2025 Mid-Year report

Characterization of microbes in the suppressive field soil against soybean cyst nematode in North Dakota

Principle Investigator: Dr. Guiping Yan
Co-investigator: Dr. Wade Webster

Research Overview and Objectives

Soybean cyst nematode (SCN) is one of the most damaging pests of soybean. There are management strategies such as crop rotation, host resistance and nematicides to manage SCN but they are either short lived as in the case of host resistance, or do not offer wide cropping options like crop rotation, or expensive and harmful to environment like chemical nematicides. Over the past years, we have been investigating the biological approach of managing SCN in North Dakota (ND) through identification and evaluation of suppressive soil to SCN from different soybean fields in ND. In our previous field survey, we observed that 10 such soybean fields which had high SCN infestation in the past showed lower SCN population at the time of sampling. From our greenhouse experiments, we identified two of those fields from Richland and Dicky counties reduced nematode activity in inoculated field soils as compared to controls, showing suppressiveness to SCN. We thus proposed to characterize the microbes present in those soils through high throughput sequencing. By sequencing the amplicons of bacteria and fungi, we characterized the microbes with biocontrol properties at higher abundance and diversity present in the rhizosphere of soybean in suppressive soils as compared to conducive soils. The findings of the research on beneficial microbes with biocontrol properties can help growers in sustainable management of SCN.

- Verify the suppressive soil from soybean fields in ND identified from our previous work.
- Evaluate the factors for suppressiveness to SCN in the suppressive field soil.
- Characterize the bacterial and fungal taxa with biocontrol properties associated with SCN-suppressiveness in the suppressive fields.

Completed Work

Four field soils (two suppressive and two conducive to SCN) identified in previous experiments were collected from different regions of North Dakota. In each field, sampling was done in similar ways; in a zig-zag pattern, by collecting and mixing the soil from different sampling points. From the sampled soils, species of plant-parasitic nematodes and number of non-plant parasitic nematodes were determined after nematode extraction. These four field soils were submitted to the Agvise Soil Laboratories for evaluating their soil physical and chemical properties to check if suppressiveness was affected by abiotic factors in the soil.

A 200 g subsample soil (fine and debris-free soil) was collected from each of four composite field soils and stored at -80 ^{o}C for preserving its DNA for microbiome work. A greenhouse experiment was set up using the soils from these four fields in order to verify our

previous findings on the suppressive soil. There are four treatments (T1: 100% autoclaved field soil with 2,000 SCN eggs inoculation, T2: 50% natural field soil mixed with 50% autoclaved field soil, inoculated with 2,000 SCN eggs, T3: 100% natural field soil with 2,000 SCN eggs inoculation, T4: 100% natural field soil without SCN eggs inoculation). Susceptible variety Barnes was used as a host. The experiment was set up in a growth chamber for 60 days at 27 ºC, and will be harvested in January 2025. SCN will be extracted and SCN population will be determined to check the suppressiveness of the soils.

DNA was extracted from 250 mg of soil taken from each of the field soil samples using DNeasy PowerSoil Pro extraction kit for microbiome analysis. After the extraction, the concentrations of DNA samples were determined using the Qubit Fluorometer with the Quanti-iT dsDNA HS Assay Kit. The DNA samples were preserved at -20°C for subsequent analyses. The DNA samples in good quality were submitted to the University of Minnesota Genomics Center for amplification of the V3-V4 hypervariable region of the bacterial 16S rRNA gene and fungal ITS1 region, library construction, and Illumina sequencing (2 × 300 paired-end with V3 chemistry). The sequencing dataset was processed for analysis using DADA2 pipeline within QIIME2. Different diversity indexes like alpha and beta diversity were measured to evaluate the diversity of fungi and bacteria associated with the suppressive soil. The fungal and bacterial taxa with potential biocontrol properties for SCN and plant-parasitic nematodes were accessed for their abundance and uniqueness. The experiment was repeated by taking field samples again and sending the samples for sequencing with similar procedures to verify the results.

