure/diversity and abundance of rhizosphere microbial communities are often used as an early and sensitive indicator for assessing the effects of transgenic crops on soil ecology (Liang *et al.* 2018).

We recently cloned a soybean cyst nematode chitin synthase gene (*SCN-CHS*), and generated transgenic soybean lines with HIGS of *SCN-CHS,* using soybean variety Jack as the wild-type. The T_2 and T_6 generations of the three homozygous *SCN-CHS* HIGS lines that we obtained all showed enhanced resistance to SCN (Kong *et al.* 2022). In the present study, the main aims were (i) to evaluate the resistance in the T_7 generation of one of the transgenic soybean lines, H57, to SCN (*H. glycines*) and its inheritance; and (ii) to analyse whether and what in detail HIGS of *SCN-CHS* alters the rhizosphere microbial community, by using high-throughput sequencing and analyses of bacterial *16S* rRNA genes and fungal internal transcribed spacer (ITS) sequences. The results obtained will provide a basis for further research on the interactions between *SCN-CHS* HIGS soybean and the rhizosphere microbial community, and for extensive planting of *SCN-CHS* HIGS soybean for effective management of SCN.

Materials and methods

Soybean lines

The sole *CHS* gene with 3984 bp (GenBank Acc. No.: OK149168) was cloned from *H. glycines* HG Type 1.2.3.5.7 and designated as *SCN-CHS*. SCN-Chs contains a typical chitin synthase catalytic domain (Chs) and seven transmembrane domains (TMs). A 420 bp cDNA fragment of the SCN-Chs core region positioned at 1936–2355 bp was cloned and induced into soybean variety Jack to generate HIGS transgenic soybeans by Dabeinong Science and Technology Group Co. Ltd., China (Kong *et al.* 2022). H57 was one of the homozygous transgenic soybean lines identified. H57 was raised to the T_7 generation by our laboratory in the greenhouse of Langfang Experimental Base of Institute of Plant Protection, Chinese Academy of Agricultural Sciences,

for the following experiments. Jack was used as the control in this study.

Soybean growth, SCN life cycle and soil sampling

Seeds of the T_7 generation of H57 and Jack (as control) were planted in six pots filled with SCN-infected soil collected from a field that was severely infected with SCN HG Type 0, with ~6000 eggs (40 cysts)/100 g dry soil, in Jiayin County, Yichun City, Heilongjiang Province, China. Plants were grown in a greenhouse under conditions of 16 h light at 28°C and 8 h dark at 26°C. A plot with six pots comprising the same soil with no planting was used as a bulk soil control.

After SCN eggs develop into first-stage juveniles (J1s) and J2s in the eggs, the parasitic J2s (par-J2s) hatch from the eggs and penetrate the soybean roots, migrating near to the vascular cylinder to induce the formation of syncytia. The par-J2s finish their life cycle through successive J3, J4 and adult (female and male) stages by obtaining nutrition from syncytia. Each female can fill up with ≥200 eggs after mating with males on the root surface, and finally, the egg-filled females die and their body wall hardens to form a tough cyst around the eggs.

At 60 days after planting, soil samples were collected from the rhizosphere of soybean-planted plots and from the bulk soil plot, as described previously (Jin *et al.* 2021). Six soybean seedlings were collected from H57 and Jack plots. Roots were shaken free of soil, and rhizosphere soil fractions were brushed and pooled into one sample (one replicate). Six replicates of soil samples were collected for each treatment (bulk soil, and rhizosphere soil of H57 or Jack); in total, 18 soil samples were obtained and used for SCN-infection phenotype evaluation and sequencing.

SCN-infection phenotype of soybeans

Cysts were collected from the rhizosphere soil samples of each treatment by suspending the soil in water and filtering the samples through nested sieves of 710 μm and 250 μm apertures. The recovered cysts were counted under an SZ61 stereo microscope (Olympus, Tokyo, Japan). The full shape of cysts was captured to measure their length and width. Ten plump cysts of similar size were randomly selected from each treatment. They were placed on a slide with a drop of water and gently punctured with tweezers, and the number of eggs in each cyst was counted under the stereo microscope.

High-throughput sequencing

Genomic DNA was extracted from each soil sample by using a Power Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the protocol of the manufacturer. Extracted DNA was quantified by ratios of 260 nm/280 nm and 260 nm/230 nm. The V3–V4 region of the bacterial *16S* rRNA gene was amplified using the common primer pair (forward primer 5′-ACTCCTACGGGAGGCAGCA-3′; reverse primer 5′-GGACTACHVGGGTWTCTAAT-3′) and the fungal ITS1 region using the primer pair (forward primer 5′-CTTGGTCATTTAGAGGAAGTAA-3′; reverse primer 5′-GCTGCGTTCTTCATCGATGC-3′) combined with adapter sequences and barcode sequences. PCR amplification was conducted in a total volume of 50 μL, which contained 10 μL buffer, 0.2 μL Q5 High-Fidelity DNA Polymerase, 10 μL High GC Enhancer (New England Biolabs, Ipswich, MA, USA), 1 μL dNTP, 10 μM each primer and 60 ng genomic DNA. Thermal cycling conditions were as follows: an initial denaturation at 95°C for 5 min, followed by 15 cycles of 95°C for 1 min, 50°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 7 min. The PCR products from the first-step PCR were purified using VAHTS DNA Clean Beads (Vazyme Biotech, Nanjing, China). A second-round PCR was then performed in a 40-μL reaction volume containing 20 μL 2× Phusion HF MM (Thermo Fisher Scientific, Waltham, MA, USA), 8 μL ddH_2O, 10 μM each primer and 10 μL PCR products from the first step. Thermal cycling conditions were as follows: an initial denaturation at 98°C for 30 s, followed by 10 cycles of 98°C for 10 s, 65°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 5 min. Finally, all PCR products were quantified using Quant-iT dsDNA HS Reagent (Thermo Fisher Scientific) and pooled. High-throughput sequencing analysis of bacterial rRNA genes and the fungal ITS region was conducted on the purified and pooled sample using the HiSeq 2500 platform (Illumina, San Diego, CA, USA) (2× 250 paired ends) at Biomarker Technologies, Beijing, China.

