NEXT-GENERATION SEQUENCING AND QUANTITATIVE PCR REVEAL PATTERNS OF CO-OCCURRENCE IN THE SOILS OF GREAT SMOKY MOUNTAINS NATIONAL PARK

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Science in Biology.

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ABSTRACT

NEXT-GENERATION SEQUENCING REVEALS PATTERNS OF CO-OCCURRENCE IN THE SOILS OF GREAT SMOKY MOUNTAINS NATIONAL PARK

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Nematodes, fungi and bacteria are highly important in the maintenance and ecology of soil communities. Bacteria and fungi are responsible for the breakdown and subsequent transformation of recalcitrant organic matter, such as cellulose and lignin, into biomass that is more readily accessible to other groups of organisms, returning this carbon to circulation. Nematodes are also responsible for a large proportion of nutrient mineralization in soils. Nematodes, while not efficient decomposers themselves, exert top-down influence on bacterial and fungal populations through predation and grazing of biofilms. It has been experimentally demonstrated that nematodes display a preference for certain taxa as food items. Many of these taxa contain members capable of essential biochemical and physical processes, such as nitrogen fixation, dissimilatory metal metabolism or enmeshment of soil particles, implying that nematode food preference may affect the chemical and trophic state of the soils they inhabit, as well as the structural properties of the soil. Bacteria and fungi parasitize or directly predate nematodes in the environment surrounding them in turn. Fungi and bacteria secrete a wide variety of compounds into the surrounding environment to inhibit each other's growth and gain the upper hand in competition. These interactions perpetuate a nuanced relationship between these three groups. Relationships between soil

nematodes, bacteria and fungi were investigated via modern, culture-free methodologies, including next-generation sequencing and qPCR, with data analyses performed using QIIME 2, Cytoscape 3 and CoNet. Inferred co-occurrence networks were used to establish possible ecological roles and relationships in the soils of Great Smoky Mountains National Park. A relationship between a family of filamentous, polymer degrading bacteria, Ktedonobacteraceae, and the fungal genus *Mortierella,* was uncovered. The nematode genus *Filenchus* was found to be negatively associated with two groups of bacteria involved in nitrogen metabolism. This information may guide future conservation and management efforts.

INTRODUCTION

Functions of nematodes in the environment

Nematodes are among the most abundant and significant groups of soil organisms. One estimate suggests that there may be as many as 4.4×10^{20} nematodes living within forest soils globally, with a total biomass of 300 million tons (van den Hoogen et al., 2019). With such a commanding presence in soil, it is reasonable to expect nematodes to have a profound influence on the ecology, health, and maintenance of terrestrial ecosystems and their soil. It has been estimated that nematodes may be responsible for up to 40% of nutrient mineralization in soils they inhabit. Nematodes may be predatory or parasitic, feeding on a domain-spanning variety of organisms, including bacteria, archaea, fungi, plants, and other nematodes (Yeates et al., 1993). Due to the wide range of food sources nematodes as a group exploit, nematodes may be viewed as linking many trophic groups together, affecting nutrient cycling in the habitat. Among all nematode feeding types, bacterivores, nematodes that consume bacteria, are the most abundant in soil, representing over half of all free-living nematodes. The abundance of nematodes, and the composition of their population, may be affected by anthropogenic factors; these may be chemical, or physical disturbances, and lead to downstream effects on soil assemblages and overall soil health. An example of this is the alteration of nematode populations due to heavy metal pollution attributable to the metal refinement process (Salamun et al., 2011).

Functions of bacteria in the environment

The extreme metabolic versatility of bacteria allows them to play various roles in soil ecology and health. Like nematodes, bacteria may be free-living, parasitic, or engaged in symbiotic interactions with other organisms (Ott et al., 2021; Dillman et al. 2021). Unlike the nematodes that graze upon them, bacteria are active and capable decomposers within soil (Raczka et al., 2021). This includes the capacity to degrade recalcitrant organic matter, such as cellulose, lignin, and keratin. These organic carbon sources are unavailable to most other soil inhabitants. Conversion of these recalcitrant organics into bacterial biomass allows carbon, that might otherwise be unreachable, to re-enter circulation throughout the soil ecosystem. Aside from acting as effective decomposers, bacteria are largely responsible for nitrogen fixation; the process in which molecular nitrogen is converted into an assimilable form such as ammonia. This process is essential for life and so bacteria are indispensable in this respect (Sepp et al., 2023). Bacteria are known to play a prominent role in all other biogeochemical cycling processes, these include iron cycling, sulfur cycling, and phosphorous cycling. Bacteria share in a complex relationship with nematodes, being grazed upon by them and parasitizing them in return (Migunova and Sasanelli, 2021).

Functions of fungi in the environment

Fungi, as decomposers in soil, are second only to bacteria in terms of efficacy and versatility. Like bacteria, fungi are capable of secreting a wide variety of extracellular enzymes and participate in the degradation of recalcitrant organic matter, rendering it available to the rest of the soil community in the form of fungal biomass. Fungi, however, play an additional role in determining and maintaining soil structure and

function. Aside from a loss of structure caused by the decomposition of organic soil particles, fungi may lend additional structure to soil. Compounds secreted into the soil environment surrounding a fungus may cause soil particles to adhere to each other. Soil particles may also become enmeshed in an expanding mycelial tapestry. Both mechanisms increase the resistance of soil to mechanical disturbances, minimizing deleterious processes such as erosion. The spatial arrangement of soil particles governs the properties of pore spaces in the soil. Pore spaces in turn govern the availability of various microhabitat types to the microbial inhabitants of soil (Ritz and Young, 2004). Fungi may also assert themselves chemically in soil. Fungi are well-known for their production of antimicrobial compounds, used to combat other microbes in their environment, including bacteria. These antimicrobials may cause downstream alterations in bacterial diversity and community composition. The presence of antibiotic resistance in the soil bacterial metagenome is evidence of this effect (Bahram et al., 2018). Similarly to bacteria, fungi engage in a complex interrelationship with nematodes. A large variety of nematophagous fungi are known and predation on nematodes is widespread, each typically producing a different type of trap. *Arthrobotrys oligospora* is a widely occurring nematophagous fungus, used as a model in the research of fungus-nematode interactions. *A. oligospora* is capable of saprotrophic growth when nitrogen is not a limiting nutrient, switching to a nematophagy when bioavailable nitrogen, particularly ammonia, becomes scarce. The production of traps, three-dimensional adhesive nets, formed from hyphae is the result of this transition (Niu and Zhang, 2011). *A. oligospora* produces traps in response to ascarosides, a group of nematode pheromones. *A. oligospora*, in turn, produces chemotactic lures to draw in potential

prey. These may mimic chemical food cues or, as demonstrated in *Caenorhabditis elegans*, pheromones involved in mating. Disruption in chemical signaling as it pertains to mating can disrupt reproductive behavior in *Caenorhabditis elegans* and results in sex-specific predation (Hsueh et al., 2017).

Interkingdom relationships

The complex predator-prey relationship bacteria and nematodes share has far-reaching implications for nutrient cycling and mineralization, the process of converting chemical resources from organic to inorganic form. The form a nutrient occupies may directly impact the availability of the nutrient to organisms in the environment. Mineralized and un-mineralized nutrients also differ in physical properties, such as solubility, that in part govern the mobility of the nutrient within the environment. These two factors have observable effects on soil health and primary productivity. It has been experimentally demonstrated that nematode grazing of soil microbes increases aboveground biomass, in a study using wheat as a model organism (Gebremikael et al., 2016). In this same study, nematode grazing was demonstrated to enrich certain bacterial taxa relative to others. The enriched groups correspond with taxa capable of symbiotic nitrogen fixation and dissimilatory metal metabolism, which is the ability to generate metabolic energy by altering the oxidation state of metals, thereby altering solubility and other mobility related factors. Iron cycling functions as an example of this process. Iron-reducing microbes, microbes that use often insoluble Fe(III) as a terminal electron acceptor, reduce iron ions in the environment to Fe(II), increasing their solubility in the process (Richter, Schicklberger and Gescher, 2012). Since nematodes exert top-down pressure on populations of bacteria, it is reasonable to suspect that they

may alter biogeochemical cycling in their surroundings and consequently soil health and function.

Bacterivorous nematodes possess the ability to differentiate between bacterial food items and display a preference for certain bacterial food items over others. Small, Gram-negative cells appear to be the preferred food source for *C. elegans* and *Cephalobus brevicauda*, two ubiquitous nematodes found in soil. This preference likely arises from anatomical constraints (Salinas et al., 2007). Some bacteria that might otherwise be choice prey items can be lethal to a hapless grazing nematode. The Gram-negative proteobacteria *Burkholderia spp*., including the opportunistic pathogen *Burkholderia cepacia*, may kill nematodes that consume them. Killing is performed via secreted toxins. Nematodes killed by consumed *Burkholderia* cells are then degraded by their would-be prey. It is believed that bacterivorous nematodes are enticed to consume the cells by a chemotactic lure (Cooper et al., 2009). Fungivorous nematodes are also common in soils. Rather than consuming their prey whole, as bacterivorous nematodes do, fungivorous nematodes tend to pierce fungal hyphae with a hollow stylet and draw off the cytoplasmic contents. Some generalist nematodes may consume whole fungal spores or yeast cells (Yeates et al.,1993). Nematode fungivory affects fungal diversity and has additional downstream effects on bacterial communities. In addition, fungivory increases carbon and nitrogen turnover (Kane et al., 2023). The differential pressure grazing nematodes place on bacterial and fungal taxa seems to suggest that key taxa, bacterial, fungal, and nematode, may be reproducibly and detectably associated with each-other.

Figure 1: The location of all 19 sampling sites within GSMNP. The park boundary is indicated in light green.

