Corn and Soybean Host Root Endophytic Fungi with Toxicity Toward the Soybean Cyst Nematode

Noah Strom,¹ Weiming Hu,² Deepak Haarith,³ Senyu Chen,⁴ and Kathryn Bushley^{1,†}

¹ Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN

² Entomology and Nematology Department, University of Florida, Gainesville, FL

³ Department of Plant Pathology, University of Minnesota, Saint Paul, MN

⁴ Southern Research and Outreach Center, University of Minnesota, Waseca, MN

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ABSTRACT

Although fungal endophytes are commonly investigated for their ability to deter microbial plant pathogens, few studies have examined the activity of fungal root endophytes against nematodes. The soybean cyst nematode (SCN; *Heterodera glycines*), the most severe yield-limiting pathogen of soybean (*Glycine max*), is commonly managed through rotation of soybean with corn (*Zea mays*), a nonhost of the SCN. A total of 626 fungal endophytes were isolated from surface-sterilized corn and soybean roots from experimental plots in which soybean and corn had been grown under annual rotation and under 1, 3, 5, and 35 years of continuous monoculture. Fungal isolates were grouped into 401 morphotypes, which were clustered into 108 operational taxonomic units (OTUs) based on 99% sequence similarity of the full internal transcribed spacer region. Morphotype representatives within each OTU were grown in malt extract broth and in a

secondary metabolite-inducing medium buffered with ammonium tartrate, and their culture filtrates were tested for nematicidal activity against SCN juveniles. A majority of OTUs containing isolates with nematicidal culture filtrates were in the order Hypocreales, with the genus *Fusarium* being the most commonly isolated nematicidal genus from corn and soybean roots. Less commonly isolated taxa from soybean roots included the nematophagous fungi *Hirsutella rhossiliensis*, *Metacordyceps chlamydosporia*, and *Arthrobotrys iridis*. Root endophytic fungal diversity in soybean was positively correlated with SCN density, suggesting that the SCN plays a role in shaping the soybean root endophytic community.

Keywords: crop rotation, endophytes, fungal communities, monoculture, soybean cyst nematode

Fungal endophytes are increasingly seen as potentially beneficial plant symbionts (Domka et al. 2019; Hardoim et al. 2015) and are a subject of current efforts in bioprospecting for sources of new secondary metabolites (Alvin et al. 2014; Strobel and Daisy 2003; Suryanarayanan et al. 2009). Endophytic fungi play diverse roles in plant health, including improving tolerance to drought (Khan et al. 2011), salt (Waller et al. 2005), cold (Redman et al. 2011), heat (Khan et al. 2012), and toxic metals (Yamaji et al. 2016); enhanced nutrient acquisition (Yadav et al. 2010); improved growth (Redman et al. 2001; Yamaji et al. 2016); disease resistance (Franken 2012; Waller et al. 2005); and herbivore resistance (Latch 1993). Given the difficulty in distinguishing truly nonpathogenic fungi from latent pathogens, we follow Hardoim et al. (2015) in defining an endophyte in this study simply as a fungus that lives at least part of its life inside the plant, a definition that may include fungi along the mutualist-pathogen continuum (Schulz and Boyle 2005).

Multiple factors may shape the composition of endophyte communities in roots. These include abiotic factors such as soil type (Bokati et al. 2016) and biotic factors relating to the plant host such as plant developmental stage (Rothen et al. 2017; Van Overbeek and Van Elsas 2008), plant tissue type (Renuka and Ramanujam 2016; Russo et al. 2016; Yang et al. 2018), and plant genotype (Lundberg et al. 2012; Russo et al. 2016). Several

culture-dependent (CD) and culture-independent (CI) studies have attempted to characterize the root endophyte community of corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.]. Among these studies, many have shown the corn community to be dominated by the genera *Fusarium* (Amin 2013; Bokati et al. 2016; Potshangbam et al. 2017; Russo et al. 2016) and *Alternaria* (Bokati et al. 2016; Orole and Adejumo 2011). Several studies also suggest that the soybean root endophyte community is dominated by the genus *Fusarium* (Fernandes et al. 2015; Russo et al. 2016; Yang et al. 2018). Some studies suggest that root tips may harbor distinct endophyte communities from other sections of the root (Compant et al. 2005; Kawasaki et al. 2016); yet to our knowledge, no endophyte studies of corn and soybean have sampled this environment.

Studies on the roles of root endophytic fungi in the antagonism of plant parasitic nematodes are scarce (Schouten 2016), but some research suggests that these fungi play significant roles in protecting plants from nematodes. For example, Hallmann and Sikora (1994) isolated several endophytic strains of *F. oxysporum* from roots of tomato plants that showed increased resistance to the root-knot nematode (*Meloidogyne incognita*) and found that many of these strains produced secondary metabolites that killed *Meloidogyne incognita* juveniles (Hallmann and Sikora 1996). The root endophyte *Piriformospora indica* was shown to antagonize the sugar beet nematode (*Heterodera schachtii*) in *Arabidopsis* species via fungal exudates and cell wall extracts (Daneshkhah et al. 2013). *Piriformospora indica* was shown to decrease egg density of the soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) in a greenhouse trial (Bajaj et al. 2015). Fungi may control nematode pathogens through direct parasitism or through production of metabolites with nematotoxic or nematostatic bioactivity (Anke et al. 1995; Degenkolb and Vilcinskis 2016a, b; Meyer 2018; Meyer et al. 1990). However, the role of naturally occurring fungal root endophytes in

[†]Corresponding author: K. Bushley; kbushley@umn.edu

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antagonizing nematodes through antibiosis has not been well explored (Schouten 2016).

The SCN is the most serious soybean pathogen, worldwide. The survival form of the SCN is an egg-filled cyst that can survive in the soil for nearly a decade in the absence of a susceptible plant host (Inagaki and Tsutsumi 1971). The infectious form of the SCN is a second-stage juvenile (J2), which emerges from eggs in SCN cysts in response to a hatching stimulus (Riga et al. 2001). In order to complete their life cycle, J2s infect the roots of susceptible plants, draining photosynthates and causing an estimated \$1.5 billion in soybean yield losses in the United States every year (Koenning and Wrather 2010). Although rotation to nonhost crops, most commonly corn, is often utilized to minimize SCN populations, the SCN is virtually impossible to eliminate from fields in which it has become established owing to the longevity of SCN cysts (Chen 2011; Inagaki and Tsutsumi 1971). While SCN-resistant varieties of soybean are available, repeated planting leads to the evolution of SCN races that can infect previously resistant varieties (Chen 2011).

With the banning of several important chemical nematicides, biological control has come to be seen as a potential alternative method to manage the SCN (Chen 2004; Kerry 2000; Preston et al. 2003; United Nations Environment Programme 2015; Warnock et al. 2017). Many studies have shown that continuous soybean monoculture leads to the development of microbial soil communities suppressive to the SCN (Hu et al. 2017a; Hussain et al. 2018). Several fungal antagonists of the SCN have been isolated from SCN cysts and juveniles collected from bulk soils (Carris et al. 1989; Chen and Chen 2002; Chen et al. 2000a; Kim and Riggs 1994). However, soybean roots may be a more attractive agroecosystem compartment in which to search for SCN biocontrol agents, because fungi living in the root are well positioned to intercept invading SCN J2s. Likewise, corn roots, although not hosts of the SCN, may nonetheless facilitate establishment of endosphere-competent microbes in the bulk soil (Bardgett and Wardle 2010; Bever 1994; van de Voorde et al. 2011). Because corn is commonly planted in rotation with soybean, it is useful to understand whether the corn endosphere harbors similar or distinct fungal antagonists of the SCN as compared with soybean.