Data analyses

The raw tags were generated by FLASH software (version 1.2.7; Center for Computational Biology, Washington, DC, USA) based on the overlap of paired-end reads, and filtered by Trimmomatic software (version 0.33; Bolger *et al.* 2014) to obtain the clean tags. Effective tags were acquired by removing chimera sequences from the clean tags using UCHIME software (version 4.2; Edgar 2017), and then clustered as operational taxonomic units (OTUs) using QIIME software (ver. 1.8.0; Caporaso *et al.* 2010). OTUs were annotated according to Silva database (https://www.arb-silva.de/), and different levels of classification tables were also generated.

Alpha diversity was applied to reflect the richness and diversity of species through five indexes: observed species, Shannon, Simpson, Chao1 and Ace. Community structure was analysed using principal components analysis (PCA). Analyses were performed using BMKCloud (http://www.biocloud.net) at the OTU level. The coordinate graphs were redrawn in RStudio (ver. 3.5.3; RStudio, Boston, MA, USA) based on the PCA coordinates and explanatory information. The relative abundance of phyla was analysed by BMKCloud to show the top 10 bacterial and fungal phyla based on their

overall abundance in the bulk soil, and the rhizosphere soils of Jack and H57. Analysis of variance was performed using SPSS Statistics 22 (IBM, Armonk, NY, USA). Duncan's Multiple-Range tests were performed to test for significant difference among treatments. All the statistical tests were performed using $P = 0.05$ as the level of significance.

Results

SCN-CHS HIGS soybean H57 showed enhanced resistance to *H. glycines*

Soybean H57 is one of three generated homozygous *SCN-CHS* HIGS lines whose T2 and T6 generations show enhanced SCN resistance over the wild-type variety Jack, with significant silencing (suppression) of *SCN-CHS* in nematodes after infection (Kong *et al.* 2022). Here, the T_7 generation of *SCN-CHS* HIGS soybean H57 was evaluated for its heritable resistance to SCN in the greenhouse. Relative to Jack, the number of *H. glycines* cysts (Fig. 1*a*), number of eggs per cyst (Fig. 1*b*) and diameter of cysts (Fig. 1*c*) in H57 were significantly ($P < 0.05$) reduced by 76.7%, 21.0% and 6.7%, respectively, at 60 days post planting. Therefore, *SCN-CHS* HIGS soybean H57 showed enhanced resistance to *H. glycines* over the variety from which it was generated.

Sequencing summary

High-throughput sequencing of 18 samples yielded 1 440 762 raw reads of the *16S* rRNA gene. After quality filtering, 1 434 149 clean reads remained. In total, 1 394 746 effective reads were obtained for further OTU clustering. Sequence clustering yielded 2034 OTUs, which were assigned to 36 phyla, 94 classes, 209 orders, 342 families, 586 genera and 636 species. On the other hand, 1 411 108 raw fungal ITS gene sequences were obtained after sequencing of 18 samples. After quality filtering, 1 390 318 clean reads remained, with 1 382 619 effective reads acquired for further OTU clustering. Sequence clustering yielded 759 OTUs, which were assigned to 11 phyla, 29 classes, 65 orders, 119 families 215 genera and 250 species. All of the rarefaction curves tended to approach a plateau, indicating that the numbers of sequences acquired were sufficient to describe the bacterial and fungal diversity and abundance within these samples (Fig. 2).

Difference in diversity and richness of microbial community between rhizosphere soil of *SCN-CHS* HIGS soybean H57 and wild-type Jack

Alpha diversity of the microbial community of rhizosphere soil of H57 and Jack, and the bulk soil, is represented by