Great Smoky Mountains National Park (GSMNP) is one of the largest contiguous pieces of federally protected land in the Eastern United States. Great Smoky Mountains National Park is also designated as an International Biosphere Reserve. The Southern Appalachians are renowned for their biodiversity, GSMNP in particular is known for very high animal and plant diversity, including members of threatened and rare taxa, such as Caudata and Myriapoda. An All-Taxa Biodiversity Inventory (ATBI) is an effort to catalog the total diversity and abundance of life in some defined zone. The GSMNP ATBI was one of the largest ATBI efforts and remains active into the present day. This project was originally conceived in 1997 to inform management and development decisions within

GSMNP, since intensification in land-use may have downstream consequences for above and belowground diversity (Thompson et al., 2015). It was soon realized that the initiative as planned was too large to be managed by one group. For this reason, a non-profit, Discover Life in America (DLIA), was created to coordinate efforts by all involved parties (White and Langdon, 2006).

Culture-free techniques in the field of soil ecology

The lion's share of microbial diversity is unculturable, this is especially true of microorganisms residing in soil (O'Connell et al., 2007). Culture-dependent methods employed in the interrogation of soil ecology, while capable of delivering high fidelity information, run the risk of magnifying the contribution and abundance of rare, ecologically unimportant taxa (O'Connell et al., 2007). Because they cannot be cultivated in a lab environment, a large part of the diversity present in these ecosystems remained undetected until the advent of culture-free techniques. Sequencing-based assays are now used to assess microbial diversity and ecological function. The current gold standard in massively parallel sequencing, Illumina sequencing, is a sequencing by synthesis technology that allows amplification and sequencing of a multitude of differing DNA fragments simultaneously (Slatko, Gardener and Ausubel, 2018). The segment of DNA to be amplified and sequenced is selected based on the choice of a primer set. The amplified segment of DNA is referred to as an amplicon. In the case of microbial ecology, when the amplicon sequencing strategy is used, the 16S rRNA and Internal transcribed spacer (ITS) genes are used to identify Bacteria/Archaea and Fungi, respectively. Raw sequencing data is then passed to a bioinformatics pipeline, where it is curated by quality control algorithms and subjected to various statistical tests.

Software used in this step of the workflow may include QIIME 2, an open-source bioinformatics software suite specifically designed for the analysis of next-generation sequencing data pertaining to microbiota (Boylen et al., 2019).

One application of count and identity data gathered through next-generation sequencing is co-occurrence network inference. Co-occurrence networks are generated by performing numerical comparisons, often pairwise, between detected operational taxonomic units (OTUs). Correlations between OTUs are then calculated (Matchado et al., 2021). A number of different correlation methods may be used including Pearson, Spearman and Kindall correlation. Permutation and bootstrapping may additionally be performed to calculate *p*-values for inferred relationships (Faust and Raes, 2016).

Project goals

The goal of this study was to characterize assemblages of soil microorganisms from various environments across GSMNP. These data were then used to infer qualitative relationships between what were discovered to be key taxa. This information may be used to develop hypotheses that direct future research efforts and enable resource managers such as in GSMNP to better understand the ecosystems they oversee.

METHODS

Sampling

During summer of 2023, soil samples were collected aseptically from 12” depth at 19 sites across GSMNP (*Figure 1*). Soil cores were kept on ice in the field, then transferred to a freezer and stored at -20 °C in preparation for shipment to Microbial Insights, Inc., (Knoxville, TN) for DNA extraction, amplification, and next-generation sequencing work. DNA Extraction, amplification, and sequencing were performed using a proprietary protocol. The soil core from the Occonaluftee site was frozen with dry-ice in the field, rather than being refrigerated using ice. Sequencing was performed on an Illumina MiSeq system (Illumina Inc., San Diego, CA).

Analysis of next-generation sequencing and qPCR data

Demultiplexed paired-end reads of both fungal ITS and bacterial 16S rRNA gene sequences, generated via next-generation Illumina sequencing, were imported into QIIME2 (Bolyen et al., 2019). DADA2 was employed to trim reads for quality, join pairs, denoise reads and remove chimeric sequences (Callahan et al., 2016). Default settings were used for all data aside from ITS data. ITS sequences were truncated at the first position with a quality score of 10 or lower. Amplicon sequence variants (ASVs) were then subjected to de-novo clustering into OTUs, at the 97% identity level, performed by QIIME2 VSEARCH (Torbjorn et al., 2016). Taxonomic assignment of OTUs produced as output was performed using the scikit-learn based Naives Bayes classifier included in QIIME2 (Pedregosa et al., 2011). Classifiers were trained on existing sequence databases, in particular UNITE version 8.0 for fungal ITS OTUs and Greengenes 2

2022.10 for bacterial 16S rRNA OTUs (Abarenkov et al., 2023; McDonald et al., 2023; Bokulich et al., 2018). A 70% confidence threshold was implemented to limit the depth of taxonomic assignment. Feature-tables were converted to relative abundance. Bar plots displaying relative abundance of fungal and bacterial taxa were constructed using Libre Office Calc 24.2.0.3. Shannon and Pielou diversity indices for bacterial and fungal assemblages were calculated using methods included in QIIME2's Diversity plugin and plotted using Libre Office Calc 24.2.0.3 (McKinney, 2010; Shannon, 1948; Pielou, 1966).

Soil nematode identity and abundance were interrogated using qPCR amplification of 18S and 28S rRNA targets, performed by Microbial Insights, Inc. This analysis was also proprietary.

Co-occurrence network inference

Feature-tables containing count data of bacterial and fungal OTUs were exported from QIIME2. These feature tables were then imported into CoNet, an ecological network inference program (Faust and Raes, 2016). OTUs in feature tables were then filtered based on occurrence and total count across samples. OTUs with values greater than 4 total occurrences and a feature count of 1000 were retained in the construction of the bacterial-fungal bipartite network. OTUs with values greater than 5 total occurrences and a feature count of 1000 were retained in the construction of the bacterial-nematode bipartite network. OTUs with values greater than 3 total occurrences and a feature count of 500 were retained in the construction of the fungal-nematode bipartite network. These settings were chosen to remove OTUs of very low abundance, improving the quality of inferred networks by reducing the likelihood of erroneously inferring strong relationships due to double absence. Creation of bipartite networks

allows relative abundances to be calculated independently. Several measures of correlation and distance were used during network inference, these being Pearson correlation, Spearman correlation, Bray-Curtis dissimilarity and Kullback-Leibler dissimilarity. Only edges generated by two or more of the four methods were retained. Since statistical distances produce unsigned values, interaction types could not be assigned to edges supported only by distances. Statistical thresholds were set to retain between 40 and 60 edges, increasing readability of generated networks, using the automatic thresholding capability of CoNet. *p*-values were also calculated for generated edges. Only edges and their associated nodes with a p-value <0.05 were plotted. Networks were organized using the “organic layout” algorithm included in Cytoscape 3. Inferred networks were then modularized to explore emergent patterns using ModuLand 2.0 (Kovacs et al., 2010).

RESULTS

Denoising statistics

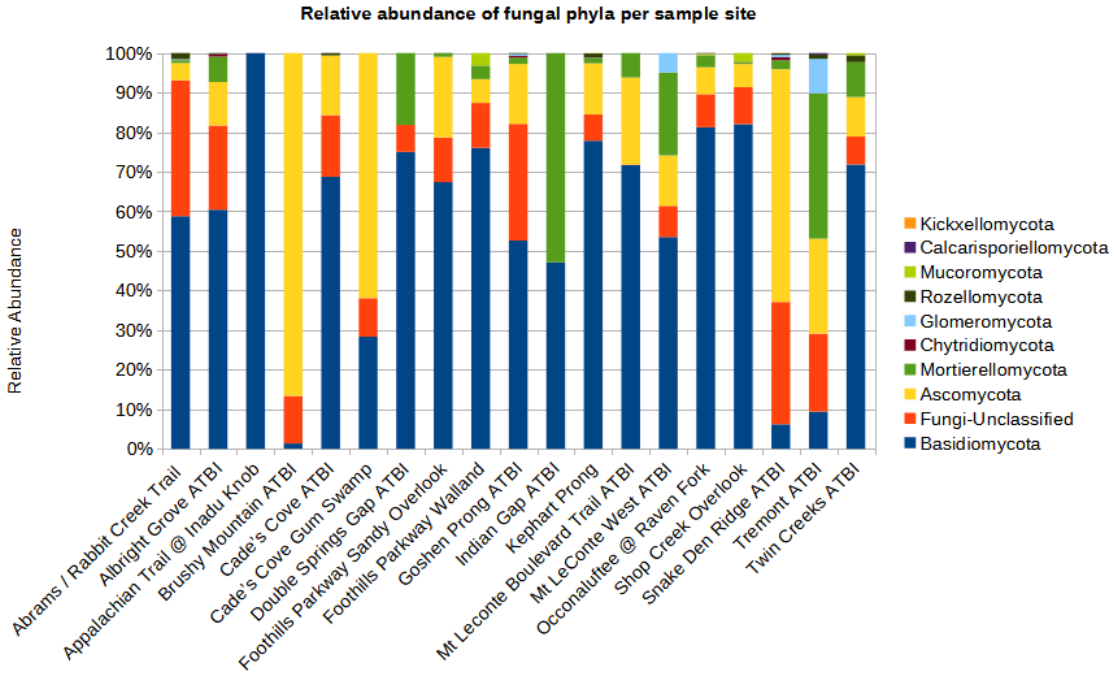

Figure 2: Relative abundance of fungal phyla across all sample sites in GSMNP, as determined through ITS region sequencing.

The total number of fungal features varied widely across samples post quality control, with 32.4% of the total feature count of 286,947 being attributed to the Albright Grove ATBI sample. A total of 1,945,121 bacterial features were observed across all samples and were distributed somewhat evenly, with the maximum observed feature

count in any single sample being 170,854. Features are observations, in this context, equivalent to inferred sequences.