Progress of Work and Results to Date

Soil samples were collected from these SCN-suppressive fields and non-suppressive fields, with subsequent profiling of bacterial and fungal communities by sequencing 16S and ITS1 rRNA genes, respectively. Various nematodes such as SCN, Dagger, Stunt, Root-lesion, Spiral, and non-plant-parasitic nematodes were observed (Field 1 to Field 4, Table 1). The initial SCN density ranged from 11 to 861 eggs and J2s per 100 cc of soil in these field soils. Field 1 and Field 4 had numerically higher SCN densities as compared to Field 2 and Field 3. Field 2 had the lowest SCN density as compared to other fields. The dagger nematodes and root-lesion nematodes were only present in Field 1 and Field 4, and stunt nematodes only in Field 4. Spiral nematodes were only present in Field 3 and Field 1. All field soils had non-plant-parasitic nematodes ranging from 450-816 per 100 cc of soil. Soil physiochemical properties were also evaluated for these field soils.

Soil fungal and bacterial communities in these fields were assayed (Figures 1-3). The analysis showed Proteobacteria and Ascomycota as the predominant bacterial and fungal groups in suppressive soils, respectively. The bacterial and fungal diversity within the soils was unlikely to contribute to the nematode suppression as alpha diversity indexes were not significantly higher in the suppressive soils than in the non-suppressive soils. However, variation in the composition of fungal and bacterial communities among the soils indicated by significant differences in beta diversity indexes, could be the key factor in suppression. The specific enrichment of bacterial and fungal taxa with potential SCN suppressiveness was identified in the suppressive soils. Among these, fungal genera such as *Lachnum, Diaporthe, Aspergillus* and *Fusarium* along with bacteria orders such as Burkholderiales and Actinomycetales, were previously documented to suppress SCN.

Work to be Completed

A greenhouse experiment has been set up using the soils from these four fields in order to verify our previous findings on the suppressive soil. This experiment will be harvested in January 2025, and SCN will be extracted and its population will be determined to check the suppressiveness of the soils. More thorough data analysis will be performed and a manuscript reporting the methodology and results will be prepared to disseminate the research findings.

Other relevant information

The initial SCN inoculum used for conducing the greenhouse experiment was not viable. The SCN population was increased on the susceptible check Barnes to obtain the fresh inoculum before the greenhouse experiment was conducted. This study first time characterized the SCN suppressive soil microbiome from fields in North Dakota, warranting further investigation into their potential nematicidal properties to advance SCN management strategies.

Summary

In summary, the number of free-living nematodes was significantly similar in suppressive soils compared to the non-suppressive soils, so they are unlikely to contribute to the nematode suppression. The metagenomic analyses indicate that bacterial and fungal communities are likely the major contributors to nematode suppression in these soils. Consistent with findings from other SCN-microbiome research, the bacterial phyla Proteobacteria and the fungal phylum Ascomycota were predominant within the microbial communities of suppressive soils. The soil bacterial and fungal diversity might not be likely to contribute to the nematode suppression as the alpha diversity indexes were not significantly higher in the suppressive soils compared to non-suppressive soils. However, variation in both fungal and bacterial community composition, indicated by significant differences in beta diversity indexes, could be the key factor in suppression. Some bacterial and fungal taxa significantly enriched in suppressive fields were prominent taxa potentially suppressing SCN. However, these taxa including numerous enriched taxa need further exploration for their roles in affecting SCN population in these soils.

Table 1. Initial plant-parasitic nematode densities per 100 cc of soil from four selected fields in North Dakota

Field ID[U]	Plant-parasitic nematodes[V]					NPN[W]
	SCN	Dagger	Stunt	Root-lesion	Spiral	
Field 1	696	19	0	9	37	484
Field 2	11	0	0	0	0	450
Field 3	429	0	0	0	257	816
Field 4	861	7	6.6	15	0	479

[U] Fields 1 to 4, soybean-corn rotated fields in North Dakota, were selected for this study based on prior observations. Previous growth chamber experiments revealed that two of these fields (Field 2 and Field 3) exhibited suppressiveness to Soybean Cyst Nematode (SCN) but Fields 1 and 4 did not demonstrate suppressiveness to SCN. [V, W]The average numbers of major plant-parasitic nematodes (PPN) and non-plant-parasitic nematodes (NPN) were quantified from nematode extractions across three replicates per field sample.