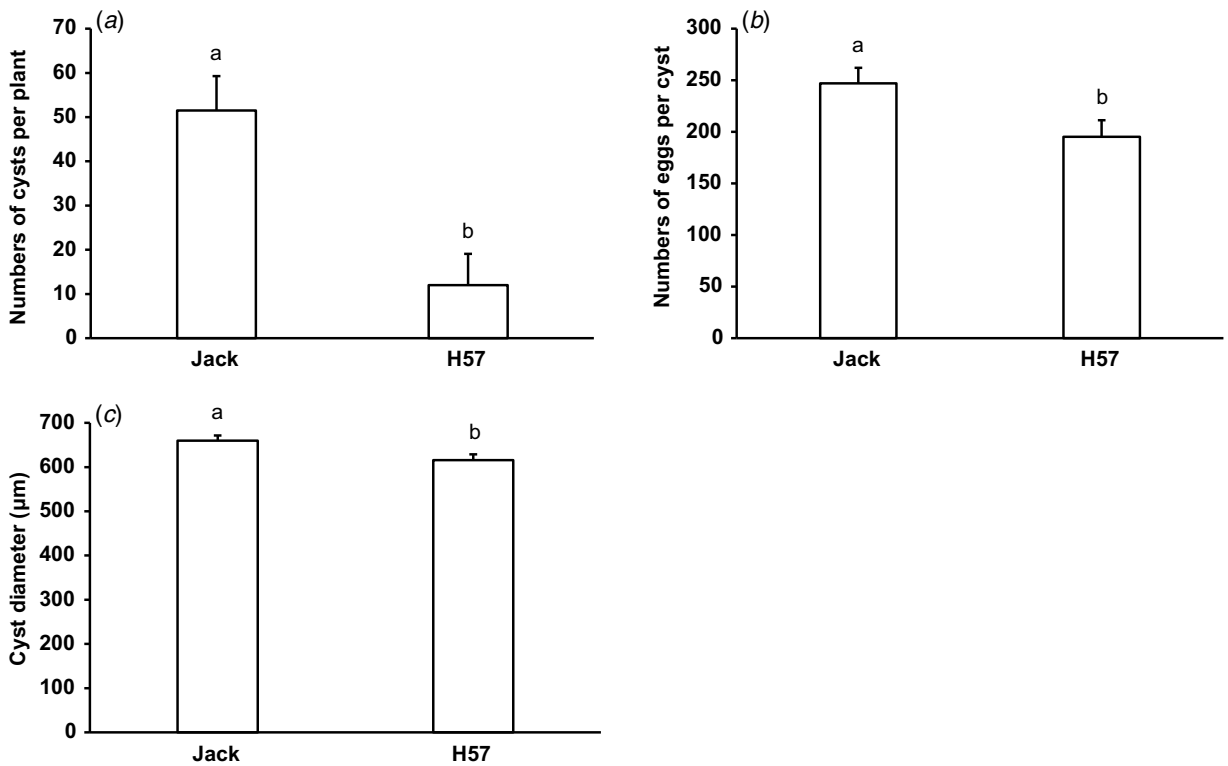

Fig. 1. SCN-infection phenotype of soybean H57 and Jack using the SCN-infected rhizosphere soil. (*a*) Number of cysts per plant, (*b*) number of eggs per cyst, (*c*) diameter of cysts. Data of six replicates were collected at 60 days post planting in the SCN-infected soil. Different letters represent significant difference ($P < 0.05$) by Duncan's multiple-range test.

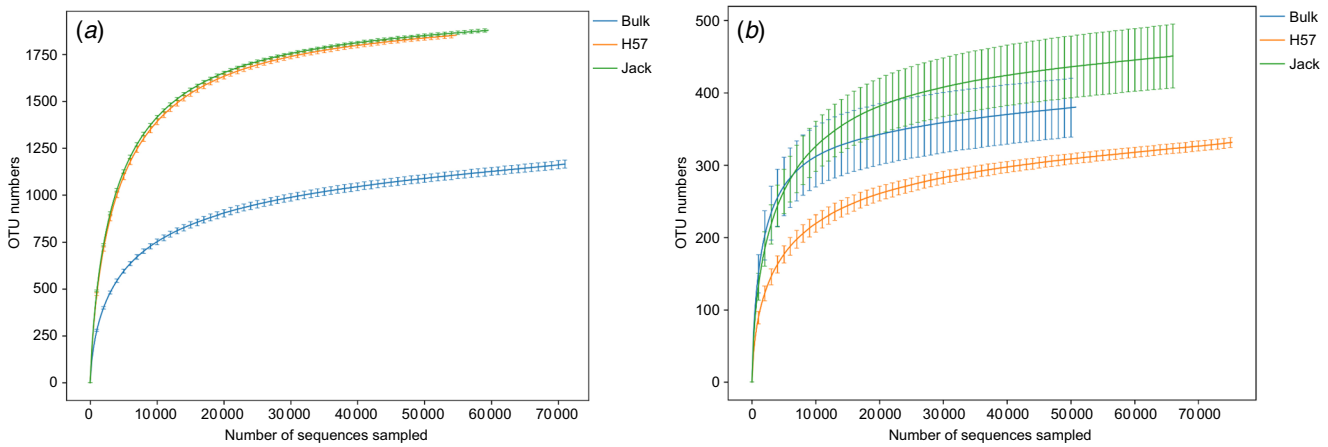

Fig. 2. Rarefaction curves of the operational taxonomic units (OTUs) obtained from sequencing of *16S* rRNA or ITS of the rhizosphere soil of H57 and Jack planted in SCN-infected soil, and the bulk SCN-infected soil without planting: (*a*) bacterial OTUs, (*b*) fungal OTUs.

five indexes; Shannon and Simpson were used to estimate the diversity, whereas observed species, Chao1 and Ace were employed to evaluate the richness (abundance), of the rhizosphere bacterial (Table 1) and fungal (Table 2) communities. The coverage of clean reads was >99% for all soil samples, indicating that depth of sequencing was sufficient to represent the majority of the microbiota in the soybean rhizosphere soil environment. Therefore, the obtained sequencing data can be used for reliably analysing the diversity and abundance of the microbial community.

The results indicate that planting soybeans (H57 or Jack) significantly increased both diversity and abundance of the rhizosphere bacterial community relative to the bulk soil; however, comparing the rhizosphere soils of H57 and Jack, both diversity and abundance of the bacterial communities were similar (Table 1). Therefore, HIGS of *SCN-CHS* did not significantly impact either the diversity or the abundance of rhizosphere bacterial community. Regarding the fungal community, planting Jack did not significantly change the diversity or abundance of the soil fungal community relative to bulk soil. On the other hand, planting H57 significantly reduced both the diversity and abundance (Chao1 and Ace, but not observed species) of the soil fungal community relative to bulk soil. Fungal

Table 1. Alpha diversity of bacterial community in the rhizosphere soil of *SCN-CHS* HIGS transgenic soybean H57 and wild-type Jack planted into SCN-infected soil, and in the bulk soil without planting.

	Coverage	Shannon	Simpson	Observed species	Chao1	Ace
Bulk	0.9974 ± 0.0002b	7.7939 ± 0.0610b	0.9893 ± 0.0008b	1168.6667 ± 47.9085b	1357.1179 ± 28.0125b	1304.6397 ± 21.2769b
Jack	0.9980 ± 0.0001a	9.4498 ± 0.0314a	0.9968 ± 0.0002a	1887.1667 ± 20.9000a	1942.5361 ± 11.3686a	1928.3748 ± 10.4906a
H57	0.9982 ± 0.0001a	9.3272 ± 0.0529a	0.9962 ± 0.0003a	1867.0000 ± 22.7303a	1924.5467 ± 13.5106a	1911.4137 ± 11.1003a

Shannon and Simpson indexes estimate the diversity, and observed species, Chao1 and Ace evaluate the richness (abundance) of the community. Coverage is coverage of reads. All data are mean of six replicates ± s.e. Within columns, means followed by the same letter are not significantly different at $P = 0.05$ based on Duncan's multiple-range test.