Abundance and diversity indices

Proteobacteria, Acidobacteria, Chloroflexota and Planctomycetota were the most abundant bacterial phyla observed across samples, as seen in *Figure 3*. Only the ten most abundant phyla are shown. The abundances of all other phyla were summed, and

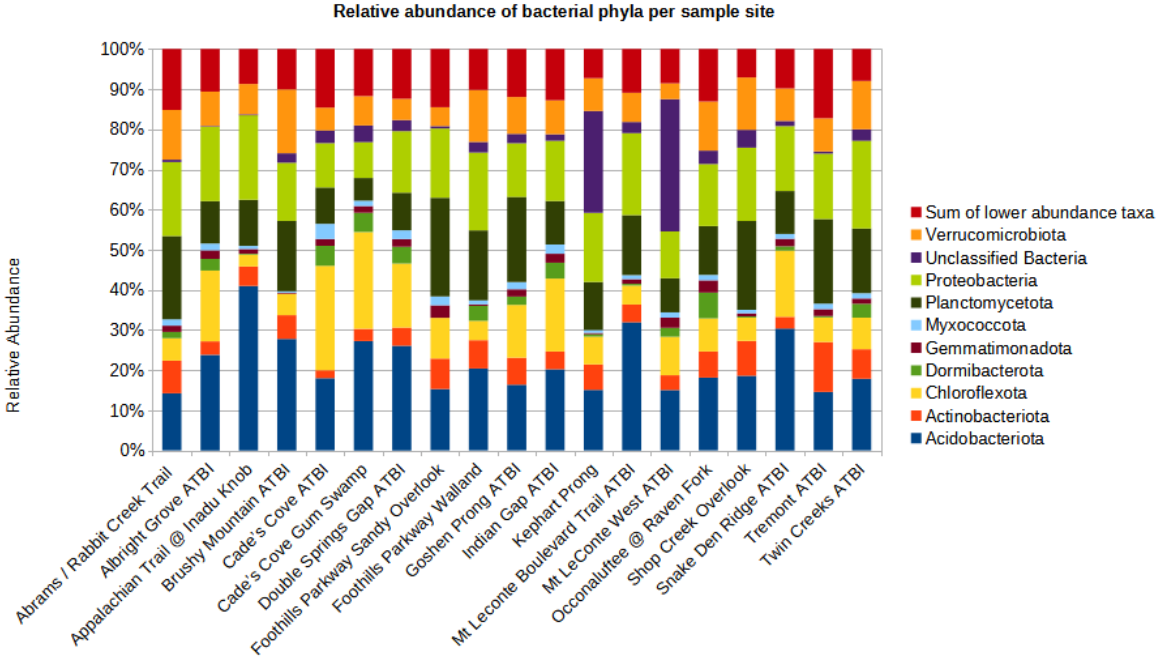

Figure 3: Relative abundance of bacterial phyla across all sample sites in GSMNP, as determined by sequencing of bacterial 16S rDNA. Only the 10 most abundant phyla are displayed separately.

their total contribution of bacterial abundance displayed as “Sum of lower abundance phyla.” Basidiomycota and Ascomycota were by far the most abundant fungal phyla observed across all samples, however, a large number of fungal OTUs remained unidentified by the classifier. This was more prominent in some samples than others, with over 34.4% of observed features being unidentifiable in the Abrams/Rabbit Creek

Trail sample. In addition, in samples from Cades Cove Gum Swamp, Kephart Prong and the Tremont ATBI site, over half of all observed features were only identified to phylum level. This artifact is observable in *Figure 2*. Several dominant nematode genera were detected (*Figure 4*). Across all but four sample sites, *Labronema* was the most abundant genus detected. *Microdorylaimus* was dominant to the point of near total

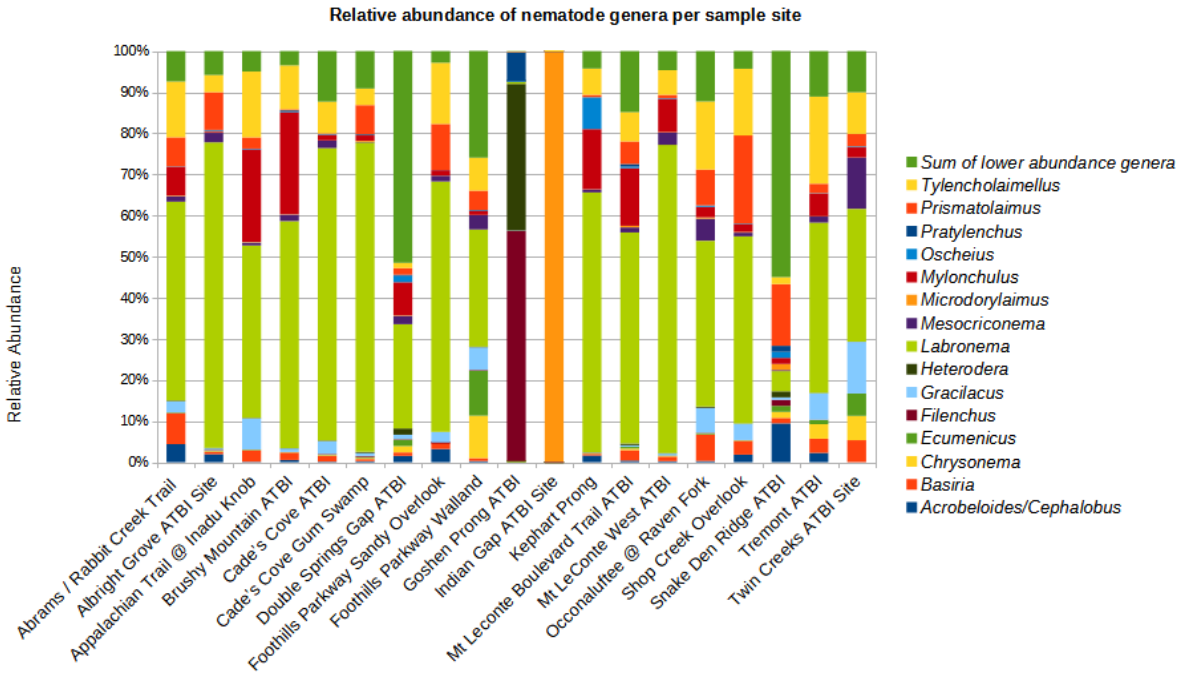

Figure 4: Relative abundance of nematode genera across all sample sites in GSMNP, as determined by qPCR amplification of the 18S and 28S rRNA genes.

exclusion of competing taxa in the sample from the Indian Gap ATBI site. *Filenchus* and *Heterodera* were dominant in the Goshen Prong ATBI sample. Fungal Shannon diversity was far more variable across samples, varying by a factor of 2.8 across all samples (*Figure 5)*. At all sample sites, fungal diversity was lower than bacterial diversity. The lowest fungal shannon index observed was 1.77, while 4.97 was the highest. The lowest bacterial shannon index observed was 7.36, while 9.70 was the highest. Bacterial Shannon diversity was more consistent across samples. Pielou

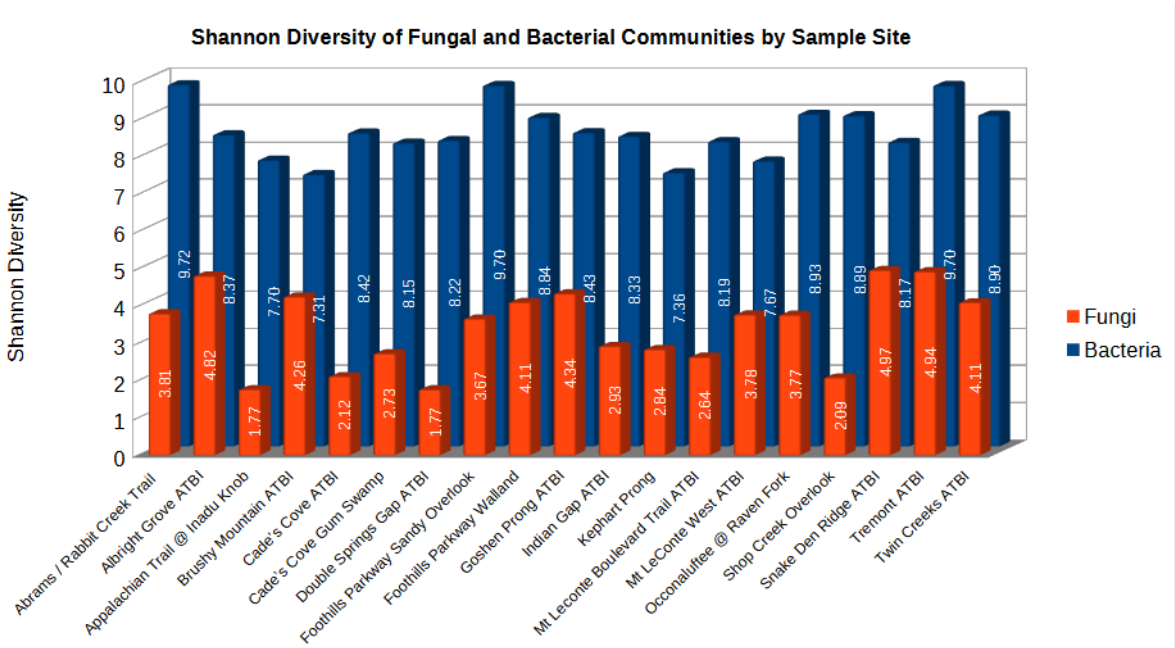

Figure 5: Shannon diversity of fungal and bacterial communities by sample site in GSMNP.