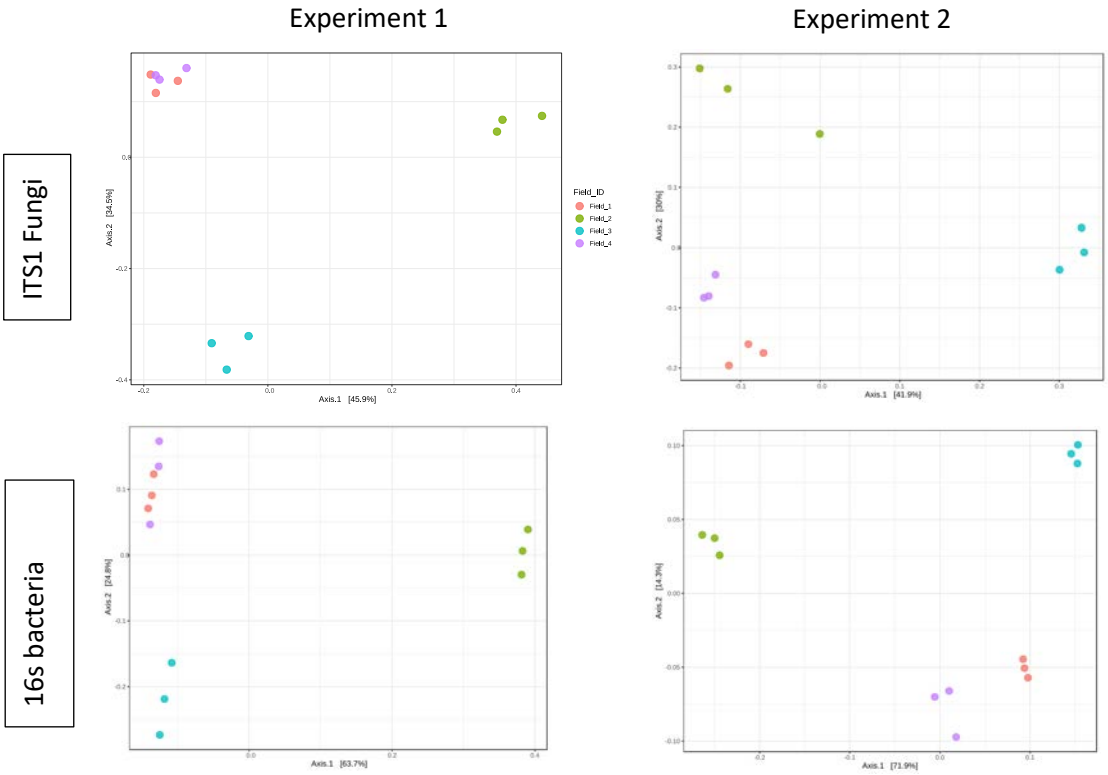

Figure 1. Principal coordinates analysis (PCoA) based on Bray-Curtis distances comparing the composition of bacterial and fungal communities across soils from four fields; two SCN-suppressive fields (Field 2 and Field 3) and two non-suppressive fields (Field 1 and Field 4)

in ND in Experiment 1 and Experiment 2. Red-, green-, blue-, and violet-colored circles indicate the composition of microbial communities of Field 1, Field 2, Field 3, and Field 4, respectively. Field 2 and Field 3 showed distinct fungal and bacterial communities compared to Field 1 and Field 4 in both experiments.