Table 2. Alpha diversity of fungal community in the rhizosphere soil of *SCN-CHS* HIGS transgenic soybean H57 and wild-type Jack planted into SCN-infected soil, and in the bulk soil without planting.

Group	Coverage	Shannon	Simpson	Observed species	Chao1	Ace
Bulk	0.9994 ± 0.0002a	5.6596 ± 0.4762a	0.9357 ± 0.0150a	396.6667 ± 81.7774ab	446.2380 ± 27.0875a	440.6907 ± 28.5766a
Jack	0.9995 ± 0.0001a	5.3596 ± 0.3717ab	0.9214 ± 0.0163ab	458.1667 ± 96.1031a	485.0321 ± 44.4550a	477.4528 ± 43.1316a
H57	0.9995 ± 0.0002a	4.3937 ± 0.2845b	0.8745 ± 0.0189b	333.6667 ± 15.0628b	371.2355 ± 8.2823b	360.6328 ± 8.2151b

Shannon and Simpson indexes estimate the diversity, and observed species, Chao1 and Ace evaluate the richness (abundance) of the community. Coverage is coverage of reads. All data are mean of six replicates ± s.e. Within columns, means followed by the same letter are not significantly different at $P = 0.05$ based on Duncan's multiple-range test.

diversity was not significantly different between plantings of H57 and Jack; however, abundance (observed species, Chao1 and Ace) of the rhizosphere fungal community was significantly lower with H57 than with Jack (Table 2). These results suggest that HIGS of *SCN-CHS* significantly decreased the abundance of rhizosphere fungal community.

The PCA indicated that the bacterial community structure/diversity was similar in the rhizosphere soil of Jack and H57, and significantly different in the bulk soil (Fig. 3*a*). The structure/diversity of the rhizosphere fungal community of H57 was different from that in the bulk soil, and that of the rhizosphere soil of Jack was partially similar to that in the bulk soil (Fig. 3*b*). A Venn diagram can be used to display the numbers of common and unique features of OTUs, and intuitively shows the coincidence of features, between samples. The number of common features of the bacterial community between the rhizosphere soil samples of H57 and Jack was 2012, with a similarity of 99.1% (Fig. 4*a*), indicating extreme similarity in the components/diversity of the bacterial community in the rhizosphere soil of H57 and Jack. The number of common features of the fungal community between the rhizosphere soil samples of H57 and Jack was 573, with a similarity of only 77.3% (Fig. 4*b*), suggesting partial differences in the diversity of the fungal

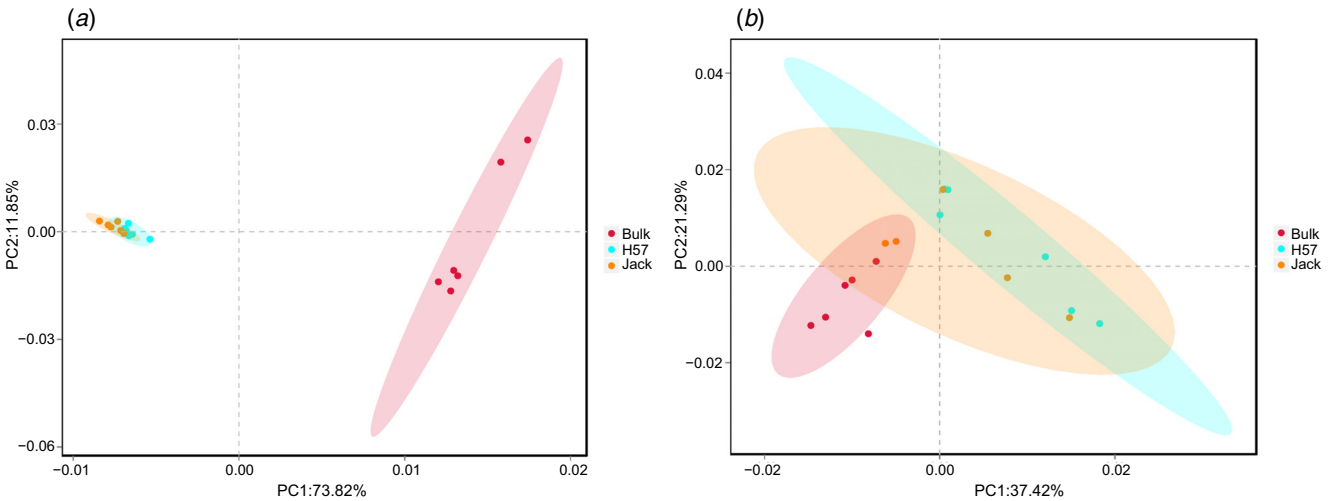

Fig. 3. Principal components analysis (PCA) on the bacterial and fungal operational taxonomic units (OTUs) obtained from sequencing of *16S* rRNA or ITS of the rhizosphere soil of H57 and Jack planted in SCN-infected soil, and the bulk soil without planting: (*a*) bacterial OTUs, (*b*) fungal OTUs.