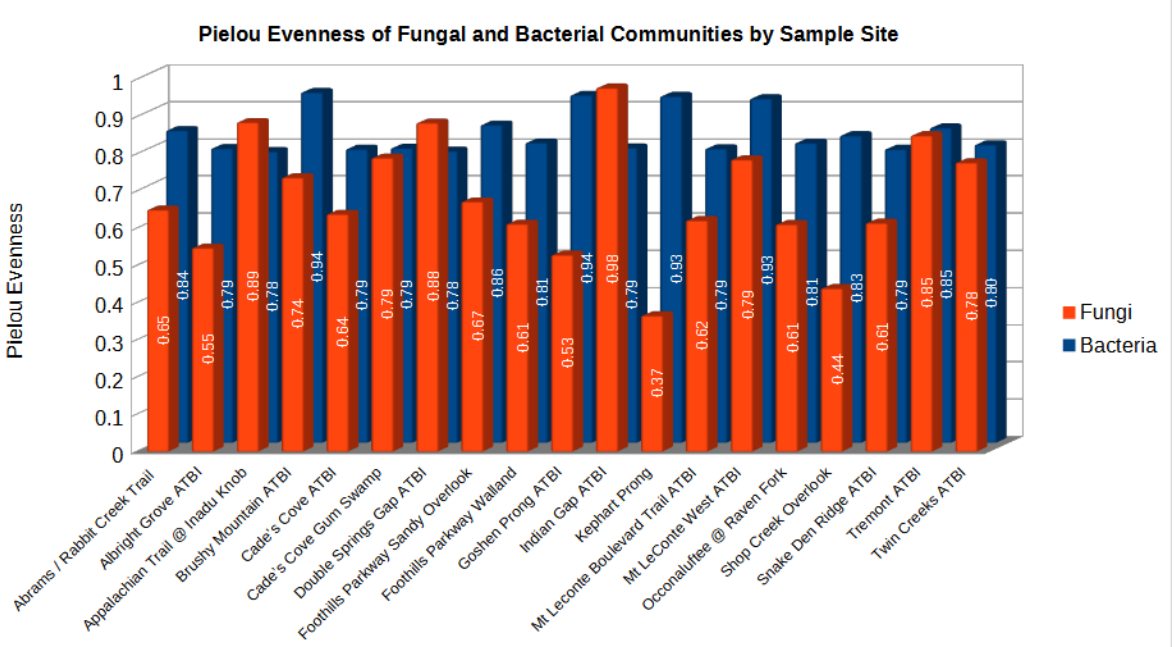

Figure 6: Pielou diversity of fungal and bacterial communities by sample site in GSMNP.

evenness of fungal assemblages was higher, relative to bacterial evenness across most samples. Fungal evenness was also higher than bacterial evenness cases, contrary to what was observed in the Shannon diversity indices. Pielou evenness was more

variable than Shannon diversity. Evenness ranged between 0.37 to 0.98 and 0.78 to 0.94 for fungal and bacterial assemblages, respectively (*Figure 6*). Pielou evenness of nematode assemblages varied between 0.00 and 0.92, this can be seen in *Figure 7.*

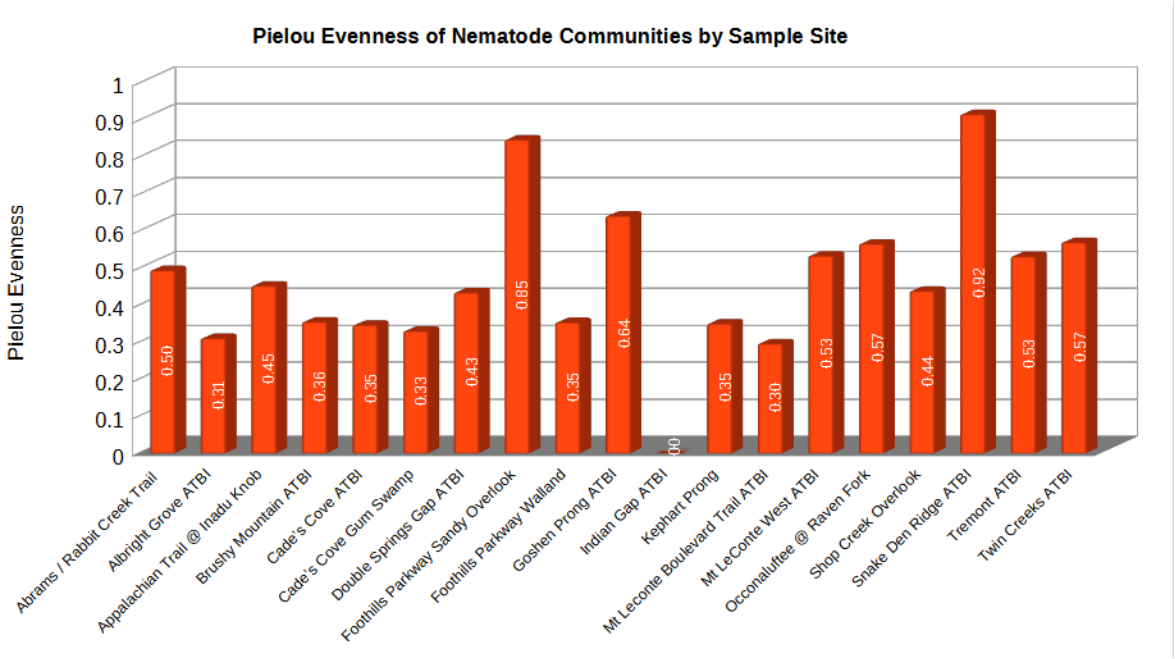

Figure 5: Pielou diversity of nematode communities by sample site in GSMNP.

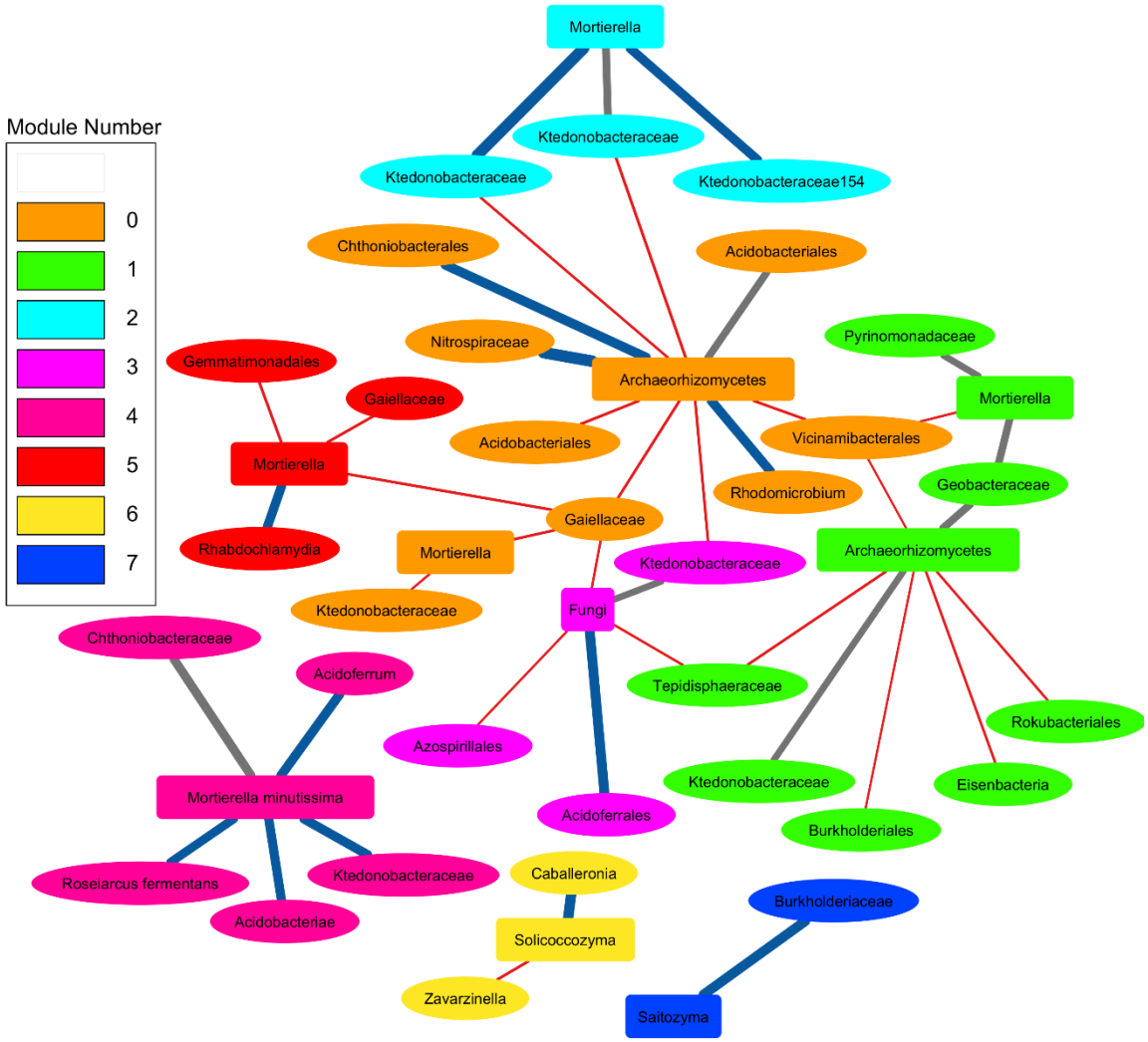

Figure 6: Bipartite co-occurrence network generated from next-generation sequencing data characterizing the identity and abundance of bacteria and fungi at 19 sites across GSMNP. Elliptical nodes represent bacterial OTUs and rectangular nodes represent fungal OTUs. Edge color represents interaction type, with blue indicating co-occurrence, red indicating mutual exclusion and grey indicating an inferred interaction of unknown

type. Node color indicates module membership, modules are enumerated in the legend at left. Width of edges represents interaction strength.