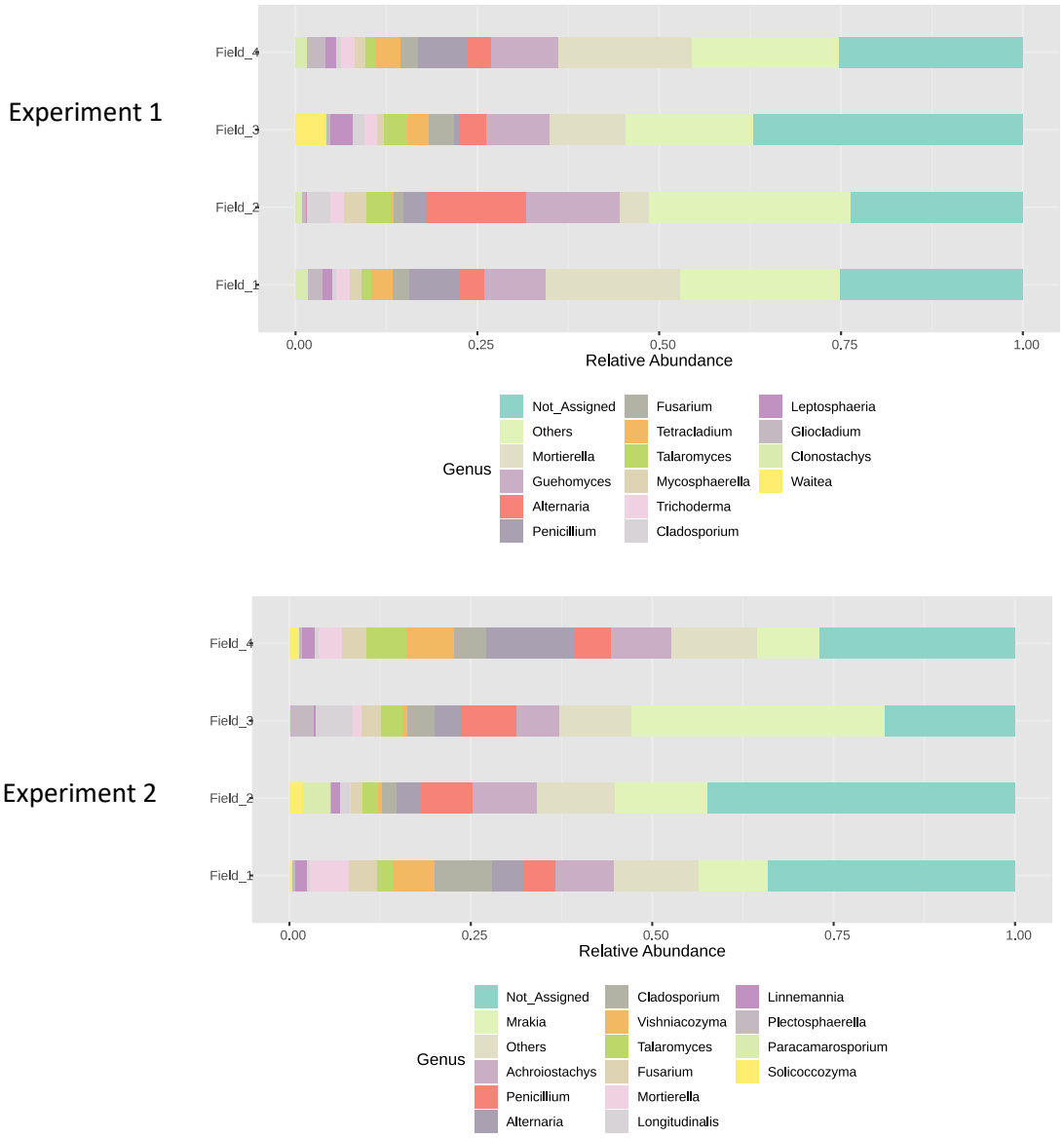

Figure 2. Relative abundance of the dominant fungal genera (top 15 in %) in SCN-suppressive field soils (Field 2 and Field 3) and non-suppressive field soils (Field 1 and Field 4) in ND in two experiments.

