Progress Report – April 30, 2024

Objective 1: Establish baseline knowledge of microbial communities associated with SCN cysts in NY state

In conjunction with the Fall 2023 SCN survey, we assisted in collecting samples and processed soil samples from 33 fields across 10 counties in NY (Table 1). The results detected SCN cysts in only 7 fields in three counties (Tioga, Montgomery, and Herkimer), with only three fields, two in Tioga county and one in Montgomery county, showing substantial egg counts above 1000. Because only a handful of cysts were isolated, these were used to reproduce the nematode on plants in order to perform HG typing for the SCN survey to identify what strains of the nematode are present in NY state. These results suggest SCN is still in the early stages of spread in NY, but has spread and reached more northern counties (Montgomery, Herkimer) in the middle of the state. This does provide an opportunity to establish a baseline of native microbial communities present in soils in grower fields in NY state before high levels of SCN develop. From each field, a subset of approximately 250g soil was sent for soil nutrient/health analysis (pH, nutrients, organic matter) at Cornell and a subsample of 10g was frozen in tubes or in glycerol solution (for culturing) for analysis of soil microbiome. We isolated DNA and sequenced barcode loci regions for bacteria (16S) and fungi (ITS) that are able to identify microbes to the genus level.

Data from microbiome sequencing was received back in April and preliminary analysis showed some interesting trends for further analysis. A principal components analysis (PCoA - unweighted unifrac) to look at the overall relationships between bacterial and fungal communities in relation to both county location and detection of SCN cysts (presence/absence) showed an overall trend of differentiation between microbial communties based on both their location and more whether the fields were infested with SCN or not. For fungi, both factors were highly significant, with county location p-value < .001 and SCN presence p-value <.001) (Fig. 1). For bacterial communities, a significant difference by county location (p-value <.05) was observed and while the difference by detection of SCN cysts was not significant (p-value = .054), the same overall trend of a difference between communities was observed between samples from fields infested with SCN versus those not infested by SCN (Fig. 2). While further analyses to identify specific taxa that may differ and a larger sample size may be needed in future research to confirm this pattern, overall these results suggest that infestation with SCN may be altering the microbial composition of soils in NY soybean fields. Soil testing results have also been received and will be analyzed in the context of microbial communities.

Analysis of specific taxa in soils in NY identified 15 bacterial taxa that were detected at greater than 2% relative abundance in any field (Fig. 3). Among these, a core microbiome or group of taxa that showed both high abundance and presence in a majority of fields across all counties included 7 taxa (*Bradyrhizobium, Hyphomicrobium*, Planococcaceae (Family), *Candidatus udaeobacter, Pseudolabrys, Pseudarthrobacter*, and Xanthobacteraceae (Family), while other taxa were more sporadically distributed across counties (Fig. 1). Some of these (*Bradyrhizobium*, Xanthobacteraceae (Family)) are known to be common symbionts of soybean and other legumes that can assist in nitrogen fixation, while others (*Candidatus udaeobacter*) are common soil fungi that we have also detected in soybean fields in the Midwest. For fungi, 29 taxa were identified

with greater than 5% relative abundance in any field. A core microbiome of 8 taxa (*Podila, Mortierella, Solicoccozyma, Gibellulopsis, Tetracladium, Cladosporium, Metarhizium*, Didymellaceae (Family)) were identified. Other fungal taxa showed high abundance in only a few fields and showed more diverse and uneven distributions than bacteria. Among these fungi, several genera (*Mortierella*, *Metarhizium*, and Didymellaceae (Family)) are known to contain species that can infect SCN eggs. Work is ongoing to isolate and identify fungal cultures for testing in bioassays in Objective 2.

Objective 2: Screen fungi for antagonism of the soybean cyst nematode

1.2 Screen fungi for production of nematicidal and hatch inhibitory metabolites

We have begun screening filtrates from fungi isolated from soil using J2 toxicity assays. We have screened raw filtrates from approximately 10 isolates to date. One isolate whose raw filtrate showed high toxicity to J2 worms in bioassays was grown up in rice media for one month. Raw extraction using a 1) water (RW), 2) a relatively polar solvent butanol (RB), and two nonpolar solvents, ethyl acetate (RE) and hexane (RH), as well as a distilled water control, were then tested in toxicity bioassays with J2 worms. Results showed that the ethyl acetate fraction (RE) had significantly higher mortality than the water extract (Fig. 5). Using this approach of bioactivity-guided fractionation, we will continue to fraction and test bioactivity to narrow down possible chemical entities involved in activity of the RE extract. Screening of other fungal filtrates is ongoing as we isolate additional strains.