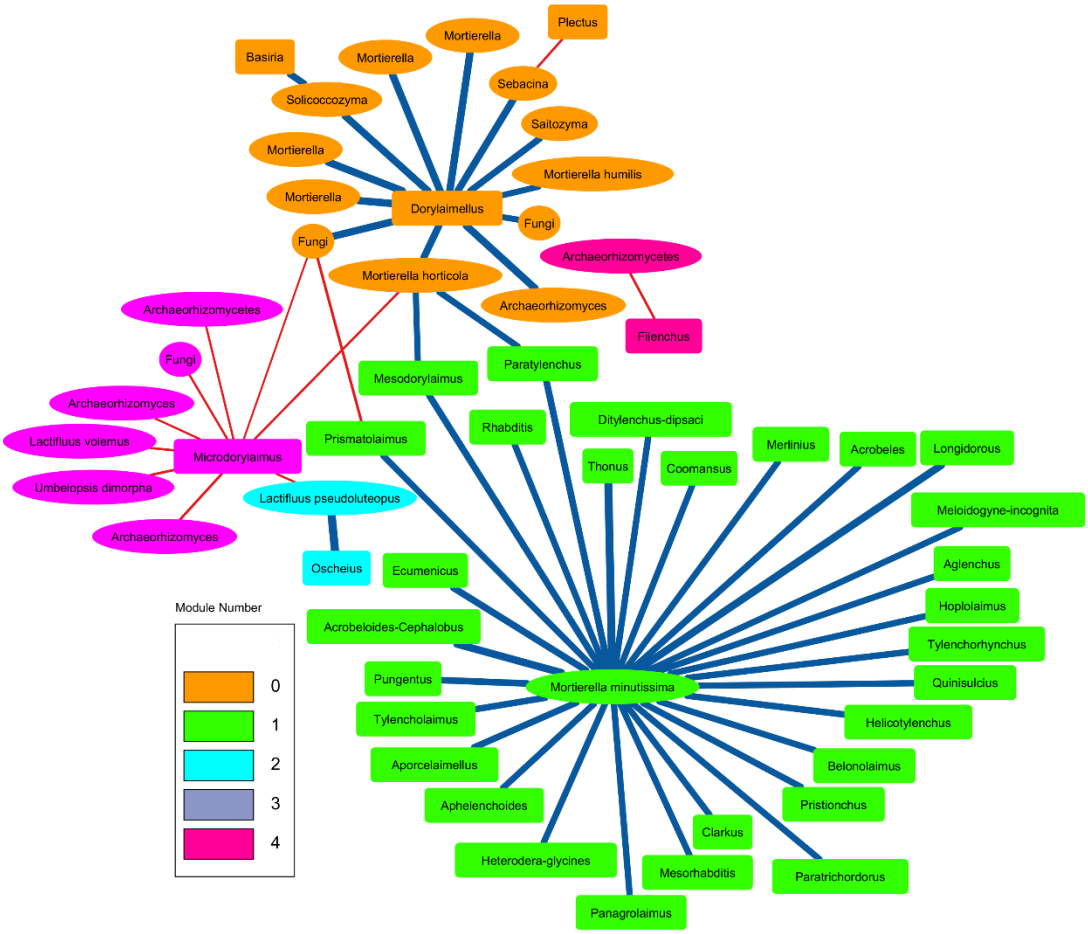

Figure 7: Bipartite co-occurrence network generated from next-generation sequencing and qPCR data characterizing the identity and abundance of fungi and nematodes at 19 sites across GSMNP. Elliptical nodes represent bacterial OTUs and rectangular nodes represent nematode Genera. Edge color represents interaction type, with blue indicating co-occurrence, red indicating mutual exclusion. Node color indicates module

membership, modules are enumerated in the legend at left. Width of edges represents interaction strength.

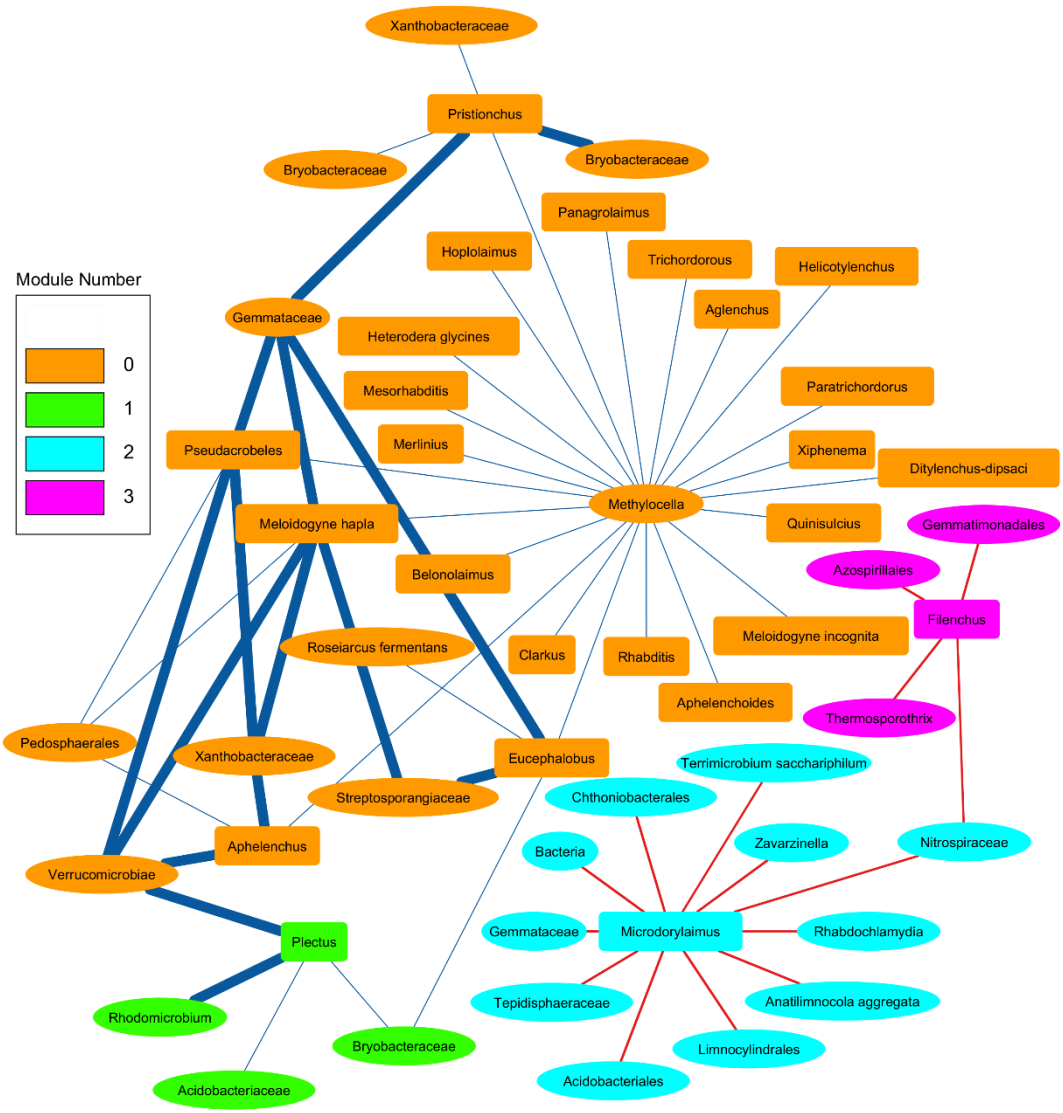

Figure 8: Bipartite co-occurrence network generated from next-generation sequencing and qPCR data characterizing the identity and abundance of bacteria and nematodes at 19 sites across GSMNP. Elliptical nodes represent bacterial OTUs and rectangular nodes represent nematode genera. Edge color represents interaction type, with blue indicating co-occurrence, red indicating mutual exclusion. Node color indicates module

membership, modules are enumerated in the legend at left. Width of edges represents interaction strength.

Network structure

The bipartite network displayed in *figure 8* consisted of 42 edges and 42 nodes. This graph is composed of one large network and three smaller disjunct satellite networks. Each of the 3 satellite networks were considered an independent module by Moduland 2. The large central network was composed of 5 modules. *Figure 9* consists of 52 nodes and 61 edges arranged into one large network flanked by a pair of linked nodes. Modularization yielded 5 modules, four are present in the main network. *Figure 10* consists of two disjunct networks of moderate size, each composed of 2 modules. There are 56 nodes and 57 edges present in this graph.

DISCUSSION

Taxa of interest

Across all samples, *Labronema* was the most abundant genus of nematodes detected, however there were other groups of varying abundance. *Tylencholaimellus* was commonly found in samples as were, *Basiria* and *Prismatolaimus*. Some genera were highly abundant in certain samples, such as *Microdorylaimus* in the sample from the Indian Gap ATBI site. Similarly, *Filenchus* and the parasitic genus *Heterodera* were highly abundant at the Goshen Prong ATBI site. According to Yeates et al., 1993, *Tylencholaimellus* is a fungivore that feeds via a stylet and *Basiria* and *Prismatolaimus* are omnivores that may feed on a variety of food sources including unicellular and multicellular fungi, plant root hairs and bacteria, via either a piercing stylet or engulfment respectively. *Labronema* is a common and versatile nematode that may feed on anything it can piece with its stylet, including microscopic animals such as rotifers tardigrades and other nematodes, as well as algae and plants such as moss (Peña-Santiago and Abolafia, 2019; Wood, 1973). *Ecumenicus* is a stylet feeding omnivore, like many of the other nematodes found in GSMNP (Yeates et al., 1993). *Oscheius* is a bacterivorous nematode that may be pathogenic to insects at points in its lifecycle, killing via entomopathogenic bacteria that it carries on and within its body. (Kumar et al., 2019).