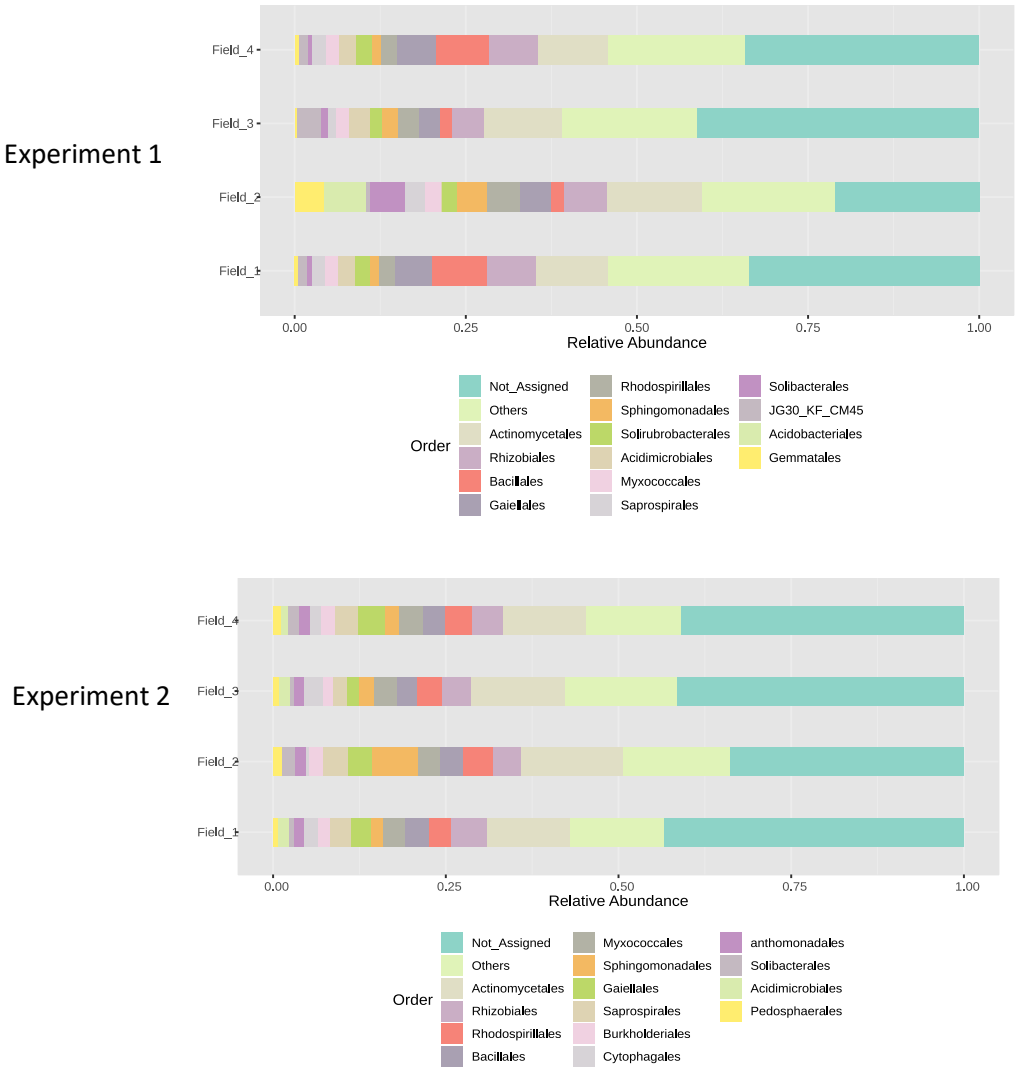

Figure 3. Relative abundance of the dominant bacterial orders (top 15 in %) in SCN-suppressive field soils (Field 2 and Field 3) and non-suppressive field soils (Field 1 and Field 4) in ND in two experiments.