Table 1. Soil sampling for SCN survey (Fall 2023) showing 33 field sampled in 10 counties across NY.

Sample number	**County**	**# Cysts**	**Viable Eggs Counts**
42H	Chautauqua		
42L	Chautauqua		
43	Chautauqua		
44	Chautauqua		
7	Chautauqua		
15	Corltand		
16	Corltand		
17	Corltand		
18	Corltand		
19	Corltand		
10	Cortland		
11	Cortland		
45	Erie		
46	Erie		
38	Herkimer		
D	Herkimer	2	44
33	Madison		
28	Montgomery	19	2080
30	Montgomery		
40	Montgomery		
41	Montgomery		
B	Montgomery	5	210
A	Montgomery		
35	Otsego		
37	Otsego		
C	Saratoga		
47	Tioga	20	1400
48	Tioga	4	91
49	Tioga	5	51
50	Tioga	87	2100
12	Tompkins		
14	Tompkins		
20	Tompkins		

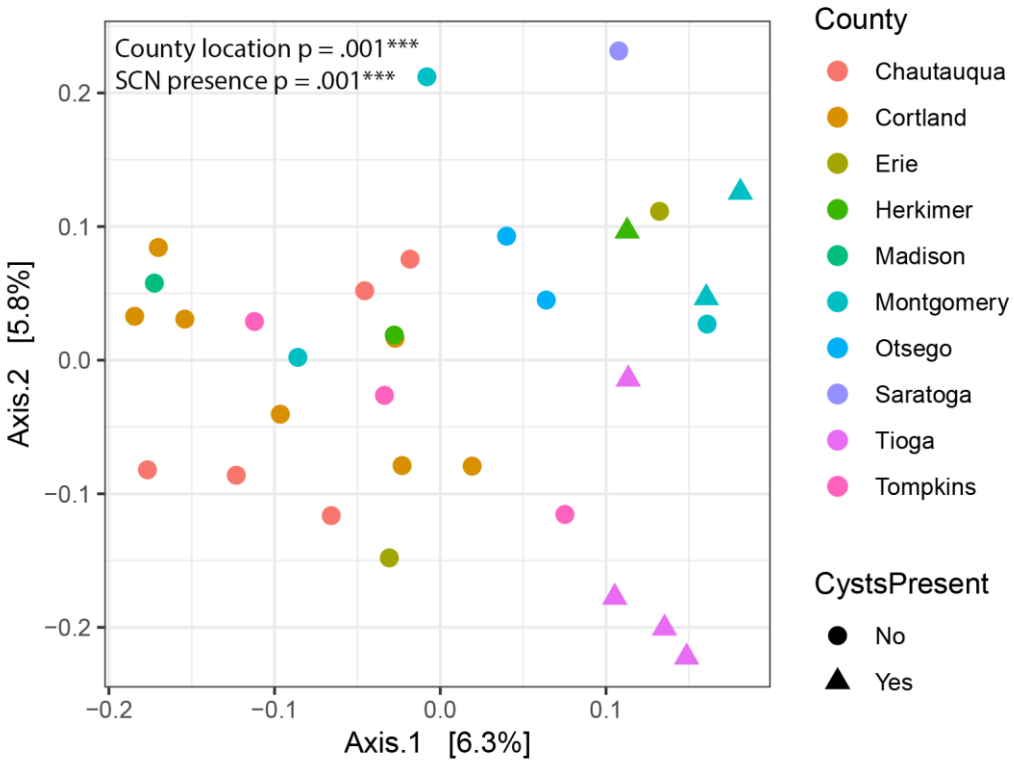

Fig. 1. Principal component analysis (PCoA – unweighted unifrac) of fungal communities in soil samples from fields in different counties (color coded) where SCN was either not detected (No = circles) or detected (Yes = triangles). Significant differences between fungal communities were observed both by county ($p < .001$) and by presence/absence of SCN ($p < .001$). The differences between communities based on SCN presence/absence can be visualized as the separation along axis 1 between samples where SCN in present (triangles), which group together on the right side of the figure, while those where SCN is absent (circles) group more on the left side of the figure.