In this study, fungal endophyte communities of roots of corn and soybean under short-term rotations and long-term monocultures were characterized using a CD method, and select isolates were screened for nematicidal activity against the SCN. The objectives of this study were (i) to examine the role of crop host, crop sequence treatment, and SCN density in shaping the culturable fungal endophyte community of corn and soybean roots; (ii) to explore endophyte communities found in the root tip; and (iii) to identify fungi that produce culture filtrates with nematicidal activity against the SCN. We hypothesized that (i) corn and soybean roots would harbor distinct root endophytic communities and (ii) soybean would host a greater number of nematicidal endophytes than corn, especially under long-term monoculture.

MATERIALS AND METHODS

Experimental design. This study utilized a long-term corn-soybean rotation experiment at the University of Minnesota Southern Research and Outreach Center in which corn and soybean have been planted continuously, since 1982, under various crop sequences (Crookston et al. 1991) on a Nicollet clay loam (fine-loamy, mixed, mesic Aquic Hapludoll) (Soil Survey Staff 2014). The crop varieties were *Bt* corn (Dekalb 50-82) and SCN-susceptible soybean (Pioneer 91Y90). The crop sequences included long-term corn and soybean monocultures (Cc and Ss), 5-year rotations in which years 1 through 5 of corn (C1 to C5) were followed by years 1 through 5 of soybean (S1 to S5), and annual rotations in which corn and soybean were alternately planted each year (Ca and Sa). There were four replicates for each crop sequence treatment, and plots were arranged in a randomized complete block

design. A complete description of the experimental plots and their management is available elsewhere (Grabau and Chen 2016a, b; Hu et al. 2018). For this study, roots were sampled from experimental plots under the following crop sequence treatments: C1, C3, C5, Cc, Ca, S1, S3, S5, Ss, and Sa. SCN density was measured at midseason as eggs/100 cm³ of soil, and yield was measured at harvest as reported in Hu et al. (2018) and Strom et al. (2019b).

Sample collection. Midseason root samples were collected on 21 July 2016. Sampling of roots followed the same protocol described in Strom et al. (2019a) and is briefly described, here. Six corn plants and six groups of three to four soybean plants were removed from evenly spaced locations in each treatment plot using a spade. Soil was removed from the root systems by hand. Three lateral roots <2 mm in diameter and approximately 6 to 9 cm in length were randomly selected and removed from each root system for a total of 18 lateral roots per plot. The remaining soil was removed by agitating lateral roots in phosphate-buffered saline (PBS) buffer (PBS-S; 130 mM of NaCl, 7 mM of Na₂HPO₄, 3 mM of NaH₂PO₄, pH 7.0, and 0.2% Tween 20) at 200 rpm for 15 min. Roots were then rinsed under tap water, agitated twice in sterile double distilled water (ddH₂O), and stored at 4°C until further processing. All roots were surface sterilized and plated within 2 days.

Isolation and culturing of root endophytes. Prior to collecting root samples for this study, the root surface sterilization protocol was optimized using roots from the same experimental plots. After agitation in PBS buffer and sterile ddH₂O, select root sections were surface sterilized in 0.6% NaOCl for between 0 s and 5 min, followed by three rinses in sterile ddH₂O. Root surface sterility was tested by briefly touching hypochlorite-treated roots to the agar surface of a Petri plate containing one-quarter strength potato dextrose agar plus 50 ppm of chlorotetracycline and 50 ppm of streptomycin (one-quarter APDA) and observing the plates for fungal growth over a period of 2 weeks. No fungal growth was observed on imprint plates for roots with as few as 10 s of soaking in 0.6% NaOCl, but fungal growth was observed on positive control plates to which root sections had been added. However, a longer soaking period of 2 min was chosen based on a literature review (Fernandes et al. 2015; Orole and Adejumo 2011) and to reduce the chance of surface contamination. Following the optimized protocol, 18 soil-free lateral roots from each experimental plot were sterilized by soaking them in 10% bleach solution (0.6% NaOCl) for 2 min followed by three rinses in sterile ddH₂O. Surface-sterilized roots were allowed to dry on a sterile paper towel for 1 min prior to plating.

A flame-sterilized scalpel was used to cut one 2- to 5-mm section from either the center or tip of each surface-sterilized lateral root. A majority of sections were taken from central regions along the length of the root, but 3 of 18 sections were taken from the tips of lateral roots from each plot. One individual root section was plated per 10-cm-diameter Petri plate containing one-quarter APDA. Plates were sealed with Parafilm and incubated at room temperature for 3 months in ambient light. The plates were checked for fungal growth every 3 days for the first 2 weeks and once per week thereafter.

Half-centimeter-wide plugs of fungal colonies growing from root sections were transferred to full-strength potato dextrose agar plus 50 ppm of chlorotetracycline and 50 ppm of streptomycin (APDA) in 10-cm-diameter Petri plates. For root sections in which more than one distinct fungal colony had grown, a mycelial plug was transferred from each colony to a new plate. When fungal colonies on APDA covered one-half to two-thirds of the plate, they were grouped by morphology into morphogroups. One representative isolate for every five isolates from each morphogroup was photographed and selected as a morphotype, and one mycelial plug from each morphotype was transferred to one-quarter APDA covered in sterile cellophane. After colonies covered one-half to two-thirds of the plate, a sterile scalpel was used to transfer half of

each colony to a cryovial containing 20% glycerol, which was stored at -80°C . The other half of each colony was transferred to a sterile microcentrifuge tube and lyophilized for 48 h prior to DNA extraction.

Root endophyte DNA extraction, amplification, and bioinformatics. Lyophilized fungal tissue was ground using a sterile pipette tip, and DNA was extracted from a small fragment of ground tissue using the Sigma REExtract-N-Amp kit, following the manufacturer's protocol. The internal transcribed spacer (ITS) region of fungal rDNA was amplified in 25- μl PCR reactions containing 13.5 μl of nuclease-free water, 10 μl of REExtract-N-Amp PCR Reaction Mix, 0.5 μl each of 10 μM ITS1F (CTTGGTCATTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATGATATGC) primers, and 0.5 μl of template DNA per sample. PCR parameters were as follows: 94°C for 10 min for initial denaturation followed by 35 cycles of 94°C for 1 min, 53°C for 40 s, and 72°C for 1 min, followed by one final extension step at 72°C for 10 min. Negative controls containing nuclease-free water in place of template DNA were used in every cycle.