Several bacterial phyla of lower abundance contributed to inferred networks. The following section will be devoted to describing them. Gemmatimonadota is a phylum of

largely Gram-negative bacilli that are often found in environmental samples (Zhang et al., 2003). Many members of the phylum are unculturable with only six culturable isolates known. These cultured isolates were purified from aquatic and soil habitats, with one member of the former group being found in a sequential batch reactor (Zeng et al., 2021; Zeng et al., 2015). Gemmatimonads appear to largely be aerobic and chemoheterotrophic in nature, however Gemmatimonadota is one of the few phyla known to have members capable of anoxygenic phototrophy. This is facilitated by the production of bacteriochlorophyll-A and the possession of type-II reaction centers. Photoautotrophy is not found among gemmatimonads, instead many are facultative photoheterotrophs (Zeng et al., 2014). This supplemental energy source allows the proportion of carbon uptake directed to catabolism to be reduced, increasing growth rates as it is instead assimilated (Koblížek et al., 2020). Gemmatimonads were detected in all samples, between 0.22% and 3.7% abundance. The detected relative abundance is comparable to values published in other literature (Delgado-Baquerizo et al., 2018; O'Connell et al., 2007).

Nitrospirota is a phylum of Gram-negative spirilla and filamentous nitrogen-oxidizing bacteria. Nitrospirota may be found in disparate habitat types including but not limited to surface waters, soil, hot springs and the subsurface (Daims, Lucker and Wagner, 2016). Some participate in symbiotic interactions with plants and participate in rhizosphere interactions. Many of these organisms are aerobic chemolithoautotrophs and some are commamox organisms capable of oxidizing ammonia completely, producing free nitrate as a result. Nitrospirota are more commonly restricted to either ammonia oxidation or nitrite oxidation (Zhang et al., 2023). An observed trend among

nitrite-oxidizing bacteria is segregation along taxonomic lines based on optimal dissolved-oxygen concentration. Nitrospirota appear to be more well adapted to lower dissolved-oxygen concentrations than their competitors, for example, *Nitrobacter*. This trend is also observed in relation to nitrite concentrations (Blackburne et al., 2007; Huang et al., 2010). These lines of evidence support the hypothesis that Nitrospirota are k-strategists, displaying slower growth than their competitors, which are suspected to be r-strategists (Andrews and Harris, 1986). This phylum represented, at most, 2.6% of features in samples collected from GSMNP.

Planctomycetes is a phylum composed of highly divergent bacteria, many of which have exotic cell plans. In what might be described as a sharp break from other, more well known, bacterial phyla, Planctomycetes may possess a primitive endomembrane system. In lieu of the typical peptidoglycan cell wall, these bacteria possess proteinaceous cell walls or none at all (Liesack et al., 1986). This renders them insusceptible to many common antibiotics, such as vancomycin, that inhibit cell wall synthesis. Some members of this phylum are capable of anaerobic ammonia oxidation (anammox). This process involves the oxidation of ammonia or ammonium ions to dinitrogen, reducing the bioavailable nitrogen in a system. This makes these organisms ecologically important and popularly applied to bioremediation of wastewater (Jetten et al., 2009). This unusual physiology is facilitated by another unique characteristic, the presence of a membrane bound ‘organelle’ called the anammoxosome. This structure is believed to be necessary in the containment of toxic intermediates formed during the anammox process, such as hydrazine (Niftrik et al., 2004).

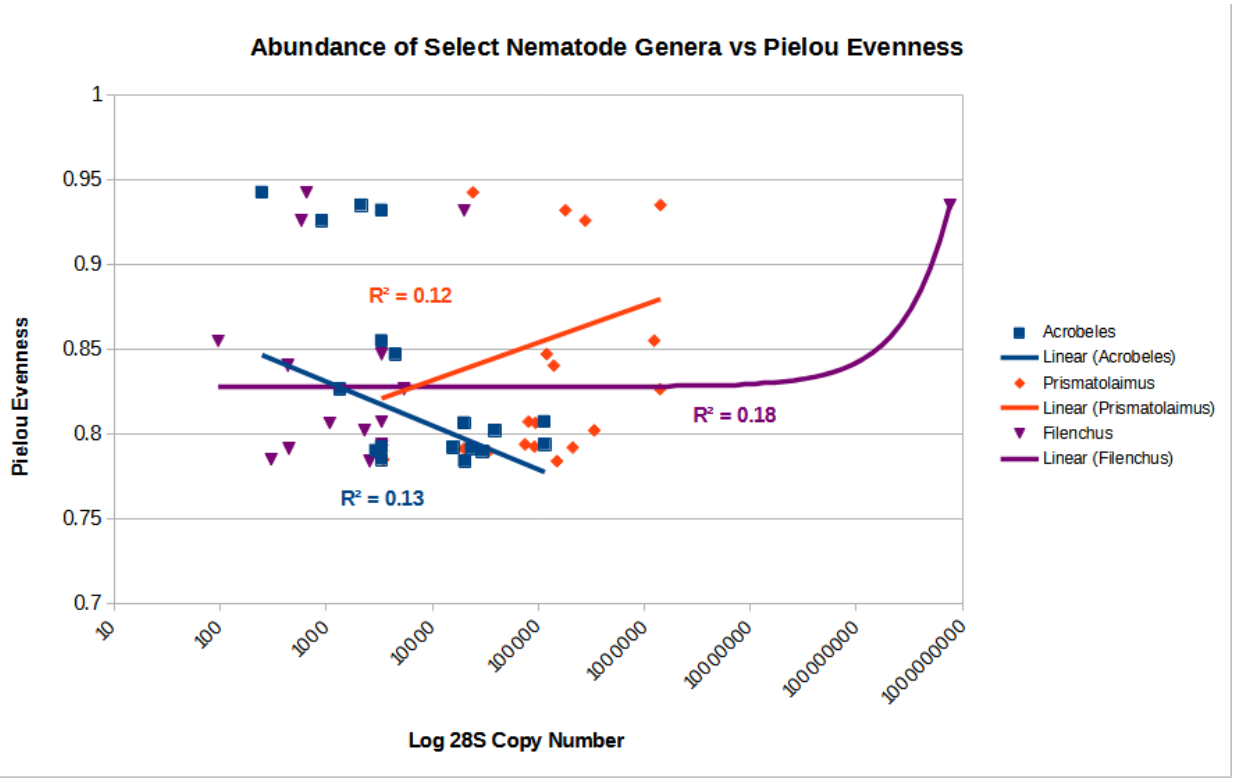

Figure 9: Relationship between select nematode genera and observed evenness of bacterial assemblages.

Chlamydiota is another highly divergent bacterial phylum, related to Planctomycetes. These organisms, along with Verrucomicrobia, and the previously discussed Planctomycetes, represent the PVC superphylum (Wagner and Horn, 2006). Chlamydiae are obligately intracellular, requiring a host cell from any branch of the eukaryotic tree of life to survive. Many of these organisms utilize nucleotide transporters to directly rob host cells of ATP and other nucleotides (Schmitz-Esser et al., 2004). Both the well-known clinical representatives, such as *Chlamydia trachomatis* and *Chlamydia pneumonia*, and environmental chlamydiae share a unique lifecycle involving infectious but metabolically inert elementary bodies and metabolically active, intracellular reticulate bodies (Bayramova, Jacquier and Greub, 2017). A member of the phylum

Chlamydiota, *Rhabdochlamydia spp.,* was placed in module 5 of *Figure 8.* Members of this genus are known to be intracellular pathogens of terrestrial arthropods (Kostanjsek et al., 2004; Corsaro et al., 2006). Given the dependence of chlamydiae on a diverse array of hosts, and with continued study of both the bacteria themselves and their individual host ranges, molecular detection of chlamydiae could be employed as an indicator of cryptic eukaryotes.

Brief investigations of hypothesized interactions

Since co-occurrence networks function as hypothesis generation tools, the following section will be devoted to exploring some of the ecological interactions suggested by generated co-occurrence networks. Module 2 of *Figure 8* is composed of an OTU belonging to the fungal genus *Mortierella* surrounded by three bacterial OTUs belonging to the genus *Ktedonobacteraceae.* Several members of the bacterial Phylum Chloroflexota display filamentous growth and are capable cellulose degraders, a trait they share with *Mortierella* (Yabe et al., 2017; Zheng et al., 2021; Ozimek and Hanaka, 2020). These organisms may represent an ecological guild of cellulose degraders. This is of interest because cellulose is a highly abundant form of recalcitrant organic matter and as such, functions as a reservoir of soil carbon.

Figure 8 suggested that the abundance of several genera of nematode fungivores may correlate with the abundance of Basidiomycetes, since Glomeromycetes are poorly detected by the sampling protocol employed. However, no meaningful correlation was observed between the aforementioned genera and the relative abundance of the fungal phylum, when regression analysis was performed manually. It was also suspected, based on module 3 in *Figure 9* that top-down pressure

exerted on bacteria by grazing nematodes might boost the diversity of detected bacterial assemblages, however this does not seem to be the case, as only an extremely week correlation was found ($r^2 = 0.13$). Bacterial evenness was compared to select nematode genera, including a genus thought to be of interest based on generated networks, *Filenchus*. However, only extremely weak correlations were found ($r^2 = 0.18$), which can be seen in *Figure 11*. In *Figure 10*, *Filenchus* was seen to be negatively correlated with the abundance of two nitrogen cycling taxa, Azospirillales and Nitrospiraceae.