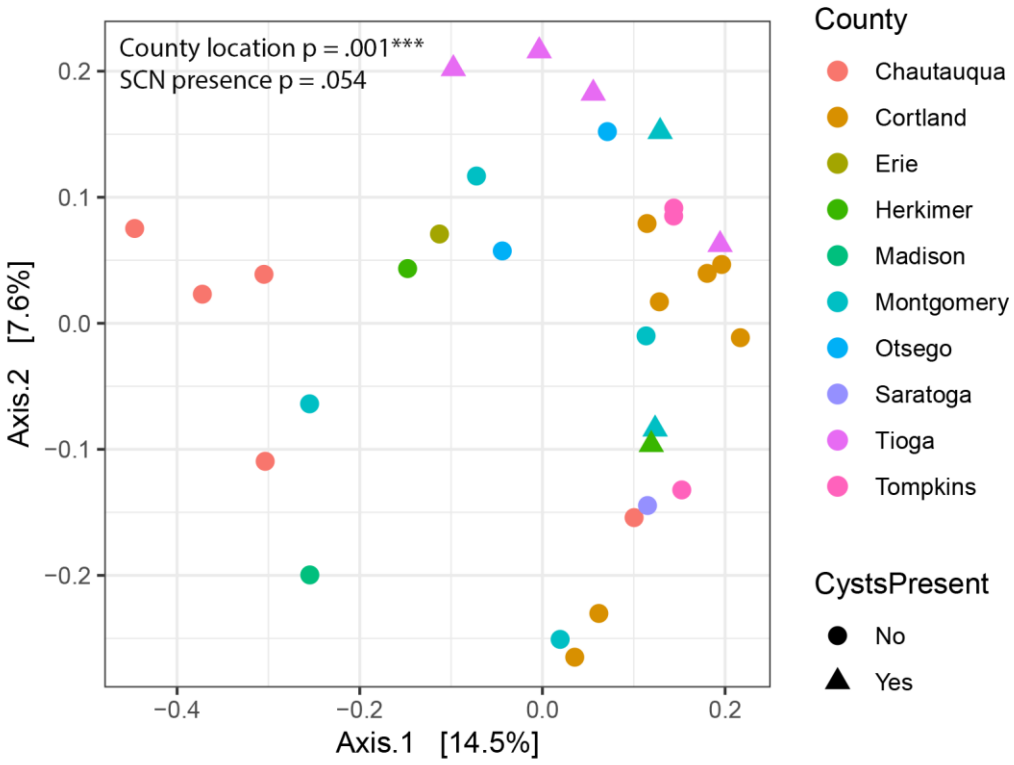

Fig. 2. Principal component analysis (PCoA – unweighted unifrac) of bacterial communities from soil samples from fields from different counties (color coded) where SCN was either no detected (No = circles) or detected (Yes = triangles). A significant difference in bacterial communities was observed by county location (p , .001) and while not statistically significant (p = .054), an overall trend was observed showing a similar differences between presence or absence of SCN which can be visualized as samples with SCN (triangles) grouping together on the right or upper right quadrant of the figure.

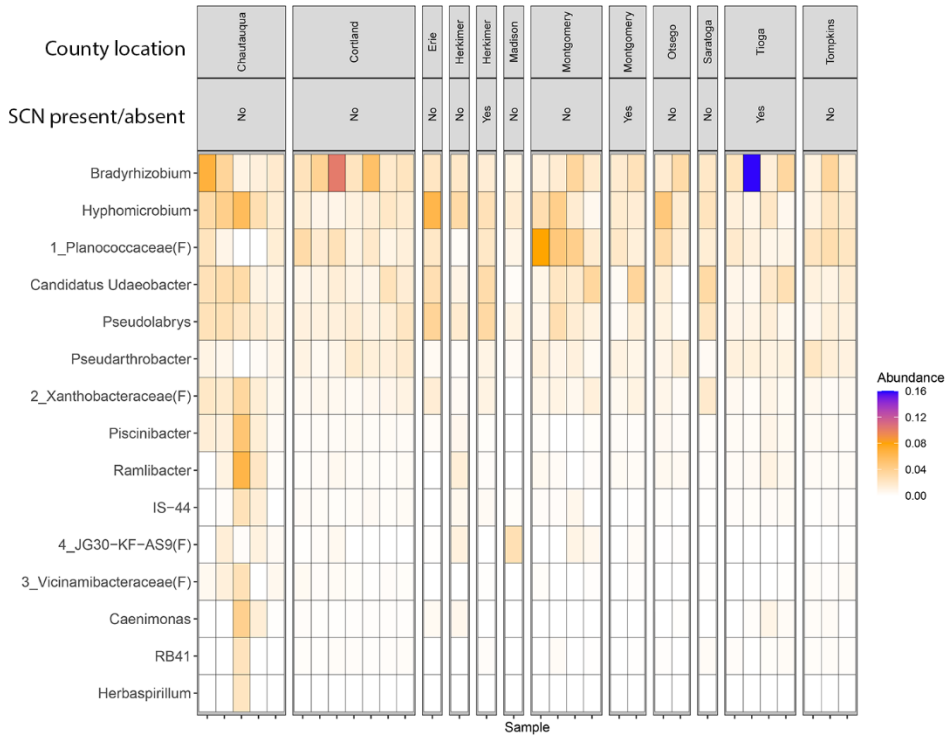

Fig. 3. Distribution of bacterial taxa with >2% relative abundance by county location and SCN presence/absence.