The success of each PCR reaction was visualized through gel electrophoresis. For failed PCR reactions, the DNA sample was diluted by factors of 10 until PCR was successful. PCR products were stored at -20°C until sequencing. Fifteen microliters of each PCR product was submitted to MCLAB (South San Francisco, CA) for cleanup and Sanger sequencing in both forward and reverse directions. Sequences were trimmed using a custom Python script, *dhtrim.py* (Byun and Haarith 2018), which trims the ends of sequences containing poor-quality base calls. Sequences that did not pass the quality controls set by *dhtrim.py* were trimmed and edited by hand in BioEdit (Hall 1999), calling ambiguous bases where possible. DNA associated with poor-quality sequences that could not be edited was resequenced. Trimmed forward and reverse reads were aligned using the "pairwise align DNA" function at <http://www.bioinformatics.org/sms2>. Aligned sequences with 99% sequence similarity were clustered into operational taxonomic units (OTUs) using USEARCH (Edgar 2010) implemented in QIIME (Caporaso et al. 2010). Species-level taxonomy was assigned to OTUs with a minimum of 97% sequence similarity, according to BLAST (Altschul et al. 1990), to reference sequences in the UNITE fungal ITS sequence database (Nilsson et al. 2019). For queried sequences matching reference sequences with $<97\%$ identity and for queried sequences matching multiple reference sequences with differing species-level taxonomy, only the genus name or higher taxonomic level is reported.

Preparation of culture filtrates of fungi. A minimum of 10% of morphotype representatives from each OTU were tested for nematicidal activity. Selected isolates were recovered from glycerol stocks by plating small amounts of preserved mycelium on one-quarter-strength cornmeal agar. After 2 weeks of growth, spores were collected from the surface of the plates using sterile 0.2% Tween, and the number of spores per milliliter was quantified using a hemacytometer. One million spores—or for nonsporulating isolates, a 1-cm² mycelial block cut into 25 smaller blocks with a sterile scalpel—were added to 125-ml flasks containing 50 ml of sterile malt extract broth (MEB) or secondary metabolite-inducing media with ammonium tartrate (SMAT) [30 g of fructose, 1.04 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.13 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 10.04 g of $(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_6$, 0.75 g of KH_2PO_4 , 1 ml of trace elements (trace element composition: 4.4 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 180 mg of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 25 mg of NaMoO_4 , 80 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 5 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 1 liter of ddH_2O), and 6 g of L-Valine per 1 liter of ddH_2O], which was modified from Lee et al. (2008) by replacing ammonium sulfate with ammonium tartrate as a pH buffer. Before inoculation with fungi, MEB media had an average pH of 5.1 and SMAT media had an average pH of 5.5.

Liquid cultures were incubated at 25°C for 2 weeks at 125 rpm in ambient light. Filtrates were collected by first pouring liquid cultures through two layers of Miracloth into 50-ml centrifuge

tubes. These were spun at $3,220 \times g$ for 20 min to settle out fungal spores. Then the liquid fraction was sterile filtered by pushing it through 0.2- μm filters using a syringe. Filtrates were stored at 4°C overnight. The pH of a 1-ml sample of each filtrate was measured using a GeneMate pH meter. After setting up a bioassay, filtrates were stored at -20°C .

In vitro screening assay of nematicidal activity of fungal culture filtrates. Cysts of SCN HG type 0 (race 3) were extracted from soybean roots from a pot soil culture in a growth room at the Southern Research and Outreach Center. Cysts were washed with running distilled water over a #60 wire filter. The cysts were then transferred to a 50-ml tube, and debris was removed by drawing it up with a disposable plastic bulb. Debris-free cysts were placed in a sterile 250-ml flask with 50 ml of sterile ddH_2O , 250 μl of Fungin, 50 ppm of chlorotetracycline, and 100 ppm of streptomycin and incubated overnight at 25°C . They were then transferred to a new 50-ml tube, rinsed three times in sterile ddH_2O , and stored at 4°C in sterile ddH_2O .

A J2 hatching apparatus was created by placing four layers of sterile coffee filters over a wire screen in a plastic container containing 4 mM of ZnCl_2 solution (Chen et al. 2000b). Several hundred sterile SCN cysts were added to the filters, and the apparatus was incubated at 25°C . J2s that emerged over 2 days were collected in two 50-ml tubes, which were spun at 1,500 rpm for 1 min to settle J2s. The supernatant was removed and replaced by 1% bleach solution (0.06% NaOCl), in which the J2s were soaked for 5 min. This J2 suspension was then poured over a 15- μm mesh, and the J2s retained by the mesh were rinsed three times with sterile ddH_2O . J2s were then transferred to a new 50-ml tube and diluted to 500 J2/ml.

Approximately 50 J2s (0.1 ml of J2 suspension) and 1 ml of fungal culture filtrate were added to each well of a 24-well plate. Four replicate wells were used for each timepoint for each filtrate. Fungal filtrate-free media spanning a broad pH range were used as negative controls. J2 viability was assessed at 6, 24, 48, and 72 h by adding two drops of 1 N of NaOH , which acts as a J2 stimulus (Chen and Dickson 2000), and observing the J2s' response under an inverted microscope. J2s that remained straight and showed no movement for up to 5 min were considered dead or paralyzed, whereas those that changed body shape to ring, hook, or S shaped were considered alive. SMAT culture filtrates that were nematicidal and whose pH was <5 or >8 were later thawed at 4°C and neutralized by adding small amounts of sterile 5 N HCl or NaOH . The thawed and neutralized filtrates were then retested against J2s at the 72-h timepoint.

These bioassays were repeated for 12 isolates that had high nematicidal activity in SMAT or MEB to assess reproducibility. For the second trial, fresh SMAT filtrates above pH 8 were not frozen and were neutralized and assayed for nematicidal activity at the same time as fresh non-neutralized SMAT filtrates.

Statistical analyses. All statistical analyses were performed in R version 3.5.0. Bray-Curtis dissimilarity between endophyte communities was calculated using Hellinger-transformed OTU counts (Legendre and Gallagher 2001). The effect of crop host and crop sequence on community dissimilarity was determined using *adonis* (Anderson 2001; Oksanen et al. 2016). OTU richness was calculated in the *Phyloseq* package (McMurdie and Holmes 2013). The abundance of taxa was compared between crop hosts using the Wilcoxon rank-sum test (Wilcoxon 1945) and across crop sequences within each host using the Kruskal-Wallis test (Kruskal and Wallis 1952). Spearman correlation tests were used to compare species' abundance to crop yield and SCN density.

Percentages of J2s killed or paralyzed by filtrates of each fungal isolate were compared with the percentage killed or paralyzed in media controls in the same pH range using the "nparcomp" function, a nonparametric equivalent of a Dunnett test, in the R package, *nparcomp* (Konietschke et al. 2015). We only considered SMAT filtrates to be nematicidal if they met three criteria: (i) $>50\%$

mortality of J2s at 72 h, (ii) a significant ($P < 0.05$) Dunnett's test comparing nematocidal activity of a filtrate to the SMAT control at the same pH range, and (iii) if originally outside a pH range of 5 to 8, some nematocidal activity was retained when the filtrate was neutralized to between pH 5 and 8. The proportion of isolates that produced nematocidal culture filtrates per plot was compared between crop hosts, across crop sequences, and against SCN density for each crop using the Wilcoxon rank-sum test, the Kruskal-Wallis test, and Spearman correlations, respectively.