Considerations when interpreting amplicon sequence data

Next-generation sequencing offers excellent insight into the microbial ecology of soils and sediments. It is, however, subject to biases and shortcomings, as any technique is. One such bias comes from target gene copy number. The bacterial 16S rRNA gene varies widely in copy number between taxa, and some taxa display greater variability (Vetrovsky, Baldrian and Neufeld, 2013; Kimbel et al., 2012). In addition, these sequences are very unlikely to be identical (Vetrovsky, Baldrian and Neufeld, 2013). This has negative downstream impacts on diversity estimates and taxonomic assignments. Since 16S rRNA gene copy number is the proxy used to estimate bacterial abundance, the detected abundance of taxa carrying multiple copies of the target gene will be inflated. This artifact can lead to taxa with greater copy numbers being attributed with greater abundance and ecological significance than they might, in reality, have. Even minute differences in sequence can have impacts on downstream feature classification. Sample inference pipelines such as DADA2 are capable of differentiating sequences that differ by as little as one nucleotide in sequence or length.

Because of this, sequence variants from the same organism may be binned into different ASVs (Callahan et al., 2016). This may be mitigated by downstream clustering into OTUs, however this may be undesirable. There exist methods to mitigate the impact of this issue. The ribosomal RNA operon copy number database (rrnDB) is an ongoing effort to catalog variations in 16S rRNA copy number across the diversity of prokaryotes, allowing abundance estimates based on 16S sequencing to be corrected based on taxonomic assignment (Stoddard, Smith and Hein, 2015). Gene copy number reflects the ecology of the organism. Many taxa known to possess high 16S rRNA gene copy numbers harbor r-strategists that are well prepared to take advantage of a sudden change in their trophic fortunes. (Klappenbach, Dunbar and Schmidt, 2000).

Sample storage and preparation may also introduce biases to NGS data. It has been demonstrated that choice of sample storage protocol can affect detected community richness and evenness, while increasing the apparent abundance of certain taxa (Pavlovska et al., 2021).

Certain bacterial taxa are harder to lyse, and so the efficiency with which their DNA is extracted from a sample for sequencing is diminished (Van Tongerson, Degener and Harmsen, 2011). This may be the case with Gram-positive taxa. It can be seen in *Figure 2* that Firmicutes did not number among the ten most abundant phyla and that Actinobacteria are the sixth most abundant in all samples. Most of the Firmicute DNA may be locked away in endospores, inaccessible and thus undetectable, unless a specialized extraction protocol was used (Delgado-Baquerizo et al., 2018; Yang et al., 2019).

There are several notable features visible in *Figure 2.* The low abundance of Glomeromycetes in most sample sites is expected. This is likely due to the sampling protocol followed. Glomeromycetes are arbuscular mycorrhizal fungi and as such, may only be found within the intercellular spaces of plants and in the rhizosphere, the zone directly adjacent to plant roots (Oehl et al., 2011). Since the sampling protocol was not suitable for collecting rhizosphere inhabitants, any Glomeromycetes detected are incidental. Of the fungal features from the Kephart prong sample, 63.38% (data not shown) were classified as belonging to the fungus *Lactifluus pseudoluteopus*, a mushroom-forming basidiomycete (De Crop et al., 2021). Features attributed to the fungus *Lactifluus volemus* represent 65.34% (data not shown) of the total feature count of the Shop Creek Overlook sample. Fungi of the ectomycorrhizal genus *Lactifluus* are commonly known as “milk caps,” for the white latex they produce when cut, and are often edible. A similar and closely related member of the family Russulaceae, *Russula crustosa* was detected at 19.28% (data not shown) abundance in the Albright Grove ATBI sample (De Crop et al., 2021). Given that these organisms are large, in relative terms, and multicellular, they contribute greatly to ITS copy number in any sample including them. This lends them a much higher apparent abundance than might be expected. In addition, since these organisms are multicellular, their distribution is likely patchy at the scale of meters (Janowshi and Leski, 2023). To avoid misrepresenting these organisms as oppressively dominant at a single site and driving down diversity estimates, it would be advantageous to collect replicate samples, separated by several meters.

Conclusions

Qualitative relationships between nematode genera, bacterial taxa and fungal taxa were uncovered, through the use of ecological network inference. These networks, when analyzed using Moduland 2, reveal guilds of organisms operating in concert and many groups in competition. Several of these groups may be involved in carbon turnover, transformation of nitrogen compounds, and other processes that are important for the maintenance and functioning of soils. Future studies may investigate these predicted relationships and guilds, by employing soil chemistry analyses and functional gene sequencing. In addition, sampling for a yet wider array of habitat types will allow additional relationships to be inferred.

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Appendix. Data reports from Microbial Insights, Inc. showing raw data and quality scoring for 53 taxonomic groups of nematodes from Great Smoky Mountains National Park.

10515 Research Drive
Knoxville, TN 37932
Phone: (865) 573-8188
Fax: (865) 573-8133

Client: Will Kuhn
Discover Life in America
1316 Cherokee Orchard Rd
Gatlinburg, TN 37738

Phone:

Fax:

Identifier: 033UH **Date Rec:** 08/09/2023 **Report Date:** 10/10/2023

Client Project #: **Client Project Name:** Sampling soil diversity in the Smokies

Purchase Order #:

Test results provided for: CENSUS

Reviewed By:

Results relate only to the items tested and the sample(s) as received by the laboratory.

MICROBIAL INSIGHTS, INC.

10515 Research Dr., Knoxville, TN 37932
Tel. (865) 573-8188 Fax. (865) 573-8133

CENSUS

Client: **Discover Life in America**
Project: Sampling soil diversity in the Smokies

MI Project Number: **033UH**
Date Received: 08/09/2023

Sample Information

Client Sample ID:		TCAS - Twin Creeks ATBI Site	IGAS - Indian Gap ATBI Site	ORRF - Oconaluftee River & Raven Fork confluence	CCAS - Cades Cove ATBI Site	CCGS - Cades Cove Gum Swamp
Sample Date:		06/22/2023	06/22/2023	06/22/2023	07/06/2023	07/06/2023
Units:		cells/g	cells/g	cells/g	cells/g	cells/g
Analyst/Reviewer:		AR/SK	AR/SK	AR/SK	AR/SK	AR/SK
Bacterivorous Nematodes						
Panagrolaimus spp.	PNGLN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Anaplectus spp.	ANPN	**8.98E+03**	**7.19E+02 (J)**	<3.33E+03	**3.09E+02 (J)**	**1.22E+02 (J)**
Plectus spp.	PLCN	**7.68E+02 (J)**	**1.28E+02 (J)**	<3.33E+03	**1.64E+02 (J)**	<3.33E+03
Wilsonema, Tylocephalus spp.	WTCN	**2.12E+04**	**2.27E+03 (J)**	<3.33E+03	<3.33E+03	**1.43E+03 (J)**
Mesorhabditis spp.	MHBN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Oscheius spp.	OSCN	**2.07E+04**	<3.33E+03	<3.33E+03	<3.33E+03	**8.80E+01 (J)**
Rhabditis spp.	RHBN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Acrobeles spp.	ACBLN	**2.64E+03 (J)**	<3.33E+03	**1.35E+03 (J)**	<3.33E+03	<3.33E+03
Eucephalobus spp.	ECPHN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Pseudacrobeles spp.	PABN	<3.33E+03	<3.33E+03	<3.33E+03	**2.65E+02 (J)**	<3.33E+03
Acrobleoides/Cephalobus spp.	ACCBN	**2.58E+03 (J)**	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Fungivorous Nematodes						
Ditylenchus spp.	BSFN	**1.86E+04**	**3.95E+03**	**4.19E+03**	<3.33E+03	<3.33E+03
Aphelenchus spp.	APHN	<3.33E+03	<3.33E+03	<3.33E+03	**2.95E+02 (J)**	<3.33E+03
Aphelenchoides spp.	ApFN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Aglenchus spp.	AGLN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Basiria spp.	BASN	**5.95E+05**	**3.33E+04**	**6.91E+04**	**3.30E+04**	**6.59E+03**
Filenchus spp.	FLCN	**2.30E+03 (J)**	<3.33E+03	**1.08E+03 (J)**	<3.33E+03	<3.33E+03
Herbivorous Nematodes						
Stem nematodes (D. dipsaci)	DtDSP	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Sting nematodes (Belonolaimus)	BLMN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Ring nematodes (Mesocriconema)	McRN	**1.39E+06**	**1.26E+04**	**5.79E+04**	**4.23E+04**	**2.06E+03 (J)**
Cyst nematodes (Heterodera)	HdCN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Soybean cyst nematode	HdGLY	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Spiral nematodes	HtSN	**1.12E+04**	<3.33E+03	**2.11E+03 (J)**	<3.33E+03	<3.33E+03
Lance nematodes (Hoplolaimus)	HpLN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Needle nematodes (Longidorous)	LGDN	**6.90E+04**	**1.09E+04**	**1.02E+02 (J)**	**1.31E+04**	**6.74E+01 (J)**
Dagger nematodes (Xiphinema)	XpDN	<3.33E+03	**2.57E+03 (J)**	<3.33E+03	<3.33E+03	<3.33E+03
Root-knot nematodes	MGRKN	**2.10E+05**	**1.26E+04**	**2.91E+03 (J)**	**5.86E+03**	<3.33E+03
Southern root-knot nematode	MgICG	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Northern root-knot nematode	MgHAP	<3.33E+03	<3.33E+03	<3.33E+03	**6.41E+02 (J)**	<3.33E+03
Pin nematodes (Paratylenchus)	PaTYN	**6.89E+04**	**1.68E+03 (J)**	**2.08E+04**	**3.02E+04**	<3.33E+03
Pin nematodes (Gracilacus)	GLPN	**1.40E+06**	**2.41E+04**	**6.51E+04**	**7.08E+04**	**9.16E+03**
Root-lesion nematodes	PRLN	**8.20E+03**	**2.19E+02 (J)**	**4.67E+02 (J)**	**3.81E+02 (J)**	<3.33E+03
Stunt nematodes	TYLRN	**4.26E+04**	<3.33E+03	**6.65E+03**	<3.33E+03	<3.33E+03