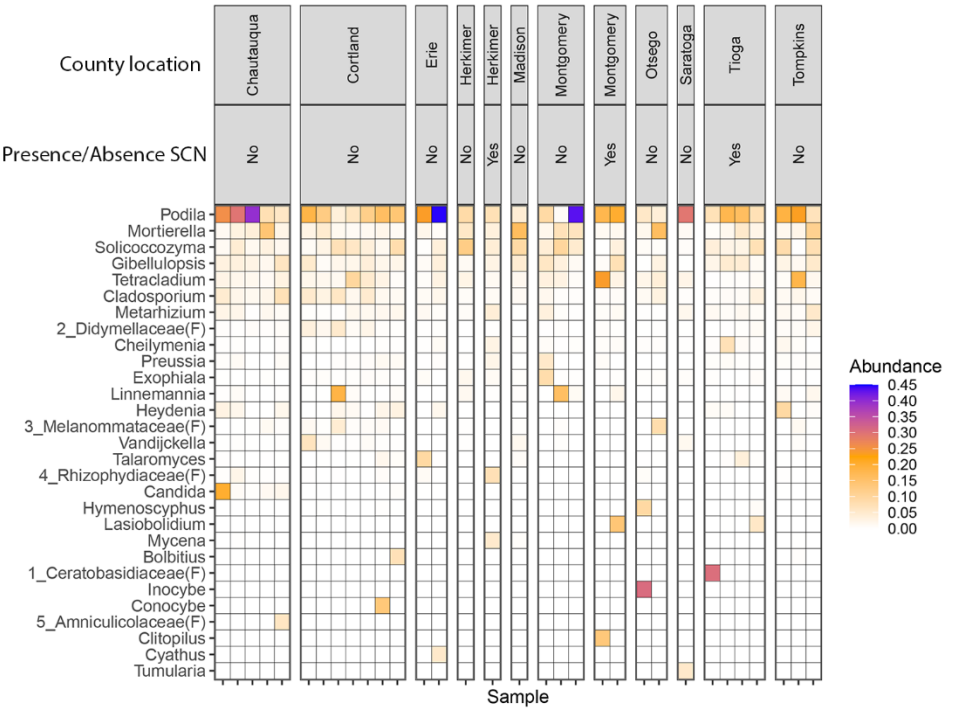

Fig. 4. Distribution of bacterial taxa with >2% relative abundance by county location and SCN presence/absence.

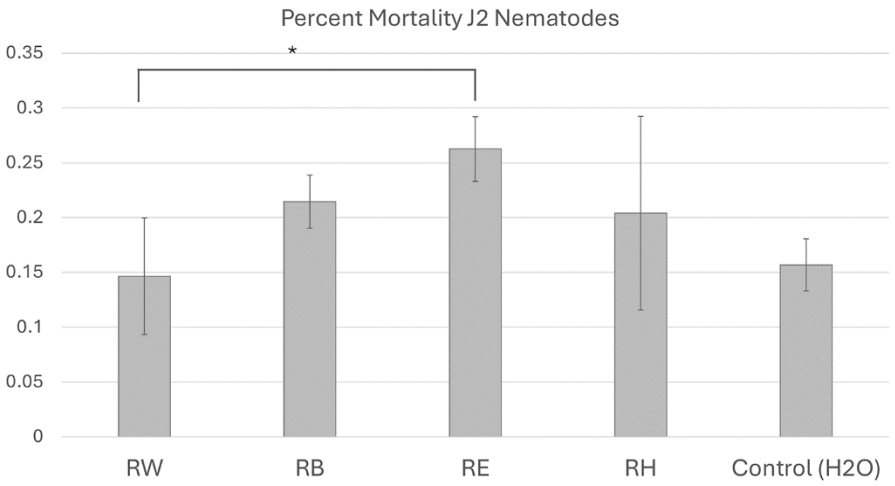

Fig. 5. Percent mortality (out of 100 J2 worms) of different chemical extracts (RW – water extract, RB, butanol extract, RE, ethyl-acetate extract, RH, hexane extract) as well as a distilled water control. The ethyl-acetate extract showed significantly higher percent mortality ($p = 0.003$) than the water extract.