RESULTS

Corn and soybean roots host distinct fungal endophyte communities. We isolated 626 individual fungal endophytes from surface-sterilized corn and soybean roots over an incubation period of 3 months. These were grouped into 401 morphogroups, which were clustered based on ITS sequencing into 108 OTUs with 69 unique taxonomic assignments. The corn root community was dominated by *Setophoma terrestris* (syn. *Pyrenochaeta terrestris*) (35% of isolates), followed by *F. oxysporum* (18%) and *Periconia macrospinoso* (16%), whereas the soybean root community was dominated by *Corynespora cassiicola* (27%), followed by *Dactylonectria macrodidyma* (12%) and *F. oxysporum* (13%) (Fig. 1; Supplementary Table S1).

Of the 69 unique taxa, 26 were present only in corn, 26 were present only in soybean, and 17 were present in both crop species. Taxa unique to corn roots included the potential plant pathogens, *Diaporthe longicolla* and *Drechslera* sp., and several species of *Trichoderma*, *Exophiala*, and *Mortierella*. Taxa unique to soybean roots included the soybean pathogen *Corynespora cassiicola* and several nematophagous taxa, including *Arthrobotrys iridis*, *Metacordyceps chlamydosporia*, and *Hirsutella rhossiliensis*. Of the taxa common to both corn and soybean roots, four had a significantly different abundance between hosts according to the Wilcoxon rank-sum test (false discovery rate [FDR]-corrected $P < 0.05$). Of these, *F. oxysporum*, *Setophoma terrestris*, and *Periconia macrospinoso*

were more abundant in corn roots, and *Dactylonectria macrodidyma* was more abundant in soybean roots.

Fungal endophytes isolated from the root tips of corn and soybean. Although they were not sampled as intensively as the central portion of the root, endophytic communities isolated from the root tips were similar in taxon composition to the central regions of lateral roots of each respective host (Fig. 1). However, some taxa were only isolated from the root tips of corn and soybean. For example, in corn roots, fungi belonging to the genera *Mortierella*, *Epicoccum*, *Diaporthe*, *Paramicrothyrium*, *Clohesyomyces*, and *Cadophora* were isolated from root tips but not from central root sections (Supplementary Table S1). In soybean, only two taxa, *Hirsutella rhossiliensis* and *Myrothecium verrucaria*, were isolated exclusively from root tips (Supplementary Table S1).

Effects of crop host, crop sequence treatment, and SCN density on fungal root endophytic communities. The adonis test showed that fungal endophyte community composition differed by crop host ($P = 0.001$) and by crop sequence within corn ($P = 0.056$) and soybean ($P = 0.012$) (Table 1). Likewise, principal coordinates analysis showed a clear separation of fungal endophyte communities based on crop host and progressive shifts by crop sequence from early to later years of monoculture within each crop host (Fig. 2). In both corn and soybean, fifth-year (C5 and S5) communities mapped close to long-term monoculture (Cc and Ss) communities associated with their respective crops. Corn root

TABLE 1. R^2 and P values calculated by the adonis test on the Bray-Curtis dissimilarity matrix for crop host and crop sequence effects on the root endophytic fungal community^a

Treatment	R^2	P value ^b
Crop host	0.24	0.001*
Corn crop sequences	0.26	0.056
Soybean crop sequences	0.25	0.012**
All crop sequences	0.44	0.001*

^a Adonis was run with 999 permutations on the models Dissimilarity ~ Block + Host and Dissimilarity ~ Block + Crop Sequence.
^b Asterisks indicate significance at * $P < 0.01$ and ** $P < 0.05$.

Fig. 1. Community profiles of cultured fungal endophytes from the center or tip of corn and soybean lateral roots. Of 18 lateral roots collected from each plot, 15 were sampled from the center and three were sampled from the tip. Genera that were isolated fewer than three times total are combined in the "rare genus" category.

Fig. 2. Principal coordinates analysis (PCoA) of culture-dependent endophyte communities of corn and soybean roots across crop sequences. R^2 and P values are reported for the Crop Sequence term of the model, Dissimilarity ~ Block + Crop Sequence, determined using adonis. Crop sequences are as follows: C1, C3, C5, S1, S3, S5 = years 1, 3, and 5 of continuous corn or soybean monoculture; Cc and Ss = 35 years of continuous corn or soybean monoculture; and Ca and Sa = corn and soybean in annual rotation, respectively.

endophyte communities were more tightly clustered overall, whereas soybean root endophyte communities shifted more in relation to crop sequence, with communities from annual rotation (Sa) and first-year monoculture (S1) plots mapping closer to corn root endophyte communities (Fig. 2). Although OTU richness did not significantly vary by crop sequence in either crop, corn roots hosted a significantly more diverse fungal community compared with soybean (Table 2).

Only one taxon, *Corynespora cassiicola*, was found to significantly differ in abundance across soybean crop sequences according to the Kruskal-Wallis test ($P = 0.04$), but this result was nonsignificant after FDR correction (FDR-corrected $P = 0.5$). *Corynespora cassiicola* was least abundant in the first year (S1) and most abundant in the long-term soybean monoculture (Ss) plots (Supplementary Table S2). No taxa were found to differ significantly in abundance across corn crop sequences.

SCN density has previously been shown to increase with increasing years of soybean monoculture and to decrease with increasing years of corn monoculture in these plots at our study site (Grabau and Chen 2016a, b) and exhibited a similar trend in our sampling year (Hu et al. 2018) (Supplementary Fig. S1). Within soybean plots, alpha diversity was significantly correlated with SCN density (Table 2). Although no OTUs or taxa in either crop host were found to be significantly correlated with SCN density or with crop yield after FDR correction, one OTU identified as *F. solani* had a positive correlation with SCN density in soybean before FDR correction ($\rho = 0.55$; $P = 0.01$, FDR-corrected $P = 0.76$), and one taxon, *Setophoma terrestris*, was negatively correlated with corn yield before FDR correction ($\rho = -0.48$; $P = 0.03$, FDR-corrected $P = 0.61$).

Nematicidal activity of fungal culture filtrates in relation to media type, pH, and SCN density. Overall, nematicidal activity was greater in filtrates of fungal cultures grown in SMAT than in MEB (Fig. 3A and B). Despite buffering with ammonium tartrate, the pH of the SMAT media was substantially altered during growth of the fungal strains, with final values ranging from 3.25 to 9.01 (Fig. 3A). The majority of nematicidal SMAT filtrates were below pH 5 or above pH 8 (Fig. 3A). Many SMAT media controls in the same pH range as some nematicidal filtrates had equal or greater nematicidal activity, especially below pH 5 (Fig. 3C). In contrast, MEB negative controls only showed significant nematicidal activity above pH 9, which was outside of the observed pH range of MEB fungal culture filtrates (Fig. 3D). All SMAT filtrates that originally had a pH <5 lost all nematicidal activity when neutralized (Supplementary Fig. S2A). Most SMAT filtrates that originally had a pH >8 retained some or all nematicidal activity when neutralized, but nematicidal activity was often less in neutralized filtrates (Supplementary Fig. S2B).