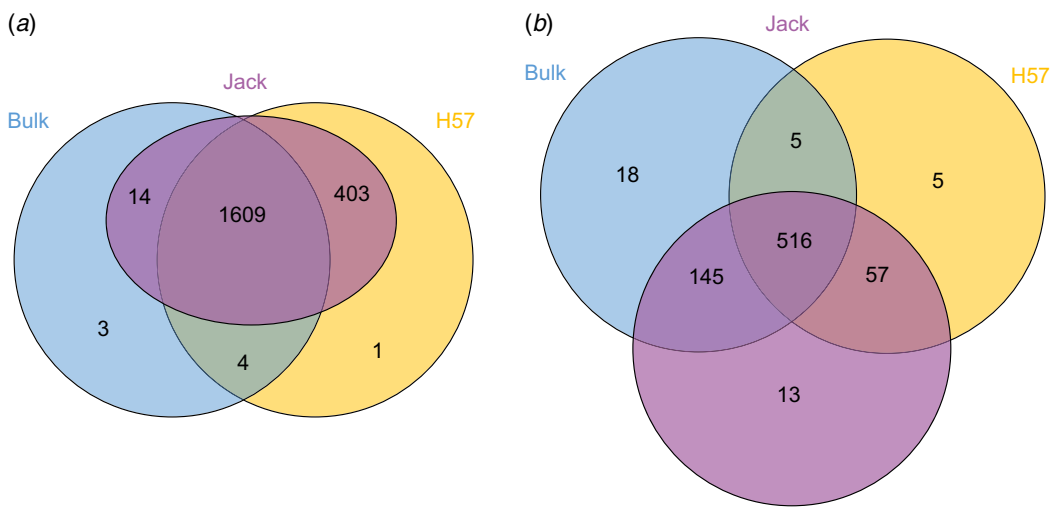

Fig. 4. Venn diagrams of bacterial and fungal operational taxonomic units (OTUs) obtained from sequencing of *16S* rRNA or ITS of the rhizosphere soil of H57 and Jack planted in SCN-infected soil, and the bulk soil without planting: (*a*) bacterial OTUs, (*b*) fungal OTUs.

community in the rhizosphere soil of H57 and Jack. All of these results further validate that planting of H57 and Jack similarly affected the structure/diversity of the rhizosphere bacterial community, but had some different impacts on the rhizosphere fungal community.

Difference in relative abundance of microbial community at phylum level between rhizosphere soil of *SCN-CHS* HIGS soybean H57 and wild-type Jack

Analyses were conducted for the rhizosphere microbial community at the phylum level, with the relative abundance of the top 10 phyla in bulk soil and rhizosphere soil of Jack and H57 presented in Fig. 5*a* and *b*, for bacteria and fungi, respectively. The relative abundance of the top 10 bacterial phyla except Chloroflexi in the bulk soil was significantly ($P < 0.05$) different from that in the rhizosphere soil of H57 and Jack (Fig. 5*c*). However, the relative abundance of only one of the top 10 fungal phyla was significantly ($P < 0.05$) altered in the rhizosphere soil of H57 compared with Jack (Fig. 5*b*). In all soil samples, Proteobacteria and Acidobacteria were the two most dominant bacterial phyla, accounting for >61% of the reads (Fig. 5*a*). Ascomycota and Basidiomycota were the two main fungal phyla, occupying >57% of the reads (Fig. 5*b*). Furthermore, after planting H57, the relative abundance of only two bacterial phyla, Acidobacteria and Actinobacteria, was significantly ($P < 0.05$) different from that in the rhizosphere soil of Jack (Fig. 5*c*); abundance of Acidobacteria was higher, and

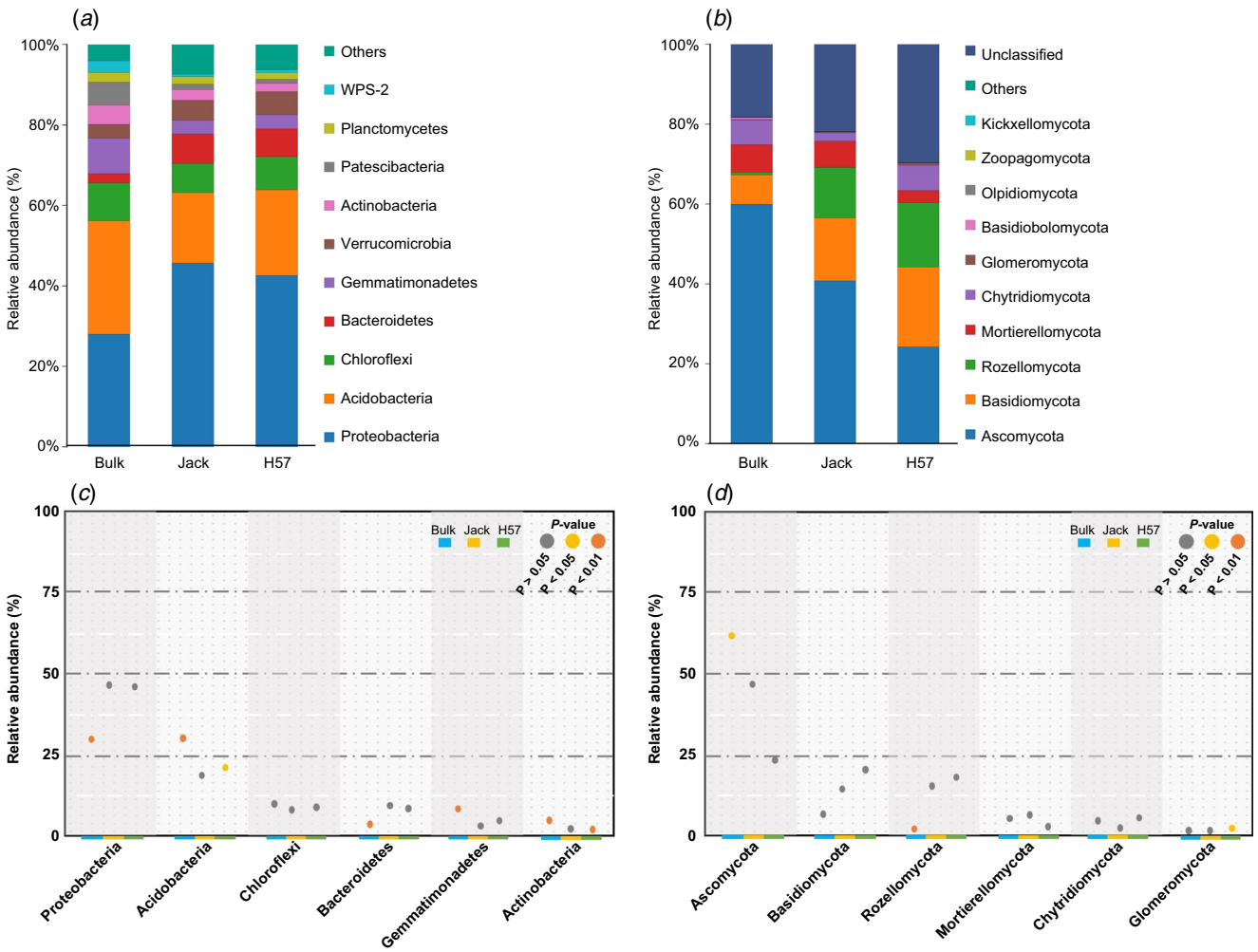

Fig. 5. Relative abundance of top 10 phyla of the bacterial and fungal communities in the rhizosphere soil of H57 and Jack planted in SCN-infected soil, and the bulk soil without planting: (*a*) relative abundance of different bacterial phyla within the different communities; (*b*) relative abundance of different fungal phyla within the different communities; (*c*) analysis of significant difference among bacterial phyla with relative abundance >0.05%, compared with Jack (six phyla are listed); (*d*) analysis of significant difference among fungal phyla with relative abundance >0.05%, compared with Jack (six phyla are listed). Sequences that could not be classified into any known groups are labelled 'unclassified'.

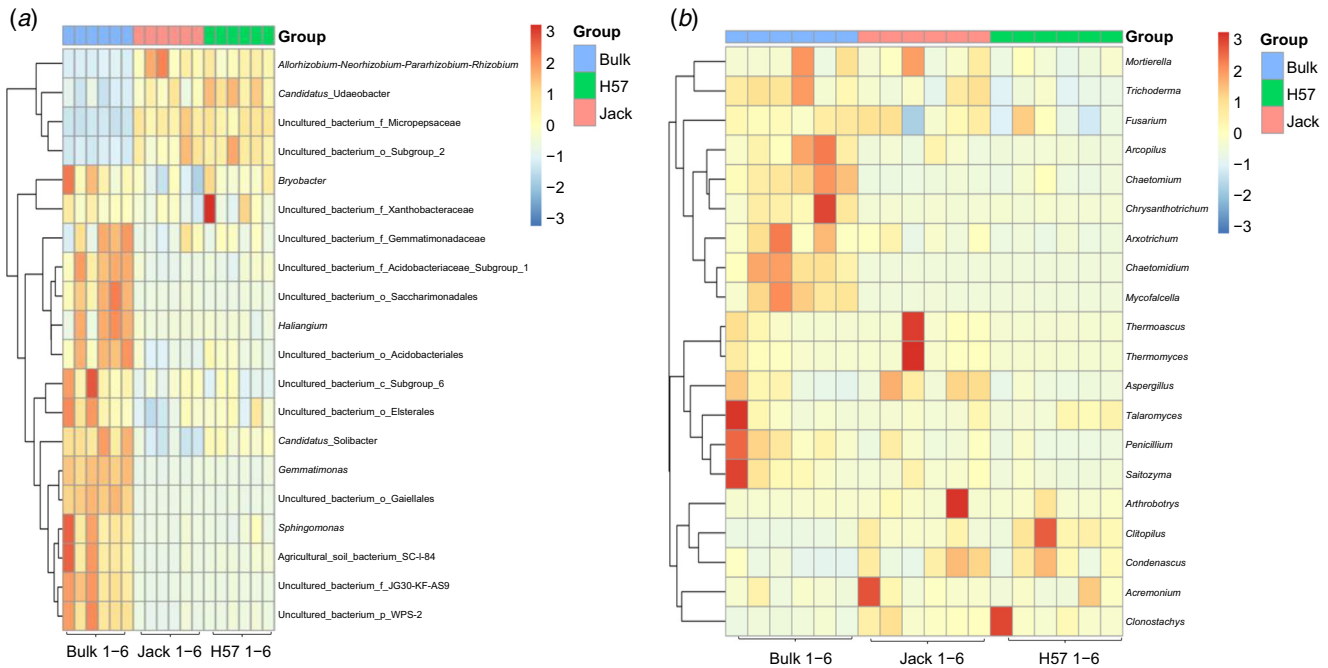

Fig. 6. Relative abundance of top 20 genera of the bacterial and fungal communities in the rhizosphere soil of Jack and H57 planted in SCN-infected soil, and the bulk soil without planting: (*a*) relative abundance of different bacterial genera within the different communities, (*b*) relative abundance of different fungal genera within the different communities.

Table 3. Relative abundance of top 20 genera of the bacterial community in the rhizosphere soil of H57 and Jack planted in SCN infected soil or bulk soil without planting.