In the first bioassay trial, 34 isolates in SMAT and 43 isolates in MEB were identified that produced significantly more J2 deaths than negative controls in the same pH range at any timepoint (Supplementary Tables S3 and S4) according to a nonparametric Dunnett's test (Konietschke et al. 2015). A majority of isolates that killed >50% of J2s at 72 h in SMAT culture filtrates in the first trial were members of the genus *Fusarium* (Table 3). Six *Fusarium* isolates and one isolate of *Epicoccum nigrum* produced basic filtrates that retained 90 to 100% nematicidal activity at 72 h when neutralized (Table 3). Additional isolates with >50% nematicidal activity at 72 h in SMAT included *Dactylonectria macrodidyma*, *Exophiala equina*, *Myrothecium verrucaria*, and *Trichoderma gamsii* (Table 3). *Hirsutella rhossiliensis* and *Waitea circinata* produced culture filtrates in MEB that resulted in >25% J2 deaths at 72 h in the first trial (Table 4).

We repeated these assays a second time for 12 isolates showing high nematicidal activity in the first trial. In the second bioassay trial, none of the MEB filtrates had nematicidal activity significantly

greater than media controls. Therefore, data are only displayed for the SMAT filtrates (Table 5). All of the SMAT filtrates in the second trial had significant nematicidal activity comparable to the activity observed for the same isolates in the first trial (Tables 3 and 5). The pH of nematicidal filtrates was similar for most isolates in the first and second trials, except for the *Myrothecium verrucaria* isolate, which produced an acidic, rather than a basic, filtrate in the second trial (Tables 3 and 5). Fresh neutralized SMAT filtrates that originally were above pH 8 had approximately the same nematicidal activity when neutralized (Table 5). The *Hirsutella rhossiliensis* isolate did not produce SCN-toxic filtrates in MEB in the second trial but did produce moderately toxic filtrates in SMAT (Table 5). No significant patterns were identified between the proportion of isolates with nematicidal culture filtrates per plot and crop host, crop sequence, or SCN density.

DISCUSSION

Corn and soybean host distinct CD endophytic communities.

Our first hypothesis, that corn and soybean would host distinct root endophyte communities, was supported by our community-level analyses (Fig. 2; Table 1) and by the isolation frequency of specific taxa from corn and soybean roots (Fig. 1). Interestingly, the CD communities of corn and soybean roots in our study differed from those reported in other culture-based studies. Previous studies of corn root endophytes have found that the community was dominated by the genera *Fusarium* and *Alternaria* in Missouri clay soil in a greenhouse experiment (Bokati et al. 2016) and on the Indian subcontinent (Potshangbam et al. 2017) with other commonly reported genera including *Acremonium*, *Aspergillus*, *Colletotrichum*, *Mucor*, *Penicillium*, *Saccharomyces*, *Sarocladium*, and *Trichoderma* (Amin 2013; Bokati et al. 2016; Orole and Adejumo 2011; Potshangbam et al. 2017; Russo et al. 2016). While our corn root endophyte community contained many *Fusarium* isolates, it was dominated by a potential corn pathogen, *Setophoma terrestris* (Mao et al. 1998), which may also explain a reddish discoloration observed in many of our corn root sections (Lević et al. 2013). The negative correlation between *Setophoma terrestris* abundance and corn yield, although not significant after FDR correction, nonetheless suggests that this fungus may be implicated in the yield declines observed under continuous corn monoculture (Seifert et al. 2017). *Setophoma terrestris* has been identified as the primary pathogen in red root rot of corn (Mao et al. 1998) and is in the order Pleosporales, which contains many plant pathogens, including the ubiquitous genus *Alternaria* (Zhang et al. 2012), suggesting that different pathogens from this order may be more or less prevalent at different sites.

Previous studies of soybean root endophytes have found that *Fusarium* isolates dominated this community (Russo et al. 2016; Yang et al. 2018), with other commonly reported genera including *Alternaria*, *Aspergillus*, *Bipolaris*, *Cadophora*, *Cladosporium*, *Clonostachys*, *Colletotrichum*, *Corynespora*, *Curvularia*, *Fusarium*, *Macrophomina*, *Mortierella*, *Mucor*, *Penicillium*, *Phaeosphaeria*, *Phoma*, *Phomopsis*, *Rhizoctonia*, *Rhizopus*, *Talaromyces*, and *Trichoderma* (Fernandes et al. 2015; Hamayun et al. 2009; Rothen et al. 2017; Russo et al. 2016; Yang et al. 2018). In contrast

TABLE 2. Patterns of root endophyte alpha diversity

Host	Mean richness (S.D.) ^a	SCN (β) ^b
Corn	10.4 (2.6)***	0.099
Soybean	7.3 (3.0)***	0.12**

^a Mean richness was compared using analysis of variance on the linear model Richness ~ Block + Host. Asterisks indicate significance at *** $P < 0.001$ and ** $P < 0.01$.

^b The coefficient (β) is reported for the soybean cyst nematode (SCN) term according to analysis of variance on the linear model Richness ~ Block + SCN Density. Asterisks indicate significance at *** $P < 0.001$ and ** $P < 0.01$.

to these studies, we found that the fungal root endophyte community in soybean was dominated by *Corynespora cassicola*. This fungus is known to be pathogenic on soybean roots and leaves without causing significant yield losses (Seaman et al. 1965). The fungus has also been isolated from SCN cysts but has little parasitic ability on SCN eggs (Chen and Chen 2002).

Many endophyte studies report on communities characterized by both CD and CI methods (Fernandes et al. 2015; Impullitti and Malvick 2013; Yang et al. 2018). Although here we only report on the CD community, we described the CI community in a previous study utilizing the same root systems (Strom et al. 2019a). Some patterns were similar across the two study types. Both methods, for example, showed that *Corynespora cassicola* and *Setophoma terrestris* were strongly associated with soybean and corn roots, respectively, and ordination plots showing the effect of crop sequence on corn and soybean endophyte communities were similar for the two methods (Fig. 2). However, the CI method captured far more fungal diversity, with a total of 221 and 189 genera (Strom et al. 2019a) compared with only 33 and 39 genera from the CD method in corn and soybean roots, respectively. These findings

agree with previous reports of fungal endophyte diversity in corn and soybean that have found that CI methods generally capture greater fungal diversity than CD methods (Bokati et al. 2016; Yang et al. 2018). However, one study of soybean stem endophytes found greater diversity using a CD method compared with a CI method (Impullitti and Malvick 2013).

Soybean root endophyte community shaped by crop sequence and the SCN. Although we did not detect many significant differences in the abundance of taxa across crop sequences, the endophyte community as a whole varied by crop sequence, especially for soybean (Fig. 2; Table 1), suggesting that some abiotic or biotic factors associated with crop sequence treatment played a role in shaping the soybean root endophyte community. The positive correlation between SCN density and endophyte alpha diversity in soybean roots (Table 2) suggests that the SCN may play a role. This increase in endophytic fungal alpha diversity could be attributable to the SCN opening up sites on the root through which fungi colonize (Westphal et al. 2014), or SCN juveniles could be transporting fungi attached to their cuticles into the roots (Chen and Dickson 2012).