Genera	Bulk	Jack	H57
Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium	0.0004 ± 0.0002b	0.0138 ± 0.0014a	0.0185 ± 0.0037a
Bryobacter	0.0193 ± 0.0014a	0.0173 ± 0.0009a	0.0138 ± 0.0010b
*Candidatus*_Solibacter	0.0423 ± 0.0022a	0.0284 ± 0.0012b	0.0176 ± 0.0015c
*Candidatus*_Udaeobacter	0.1000 ± 0.0007c	0.0246 ± 0.0016a	0.0181 ± 0.0015b
Gemmatimonas	0.0541 ± 0.0013a	0.0109 ± 0.0006b	0.0089 ± 0.0006b
Haliangium	0.0190 ± 0.0040a	0.0074 ± 0.0001b	0.0078 ± 0.0003b
Sphingomonas	0.0445 ± 0.0063a	0.0135 ± 0.0022b	0.0117 ± 0.0008b
Agricultural_soil_bacterium_SC-I-84	0.0234 ± 0.0038a	0.0062 ± 0.0004b	0.0047 ± 0.0033b
Uncultured_bacterium_c_Subgoup_6	0.0302 ± 0.0028a	0.0200 ± 0.0013b	0.0215 ± 0.0011b
Uncultured_bacterium_f Acidobacteriaceae_Subgroup_I	0.0249 ± 0.0027a	0.0101 ± 0.0001b	0.0089 ± 0.0007b
Uncultured_bacterium_f_Gemmatimonadaceae	0.0326 ± 0.0045a	0.0327 ± 0.0012a	0.0238 ± 0.0025a
Uncultured_bacterium_f_JG30-KF-AS9	0.0557 ± 0.0063a	0.0058 ± 0.0004b	0.0043 ± 0.0005b
Uncultured_bacterium_f_Micropepsaceae	0.0040 ± 0.0002b	0.0167 ± 0.0008a	0.0170 ± 0.0092a
Uncultured_bacterium_f_Xanthobacteraceae	0.0223 ± 0.0011a	0.0242 ± 0.0045a	0.0177 ± 0.0013a
Uncultured_bacterium_o_Acidobacteriales	0.1310 ± 0.0134a	0.0763 ± 0.0055b	0.0592 ± 0.0044b
Uncultured_bacterium_o_Elsterales	0.0153 ± 0.0013a	0.0119 ± 0.0008b	0.0097 ± 0.0008b
Uncultured_bacterium_o_Gaiellales	0.0269 ± 0.0007a	0.0039 ± 0.0002b	0.0042 ± 0.0001b
Uncultured_bacterium_o_Saccharimonadales	0.0379 ± 0.0061a	0.0039 ± 0.0005b	0.0047 ± 0.0005b
Uncultured_bacterium_o_Subgeoup_2	0.0029 ± 0.0002b	0.0190 ± 0.0018a	0.0160 ± 0.0024a
Uncultured_bacterium_p_WPS-2	0.0294 ± 0.0029a	0.0072 ± 0.0004b	0.0059 ± 0.0006b

All data are mean of six replicates ± s.e. Within rows, means followed by the same letter are not significantly different at $P = 0.05$ by Duncan's multiple-range test.

that of Actinobacteria was much lower, in the rhizosphere soil of H57. Similarly, the relative abundance of only one fungal phylum, Glomeromycota, showed significant difference between the rhizosphere soils of H57 and Jack (Fig. 5*d*).

Difference in relative abundance of microbial community at genus level between rhizosphere soil of *SCN-CHS* HIGS soybean H57 and wild-type Jack

At the genus level, the relative abundance of the bacteria *Candidatus*_Solibacter, *Candidatus*_Udaeobacter and *Bryobacter* in the rhizosphere soil of H57 was different from that of Jack (Fig. 6*a*, Table 3). Meanwhile, the relative abundance of fungal genera *Arcoplius, Arxotrichum, Chaetomidium, Chaetomium, Chrysanthotrichum, Condenascus*, *Mycofalcella* and *Penicillium* was significantly changed in the rhizosphere soil of Jack, whereas that of fungal *Aspergillus* was not significantly altered, compared with the bulk soil (Fig. 6*b*, Table 4). However, after planting H57, the relative abundance of rhizosphere *Aspergillus* was considerably lower than after planting Jack. *Aspergillus* is the sole genus of the top 20 fungal genera presenting significant ($P < 0.05$) abundance difference between the rhizosphere soils of Jack and H57 (Fig. 6*b*, Table 4).

Discussion

In this study, a 76.7% decrease of *H. glycines* cyst numbers and significant reductions in numbers of eggs per cyst and cyst diameters in the plots with T_7 generation *SCN-CHS* HIGS transgenic soybean H57 (Fig. 1) indicate its significantly enhanced resistance. In addition to enhanced SCN resistance phenotype results for T_2 and T_6 generations of *SCN-CHS* HIGS soybean lines (Kong *et al.* 2022), this clearly indicates that the enhanced resistance to SCN is highly heritable in HIGS soybean H57, establishing a strong basis for planting H57 for effective management of SCN. Meanwhile, planting *SCN-CHS* HIGS soybean H57 and the wild-type Jack almost equally significantly enhanced the diversity and richness (abundance) of the rhizosphere bacterial community (Table 1, Fig. 3*a*), whereas planting H57 rather than Jack greatly decreased the abundance of the rhizosphere fungal community, and planting soybean H57 significantly reduced its diversity compared with bulk soil (Table 2), suggesting that

Table 4. Relative abundance of top 20 genera of the fungal community in the rhizosphere soil of H57 and Jack planted in SCN infected soil or bulk soil without planting.

Genera	Bulk	Jack	H57
Acremonium	0.0080 ± 0.0029a	0.0198 ± 0.0099a	0.0118 ± 0.0057a
Arcopilus	0.0142 ± 0.0042a	0.0026 ± 0.0014b	0.0012 ± 0.0002b
Arthrobotrys	0.0012 ± 0.0006a	0.0182 ± 0.0146a	0.0660 ± 0.0051a
Arxotrichum	0.0101 ± 0.0028a	0.0037 ± 0.0011b	0.0014 ± 0.0003b
Aspergillus	0.0069 ± 0.0026ab	0.0117 ± 0.0003a	0.0030 ± 0.0004b
Chaetomidium	0.0233 ± 0.0047a	0.0000 ± 0.0000b	0.0000 ± 0.0000b
Chaetomium	0.0466 ± 0.0075a	0.0037 ± 0.0007b	0.0007 ± 0.0029b
Chrysanthotrichum	0.0153 ± 0.0057a	0.0004 ± 0.0000b	0.0007 ± 0.0002b
Clitopilus	0.0041 ± 0.0004b	0.0620 ± 0.0169ab	0.0133 ± 0.0442a
Clonostachys	0.0027 ± 0.0009a	0.0167 ± 0.0038a	0.0199 ± 0.0104a
Condenascus	0.0016 ± 0.0008b	0.0085 ± 0.0025a	0.0071 ± 0.0022ab
Fusarium	0.0659 ± 0.0032a	0.0623 ± 0.0094a	0.0511 ± 0.0084a
Mortierella	0.0665 ± 0.0256a	0.0664 ± 0.0226a	0.0265 ± 0.0066a
Mycofalcella	0.1099 ± 0.0236a	0.0016 ± 0.0005b	0.0015 ± 0.0003b
Penicillium	0.0287 ± 0.0083a	0.0098 ± 0.0035b	0.0035 ± 0.0013b
Saitozyma	0.0155 ± 0.0006a	0.0059 ± 0.0017ab	0.0023 ± 0.0006b
Talaromyces	0.0087 ± 0.0058a	0.0027 ± 0.0012a	0.0046 ± 0.0017a
Thermoascus	0.0210 ± 0.0122a	0.0381 ± 0.0288a	0.0014 ± 0.0002a
Thennomyces	0.0114 ± 0.0055a	0.0267 ± 0.0204a	0.0013 ± 0.0003a
Trichoderma	0.0301 ± 0.0051a	0.0179 ± 0.0048ab	0.0071 ± 0.0019b

All data are mean of six replicates ± s.e. Within rows, means followed by the same letter are not significantly different at $P = 0.05$ by Duncan's multiple-range test.

HIGS of *SCN-CHS* mainly altered the abundance of the rhizosphere fungal community.

After planting H57, only two rhizosphere bacterial phyla, Acidobacteria and Actinobacteria, were significantly altered compared with planting Jack; relative abundance of Acidobacteria was significantly increased, and relative abundance of Actinobacteria was greatly decreased. Acidobacteria was one of the two most predominant bacterial phyla, and was much more abundant than Actinobacteria in all soil samples (Fig. 5*a*, *c*). Thus, Acidobacteria should contribute more to differences in the bacterial community between the rhizosphere soils of H57 and Jack. Furthermore, at the genus level, the abundance of *Bryobacter*, which belongs to Acidobcateria phylum, was also much lower in the rhizosphere soil of H57 than Jack (Fig. 6*a*, Table 3). These results indicate that HIGS of *SCN-CHS* stimulates the growth of Acidobacteria while suppresses *Bryobacter*. The ubiquity of Acidobacteria in soil indicates their importance in the functioning of the soil ecosystem (Ward *et al.* 2009); for example, they might be responsible for degrading polysaccharides of plants and fungi in the ecosystems of acidic coniferous forests (Lladó *et al.* 2016). Genomic and metagenomic data predict a number of ecologically relevant capabilities of some Acidobacteria, which include the ability to use nitrite as a nitrogen source, respond to soil macro- and micro-nutrients and soil acidity, express multiple active transporters, degrade gellan gum, and produce exopolysaccharide (Kielak *et al.* 2016). Therefore, we propose that higher abundance of Acidobacteria in the rhizosphere soil of H57 might degrade more polysaccharides of fungal cell walls, resulting in suppression of the rhizosphere fungal community compared with the rhizosphere soil of Jack.

At the fungal phylum level, only Glomeromycota was significantly different between the rhizosphere soils of H57 and Jack. Fungi in the phylum Glomeromycota include the arbuscular mycorrhizal fungi (AMF), which are are symbiotic with plant roots, and therefore the most common fungi in the terrestrial ecosystem (Lee *et al.* 2018). AMF are also associated with vascular plants and thalloid bryophytes (Brundrett and Tedersoo 2018). In addition, the species richness of sporulating AMF is high in Roraima savannas of Brazil, indicating that AMF may play a key role in plant nutrition and soil structure improvement in the presence of poor soil nutrients or other harsh conditions (Stürmer *et al.* 2018). However, in our study, the relative abundance of Glomeromycota in each soil sample was ≤1% (Fig. 5*b*, *d*). Thus, Glomeromycota is a minor fungal phylum in all soil samples in this study. Nonetheless, the functions of fungal Glomeromycota in *SCN-CHS* HIGS soybean H57 and the rhizosphere microbial community interaction system are worthy of further investigation.

At the fungal genus level, *Aspergillus* was the sole genus that showed significant difference between the rhizosphere soil of H57 and Jack (Fig. 6*b*, Table 4). Some *Aspergillus* spp. such as *A. welwitschiae* (Liu *et al.* 2019) and *A. niger* NBC001 (Jin *et al.* 2019) have been isolated and can be used as biocontrol reagents for effectively controlling root-knot nematode (*Meloidogyne graminicola*) (Shemshura *et al.* 2016; Liu *et al.* 2019), and SCN (Jin *et al.* 2019, 2021), but most *Aspergillus* spp. are pathogenic. The much lower abundance of *Aspergillus* in the rhizosphere soil of H57 than Jack (Fig. 6*b*, Table 4) indicates that HIGS of *SCN-CHS* inhibits rhizosphere *Aspergillus*. Our recent study demonstrated that HIGS of *SCN-CHS* could significantly suppress soybean infection by *Fusarium oxysporum*, which causes Fusarium wilt disease in soybean (Kong *et al.* 2022). The present results suggest that HIGS of *SCN-CHS* can suppress the relative abundance of pathogenic fungi, leading to decreased abundance of the rhizosphere fungal community (Table 2) while enhancing the resistance of soybean to SCN (Fig. 1). The significantly increased relative abundance of the bacterial phylum Acidobacteria under planting of *SCN-CHS* HIGS H57 may increase degradation of polysaccharides in fungal cell walls and result in reduced abundance of the rhizosphere fungal community. Therefore, we hypothesise that HIGS of *SCN-CHS* compromises the abundance of the rhizosphere fungal community, likely through stimulation of the diversity and abundance of the rhizosphere bacterial community to enhance the resistance of soybean to SCN (*H. glycines*), but the mechanisms require further study.

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