Fig. 3. Percentage of *Heterodera glycines* second-stage juveniles (J2s) killed or paralyzed in fresh non-neutralized filtrates of fungal cultures and media controls in **A**, secondary metabolite-inducing media buffered by ammonium tartrate (SMAT) and **B**, malt extract broth (MEB) and in **C**, SMAT and **D**, MEB media controls with no fungal growth across a pH range. Symbols represent the percentage of J2s killed or paralyzed in individual wells on a 24-well plate. A jitter effect was used to slightly displace some of the symbols to avoid overlap.

The root endophyte community of several plant species has been hypothesized to be composed of a subset of the rhizosphere microbial community (Bulgarelli et al. 2013; Edwards et al. 2015; Sasse et al. 2018; Strom et al. 2019a), which may be altered in response to pathogen attack (Lareen et al. 2016). In a greenhouse experiment in which soybean plants were challenged with the SCN, the soybean rhizosphere became enriched in several bacterial taxa with putative roles in SCN suppression (Hussain et al. 2018). This study also showed that the SCN cyst bacterial community was closely related to the soybean root endosphere bacterial community and was initially assembled through the selection of bacterial taxa from the soybean root endosphere. Carris et al. (1986, 1989) hypothesized that soybean root endophytic fungi may grow into SCN cysts attached to the root. It was later shown that several species of fungi, including several species of nematophagous fungi, isolated from SCN cysts can in fact colonize soybean roots endophytically (Stiles and Glawe 1989). A study of fungi using CI approaches at our study site found a similar pattern in which fungal taxa inhabiting SCN cysts showed greater overlap with taxa found in soybean roots compared with corn roots (Strom et al. 2019a). This study builds on these findings by showing that many nematophagous fungi, including *Arthrobotrys iridis*, a nematode-trapping fungus (Yu et al. 2014), *M. chlamydosporia* (syn. *Pochonia chlamydosporia*), a nematode egg parasite (Manzanilla-Lopez et al. 2013), and *Hirsutella rhossiliensis*, a nematode endoparasite (Wang et al. 2016), can grow endophytically in soybean roots. Collectively, these results suggest that pathogen pressure from the SCN may alter the root endophyte mycobiota of SCN-susceptible soybean.

Fungal endophytes from the root tip. Most culture-based studies do not indicate that the root tip is a common sampling site for fungal endophytes (Potshangbam et al. 2017; Russo et al. 2016; Yang et al. 2018). The root tip is a site of active growth and secretion of mucilage and other exudates (Badri and Vivanco 2009). These secretions attract fungi (Graham et al. 1981), bacteria (Bulgarelli et al. 2013), and nematodes (Curtis 2008), including SCN juveniles (Papademetriou and Bone 1983), resulting in rhizosphere microbial communities distinct from more mature root regions (Kawasaki et al. 2016; Massalha et al. 2017; Sasse et al. 2018). SCN juvenile density has been shown to be highest surrounding soybean root tips (Hu et al. 2017b).

Overall, the fungal communities recovered from the root tip were similar in composition to those in central sections of the root (Fig. 1; Supplementary Table S1), but because roots tips were sampled with less frequency than central portions of the root, we did not quantitatively compare endophyte communities isolated from these two different regions of the root. However, despite five-fold greater sampling of central sections of roots, several fungi of interest for use in biocontrol or nutrient acquisition were only isolated from the root tip sections. For example, the only isolate of *Hirsutella rhossiliensis*, a potential biocontrol agent of the SCN (Zhang et al. 2008), and the only isolate of *Myrothecium verrucaria*, which has been developed into a commercial nematicide (Li et al. 2015; Valent BioSciences 2010), were isolated only from soybean root tips. Furthermore, the corn root tip hosted the only two isolates of *Mortierella*, a genus with roles in soil phosphorus solubilization (Osorio and Habte 2014). Some *Mortierella* isolates have been

TABLE 3. Percentage of second-stage juveniles (J2s) killed or paralyzed in nematicidal filtrates of isolates cultured in secondary metabolite-inducing media buffered with ammonium tartrate (SMAT)^a

OTU ID ^b	Isolate ID	Taxonomy	Incubation period (h) ^c						SMAT pH	Neutralized pH	Host
			6	24	48	72	72 (thawed)	72 (neutralized)			
1	205A_c9.1	<i>Dactylonectria macrodidyma</i>	0	3.7	5.3*	7.1*	100*	49.8*	8.23	5.32	Corn
1	305A_c11.1	<i>Dactylonectria macrodidyma</i>	31.2*	49.5*	60.6*	83.5*	NA	NA	7.97	NA	Soybean
1	308B_c4.1	<i>Dactylonectria macrodidyma</i>	0.4	8.5*	22.4*	24.8*	87.5*	4.4	8.24	5.5	Soybean
36	302B_c7.1	<i>Epicoccum nigrum</i>	0	34.5*	33.8*	18.5*	100*	98*	8.54	7.46	Soybean
32	403A_T17.2	<i>Exophiala equina</i>	2	77.3*	91.1*	89.8*	100*	30.1*	8.78	6.31	Corn
44	412B_c13.1	<i>Fusarium dimerum</i>	0.5	50.4*	95.1*	100*	NA	NA	7.6	NA	Soybean
18	207A_c7.2	<i>F. acuminatum</i>	38.6*	49.5*	60.8*	75.6*	NA	NA	7.93	NA	Corn
26	212A_T16.2	<i>F. oxysporum</i>	0	4.4*	14.4*	31.7*	100*	82.5*	8.53	6.59	Corn
26	401B_T17.1	<i>F. oxysporum</i>	0	24.3*	21.5*	19.9*	100*	7.1	8.47	7.83	Corn
26	403A_c5.1	<i>F. oxysporum</i>	17.4*	85.8*	79.7*	85.3*	100*	100*	8.74	5.87	Corn
26	404A_c1.2	<i>F. oxysporum</i>	1	15.8*	28.6*	21.7*	95.5*	21*	8.21	7.18	Corn
26	302B_c13.1	<i>F. oxysporum</i>	0	18.1*	22.9*	30.3*	100*	91.6*	8.02	6.84	Soybean
3	110A_c3.1	<i>F. oxysporum</i>	0.4	100*	95.6*	100*	100*	100*	8.27	5.71	Corn
3	207A_c10.1	<i>F. oxysporum</i>	0	26.4*	97.9*	100*	86.9*	13.3*	8.59	7.38	Corn
8	110A_c18.2	<i>F. oxysporum</i>	20.9*	86.2*	91.7*	90.1*	100*	100*	8.82	6.84	Corn
8	105B_c14.1	<i>F. oxysporum</i>	1.8	24.6*	47.5*	62.4*	NA	NA	8.14	NA	Soybean
16	303A_c10.1	<i>F. solani</i>	0	15.8*	68.9*	80*	100*	100*	8.09	6.94	Soybean
78	206A_T16.1	<i>F. solani</i>	2.7*	48.1*	99*	98.9*	100*	60.2*	8.22	7.28	Soybean
101	212A_c9.1	<i>Fusarium</i> sp.	25.4*	58*	55.2*	80.2*	NA	NA	7.82	NA	Corn
12	407B_c13.1	<i>Fusarium</i> sp.	0	100*	57.6*	70.7*	100*	100*	8.26	6.24	Soybean
102	111A_T4.1	<i>Myrothecium verrucaria</i>	0	75.2*	87.1*	82.5*	100*	26.4*	8.46	5.98	Soybean
74	410B_T17.1	<i>Trichoderma gamsii</i>	0	92.4*	41.4*	82.9*	NA	NA	6.14	NA	Corn
Negative controls											
SMAT (pH)											
3–4			0	78.9	95.7	100	100	NA	3.4		
4–5			0	0	25.6	77.8	77.8	NA	4.04		
5–6			0	0	0.9	1.4	1.4	1.4	5.05		
6–7			0	0.6	0	0	0	0	6.8		
7–8			0	0	0	0.7	0	0	7.75		
8–9			0	2	2	1.7	10	NA	8.95		
Water			0	0	0	0	0	0			

^a Filtrates that killed or paralyzed a minimum of 50% of J2s at 72 h are included in this table. SMAT culture filtrates with a pH <5 or >8 were thawed and neutralized such that the neutralized pH was between pH 5 and 8. These thawed and neutralized filtrates were retested at the 72-h timepoint.

^b OTU = operational taxonomic unit and ID = identifier.

^c Asterisks indicate that the percentage of J2s killed or paralyzed was greater than in media controls in the same pH range according to a nonparametric Dunnett's test (false discovery rate-corrected $P < 0.05$). NA = not applicable.

shown to degrade toxic compounds in the soil, increase soil organic matter, and promote growth in corn (Li et al. 2018). It is possible that such isolates could ultimately be developed into biofertilizers that could reduce reliance on inorganic sources of phosphorus and improve crop yields (Li et al. 2018). Recovery of endophytes from plant tissues is highly variable and influenced by many factors, and more intensive sampling is required to assess the distribution of these fungi spatially within roots. Nonetheless, these observations suggest that root tips may harbor unique taxa and should be sampled along with other root sections in bioprospecting studies seeking to isolate fungi with potential biocontrol and biofertilizer applications.

Nematicidal activity of fungal culture filtrates in relation to media type, pH, and SCN density. We hypothesized that more endophytes isolated from long-term soybean monoculture plots would produce nematicidal culture filtrates. Although this hypothesis was not supported, we did identify a number of

fungal isolates that produced culture filtrates with nematicidal activity against the SCN. Previous studies testing culture filtrates of fungi for activity against the SCN have primarily relied on rich media, like potato dextrose broth (PDB) (Juba et al. 2004) or MEB (Chen et al. 2000b) for induction of secondary metabolite production. In a pilot study, we tested PDB for the induction of nematicidal compound production by fungi but found that none of the PDB filtrates had any nematicidal activity against the SCN. Here, we show that a synthetic medium (SMAT) induced nematicidal compound production in a greater number of fungal isolates than MEB and that nematicidal activity was generally greater in this synthetic media than in MEB (Fig. 3). However, very slow-growing isolates, like *Hirsutella rhossiliensis*, had limited growth in SMAT but had robust growth and produced nematicidal compounds in MEB in the first trial. These results suggest that a best practice for screening fungi for toxic compound production may be to utilize two contrasting types of media, one to induce secondary

TABLE 4. Percentage of second-stage juveniles (J2s) killed or paralyzed in nematicidal filtrates of isolates cultured in malt extract broth (MEB)^a

OTU ID ^b	Isolate ID	Taxonomy	Incubation period (h) ^c				MEB pH	Host
			6	24	48	72		
3	207A_c10.1	<i>Fusarium oxysporum</i>	0	5*	97.5*	98.6*	7.42	Corn
71	206A_T18.1	<i>Hirsutella rhossiliensis</i>	0	23.2*	16*	29.3*	7.57	Soybean
49	212A_c4.1	<i>Waitea circinata</i>	0	17.5*	16.2*	31.5*	7.75	Corn
Negative controls								
MEB			0	0	0.5	0	5.21	
Water			0	0	0	0		

^a Only filtrates that killed or paralyzed a minimum of 25% of J2s at 72 h are included in this table.

^b OTU = operational taxonomic unit and ID = identifier.

^c Asterisks indicate that the percentage of J2s killed or paralyzed was greater than in media controls according to a nonparametric Dunnett's test (false discovery rate-corrected $P < 0.05$).

TABLE 5. Percentage of second-stage juveniles (J2s) killed or paralyzed in the second trial of isolates cultured in secondary metabolite-inducing media buffered with ammonium tartrate (SMAT)^a

OTU ID ^b	Isolate ID	Taxonomy	Incubation period (h) ^c				pH	Host
			6	24	48	72		
Original pH filtrate								
1	305A_c11.1	<i>Dactylonectria macrodidyma</i>	0	5.1*	97.3*	100*	7.95	Soybean
36	302B_c7.1	<i>Epicoccum nigrum</i>	0	13.1*	21.6*	100*	7.12	Soybean
32	403A_T17.2	<i>Exophiala equina</i>	0	22.4*	76.4*	100*	7.91	Corn
18	207A_c7.2	<i>Fusarium acuminatum</i>	0	22.4*	98.3*	100*	8.55	Corn
44	412B_c13.1	<i>F. dimerum</i>	2.3	33.1*	100*	100*	8.7	Soybean
3	207A_c10.1	<i>F. oxysporum</i>	2.8	25.9*	97.3*	100*	8.85	Corn
8	110A_c18.2	<i>F. oxysporum</i>	0	15.3*	94.5*	100*	8.38	Corn
78	206A_T16.1	<i>F. solani</i>	0	7.0*	23.2*	100*	9.01	Soybean
12	407B_c13.1	<i>Fusarium</i> sp.	0	8.1*	98.6*	100*	8.68	Soybean
71	206A_T18.1	<i>Hirsutella rhossiliensis</i>	0	0.0	26.7*	21.4*	5.01	Soybean
102	111A_T4.1	<i>Myrothecium verrucaria</i>	0	14.6*	10.3*	88.3*	5.00	Soybean
74	410B_T17.1	<i>Trichoderma gamsii</i>	0	42.9*	98.4*	100*	5.07	Corn
Neutralized filtrate								
18	207A_c7.2	<i>F. acuminatum</i>	0	19.6*	46.8*	100*	7.93	Corn
44	412B_c13.1	<i>F. dimerum</i>	0	32.5*	98.7*	100*	7.97	Soybean
3	207A_c10.1	<i>F. oxysporum</i>	0	34.2*	46*	100*	7.32	Corn
8	110A_c18.2	<i>F. oxysporum</i>	0	32.4*	100*	100*	7.85	Corn
78	206A_T16.1	<i>F. solani</i>	0	24.9*	14.6*	100*	7.83	Soybean
12	407B_c13.1	<i>Fusarium</i> sp.	0	13.0*	23.7*	100*	7.65	Soybean
Negative controls								
SMAT (pH)								
5–6			0	0	0	0	5.51	
6–7			0	0	0	0	6.70	
7–8			0	0	0	0.7	7.75	
8–9			0	0	0	6.8	8.64	
Water			0	0	0	0	6	

^a Fresh SMAT culture filtrates with a pH <5 or >8 were neutralized such that the neutralized pH was between pH 5 and 8 and tested at the same time as non-neutralized filtrates.

^b OTU = operational taxonomic unit and ID = identifier.

^c Asterisks indicate that the percentage of J2s killed or paralyzed was greater than in media controls in the same pH range according to a nonparametric Dunnett's test (false discovery rate-corrected $P < 0.05$).

metabolite production in fast-growing isolates and one to encourage growth of slow-growing toxin-producing isolates.

Our research also points to the need to investigate the effect of pH on nematicidal activity of culture filtrates, given that the pH of SMAT was a strong determinant of J2 deaths. Many species of nematophagous fungi are known to produce organic acids with nematicidal activity (Degenkolb and Vilcinskas 2016a). For example, the nematode-trapping fungus *Arthrobotrys* and nematode egg-parasitic fungus *Purpureocillium lilacinum* have been shown to produce nematicidal amounts of linoleic acid (Anke et al. 1995; Park et al. 2004). Furthermore, *Purpureocillium lilacinum* and a non-nematophagous fungus, *Trichoderma longibrachiatum*, have been shown to produce acetic acid, which was nematicidal principally in its nondissociated form below pH 5 (Favre-Bonvin et al. 1991). However, in our study, while it is possible that SMAT culture filtrates with a pH <5 contained nematicidal compounds that were active only at an acidic pH, it was impossible to separate their effects from those of the acidic media.

Previous studies have shown that secondary metabolite production by fungi increases in acidic media (Calvo et al. 2002; Chen et al. 2000b; Cotty 1988; Gardiner et al. 2009). However, we found that most SMAT filtrates that killed a significant percentage of J2s were originally in a basic pH range. Several of these filtrates killed 100% of J2s at 72 h, even when neutralized, suggesting that pH was not a significant factor in their activity. These filtrates, as well as those that had nematicidal activity in a pH range of 5 to 8, may prove to be sources of new nematicidal compounds active against the SCN, and the fungi that produced them should be investigated for their biocontrol potential in planta.

Prospects for biocontrol of the SCN with fungal isolates and culture filtrates. Several isolates produced culture filtrates that exhibited significant activity against the SCN (Tables 3 and 5). However, the ability of these isolates to be developed into biocontrol agents depends on many factors. One factor is the pathogenicity of an isolate on its host plant. Of the isolates that produced culture filtrates exhibiting high nematicidal activity, members of the genera *Dactylonectria*, *Epicoccum*, *Fusarium*, and *Myrothecium* are all potential plant pathogens (Chen et al. 2016b; Mahadevakumar et al. 2014; Malapi-Wight et al. 2015; Wilman et al. 2014). Various formae speciales of the *F. oxysporum* species complex and the *F. solani* species complex are pathogenic on a broad range of host plants, including soybean (Coleman 2016; Cruz Jimenez 2017). Other strains of *F. oxysporum* and *F. solani*, however, are nonpathogenic (Fravel et al. 2003; Siddiqui and Shaikat 2003). Given the difficulty in distinguishing formae speciales using ITS sequencing alone (O'Donnell et al. 2015), tests for the pathogenicity of *Fusarium* species and other potentially pathogenic isolates on soybean and corn should be conducted before considering them as candidates for biocontrol.

It is possible, however, to develop chemical nematicides from isolates that are pathogenic on plants, provided no living fungal inoculum is present. Such a nonliving product, marketed as DiTerra, has been developed from the plant pathogen, *Myrothecium verrucaria* (Wilson and Jackson 2013), and the culture filtrates of the only isolate of *Myrothecium verrucaria* in our study also showed high nematicidal activity against the SCN. Plant pathogenic *Fusarium* or *Dactylonectria* isolates with nematicidal culture filtrates could potentially be developed into similar products. Some research suggests that nematicidal endophytes can produce toxins at a level that is nematicidal in plant roots (Bacetty et al. 2009), but for the most part, this question is unresolved (Schouten 2016). Fungal secondary metabolites have been shown to have nematicidal activity at similar concentrations in previous in vitro and in vivo assays (Mayer et al. 1999), suggesting that direct application of fungal culture filtrates may be effective in agricultural settings.

A second consideration for biocontrol agents is that they should not be dangerous to humans or other organisms, aside from their target organism. Isolates of several *Fusarium* spp., including those

in the *F. solani*, *F. oxysporum*, and *F. dimerum* species complexes can cause diseases of the cornea (Hassan et al. 2016) and may pose a greater threat to immunocompromised individuals (Zhang et al. 2006). *Epicoccum nigrum*, one isolate of which produced culture filtrates with high nematicidal activity, is also implicated in some human respiratory diseases, including allergic asthma (Summerbell 2001). Likewise, *Exophiala equina* may opportunistically infect human skin (Najafzadeh et al. 2013). Although many strains of these fungi may not pose a threat, fungi for which human or animal pathogenicity is a concern should not be considered for biocontrol.

Of the isolates that produced culture filtrates with high nematicidal activity, those that have previously been identified as potential biocontrol agents of nematodes or other organisms may hold the greatest potential. *Trichoderma gamsii*, one isolate of which showed high nematicidal activity in this study, is a fast-growing nonpathogenic endophyte that can antagonize a variety of fungal pathogens as well as promote plant growth in corn, soybean, wheat, and lentil (Chen et al. 2016a; Rinu et al. 2014). While to our knowledge, *Trichoderma gamsii* has not been tested for the biocontrol of nematodes, related *Trichoderma* species, such as *Trichoderma virens* and *Trichoderma harzianum*, have shown some potential in controlling populations of the root-knot nematode (*Meloidogyne incognita* and *Meloidogyne javanica*) in greenhouse studies, mostly through parasitism of nematode eggs (Mascarin et al. 2012; Massoud et al. 2000; Sahebani and Hadavi 2008; Sharon et al. 2007). Our isolate of *Trichoderma gamsii* has the potential to be a novel biocontrol agent of the SCN.

Hirsutella rhossiliensis may also hold potential as a biocontrol agent of the SCN. Although our *Hirsutella rhossiliensis* isolate showed lower nematicidal activity compared with other nematicidal isolates, this could be attributable to the synthetic media used. As a near-obligate parasite of nematodes, it has several beneficial traits for biocontrol, such as better growth in the presence of high population levels of the SCN (Chen and Liu 2005; Zhang et al. 2008) and a lower likelihood to affect nontarget organisms. However, it may be difficult to mass produce, given its slow growth rate in most synthetic media types (Liu and Chen 2002).

Conclusions. Here, we show that roots of the corn and soybean varieties planted at this study site host distinct communities of root endophytic fungi and that both crop sequence and the SCN play a role in shaping these communities. Several species of nematophagous fungi were isolated from the soybean root, suggesting that this agroecosystem compartment hosts fungi with potential roles in parasitizing the SCN. Isolates from both plant hosts produced compounds toxic to the SCN in vitro and may hold promise for the biocontrol of the SCN. Future research should focus on characterizing metabolites produced by these fungi and on testing their ability to control the SCN in the greenhouse and field. Sustainable approaches to control the SCN are needed, and endophytes may offer one part of the solution.

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