Herbivorous Nematodes

Stunt nematodes	QNSN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Stunt nematodes	MRLN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Stubby-root nematodes	TRDN	**1.16E+03 (J)**	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Stubby-root nematodes	PTDN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03

Nematodes (other)

Pristionchus spp.	PSTCN	**3.42E+02 (J)**	<3.33E+03	**1.86E+02 (J)**	<3.33E+03	<3.33E+03
Clarkus spp.	CLKN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Coomansus spp.	CMSN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Mylonchulus spp.	MLCN	**2.72E+05**	**2.34E+04**	**2.76E+04**	**3.21E+04**	**1.69E+04**
Prismatolaimus spp.	PRSMN	**3.37E+05**	**7.50E+04**	**9.42E+04**	<3.33E+03	**9.21E+04**

Omnivorous Nematodes

Aporcelaimellus spp.	APCLN	**3.59E+05**	**9.07E+01 (J)**	**4.38E+03**	<3.33E+03	<3.33E+03
Dorylaimellus spp.	DLMN	**4.62E+03**	**6.63E+03**	**9.63E+02 (J)**	**1.20E+05**	<3.33E+03
Mesodorylaimus spp.	MDLN	**2.23E+05**	**2.52E+04**	**1.38E+03 (J)**	**1.77E+04**	<3.33E+03
Pungentus spp.	PNGN	<3.33E+03	**3.96E+04**	<3.33E+03	<3.33E+03	<3.33E+03
Chrysonema spp.	CHRSN	**6.60E+05**	**5.04E+05**	**8.60E+02 (J)**	<3.33E+03	<3.33E+03
Ecumenicus spp.	ECMCN	**6.05E+05**	**1.86E+05**	<3.33E+03	<3.33E+03	<3.33E+03
Microdorylaimus, Eudorylaimus spp.	MAEN	<3.33E+03	**4.75E+10**	<3.33E+03	**1.94E+02 (J)**	<3.33E+03
Labronema spp.	LBMN	**3.61E+06**	**9.19E+05**	**4.36E+05**	**1.63E+06**	**9.75E+05**
Thonus spp.	THNN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Tylencholaimus spp.	TYLMN	<3.33E+03	<3.33E+03	**7.66E+02 (J)**	<3.33E+03	<3.33E+03
Tylencholaimellus spp.	TLMLN	**1.13E+06**	**2.84E+05**	**1.79E+05**	**1.77E+05**	**5.21E+04**

Legend:

NA = Not Analyzed NS = Not Sampled J = Estimated gene copies below PQL but above LQL I = Inhibited

< = Result not detected

MICROBIAL INSIGHTS, INC.

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CENSUS

Client: **Discover Life in America**
Project: Sampling soil diversity in the Smokies

MI Project Number: **033UH**
Date Received: 08/09/2023

Sample Information

Client Sample ID:		ACRC - Abrams Creek Rabbit Creek Trail	FPSO - Foothills Parkway Sandy Overlook	CFSC - Cliff face at HWY 129 & Shop Creek	FPWA - Foothills Parkway Walland	TRAS - Tremont ATBI Site
Sample Date:		07/05/2023	07/05/2023	07/05/2023	07/05/2023	07/05/2023
Units:		cells/g	cells/g	cells/g	cells/g	cells/g
Analyst/Reviewer:		AR/SK	AR/SK	AR/SK	AR/SK	AR/SK
Bacterivorous Nematodes						
Panagrolaimus spp.	PNGLN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Anaplectus spp.	ANPN	**4.48E+02 (J)**	**1.45E+04**	<3.33E+03	**1.30E+04**	**5.14E+04**
Plectus spp.	PLCN	**1.04E+02 (J)**	**1.76E+02 (J)**	<3.33E+03	**2.30E+03 (J)**	**1.94E+04**
Wilsonema, Tylocephalus spp.	WTCN	**6.64E+02 (J)**	**3.11E+04**	<3.33E+03	<3.33E+03	**1.53E+04**
Mesorhabditis spp.	MHBN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Oscheius spp.	OSCN	**3.68E+02 (J)**	<3.33E+03	<3.33E+03	**1.50E+02 (J)**	<3.33E+03
Rhabditis spp.	RHBN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Acrobeles spp.	ACBLN	**1.57E+04**	**1.14E+05**	**2.98E+04**	**2.13E+03 (J)**	**3.90E+04**
Eucephalobus spp.	ECPHN	**1.49E+02 (J)**	**1.71E+03 (J)**	**6.16E+02 (J)**	<3.33E+03	**1.14E+03 (J)**
Pseudacrobeles spp.	PABN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Acrobleoides/Cephalobus spp.	ACCBN	**8.72E+04**	**3.57E+05**	**1.21E+05**	**4.98E+03**	**1.18E+05**
Fungivorous Nematodes						
Ditylenchus spp.	BSFN	**3.00E+03 (J)**	**9.41E+03**	**2.13E+04**	**5.45E+03**	**6.06E+03**
Aphelenchus spp.	APHN	**4.99E+03**	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Aphelenchoides spp.	ApFN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Aglenchus spp.	AGLN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Basiria spp.	BASN	**1.49E+05**	**1.70E+05**	**2.16E+05**	**1.20E+04**	**1.79E+05**
Filenchus spp.	FLCN	**4.35E+02 (J)**	**9.59E+01 (J)**	**5.42E+03**	<3.33E+03	<3.33E+03
Herbivorous Nematodes						
Stem nematodes (D. dipsaci)	DtDSP	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Sting nematodes (Belonolaimus)	BLMN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Ring nematodes (Mesocriconema)	McRN	**2.65E+04**	**1.56E+05**	**5.85E+04**	**6.03E+04**	**7.58E+04**
Cyst nematodes (Heterodera)	HdCN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Soybean cyst nematode	HdGLY	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Spiral nematodes	HtSN	**5.60E+03**	<3.33E+03	<3.33E+03	<3.33E+03	**8.92E+01 (J)**
Lance nematodes (Hoplolaimus)	HpLN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Needle nematodes (Longidorous)	LGDN	<3.33E+03	**3.30E+02 (J)**	**4.30E+04**	**4.09E+03**	**1.08E+04**
Dagger nematodes (Xiphinema)	XpDN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Root-knot nematodes	MGRKN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Southern root-knot nematode	MgICG	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Northern root-knot nematode	MgHAP	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Pin nematodes (Paratylenchus)	PaTYN	**7.38E+01(J)**	**8.61E+03**	<3.33E+03	**1.40E+05**	**1.92E+04**
Pin nematodes (Gracilacus)	GLPN	**5.32E+04**	**2.83E+05**	**2.64E+05**	**9.49E+04**	**3.37E+05**
Root-lesion nematodes	PRLN	**1.95E+03 (J)**	**2.08E+03 (J)**	**1.04E+04**	<3.33E+03	**5.75E+03**
Stunt nematodes	TYLRN	**1.55E+04**	**1.42E+04**	**1.24E+04**	<3.33E+03	**6.25E+04**

Herbivorous Nematodes

Stunt nematodes	QNSN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Stunt nematodes	MRLN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Stubby-root nematodes	TRDN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Stubby-root nematodes	PTDN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03

Nematodes (other)

Pristionchus spp.	PSTCN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Clarkus spp.	CLKN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Coomansus spp.	CMSN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Mylonchulus spp.	MLCN	**1.38E+05**	**1.45E+05**	**1.23E+05**	**1.76E+04**	**2.82E+05**
Prismatolaimus spp.	PRSMN	**1.40E+05**	**1.24E+06**	**1.41E+06**	**8.10E+04**	**1.20E+05**

Omnivorous Nematodes

Aporcelaimellus spp.	APCLN	<3.33E+03	<3.33E+03	**4.35E+03**	**9.19E+04**	**6.59E+04**
Dorylaimellus spp.	DLMN	<3.33E+03	**1.80E+04**	**1.05E+04**	**6.65E+02 (J)**	**1.05E+05**
Mesodorylaimus spp.	MDLN	**1.25E+03 (J)**	**1.57E+04**	**5.66E+04**	**9.02E+04**	**9.94E+04**
Pungentus spp.	PNGN	**1.26E+04**	**2.38E+02 (J)**	**8.96E+03**	<3.33E+03	**9.40E+02 (J)**
Chrysonema spp.	CHRSN	**2.16E+02 (J)**	**1.04E+03 (J)**	**1.01E+04**	**1.78E+05**	**1.84E+05**
Ecumenicus spp.	ECMCN	<3.33E+03	<3.33E+03	<3.33E+03	**1.88E+05**	**5.33E+04**
Microdorylaimus, Eudorylaimus spp.	MAEN	<3.33E+03	<3.33E+03	**1.12E+04**	**4.71E+02 (J)**	**4.55E+03**
Labronema spp.	LBMN	**9.59E+05**	**6.75E+06**	**3.00E+06**	**4.90E+05**	**2.15E+06**
Thonus spp.	THNN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Tylencholaimus spp.	TYLMN	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03	<3.33E+03
Tylencholaimellus spp.	TLMLN	**2.69E+05**	**1.65E+06**	**1.07E+06**	**1.38E+05**	**1.10E+06**

Legend:

NA = Not Analyzed NS = Not Sampled J = Estimated gene copies below PQL but above LQL I = Inhibited

< = Result not detected

MICROBIAL INSIGHTS, INC.

10515 Research Dr., Knoxville, TN 37932
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CENSUS

Client: **Discover Life in America**
Project: Sampling soil diversity in the Smokies

MI Project Number: **033UH**
Date Received: 08/09/2023

Sample Information

Client Sample ID:		DSAS - Double Springs Gap ATBI Site	MBAS - Mt LeConte Boulevard Trail ATBI Site	AGAS - Albright Grove ATBI Site	ATIK - App Trail @ Inadu Knob	SDAS - Snake Den Ridge ATBI Plot
Sample Date:		07/11/2023	07/21/2023	07/25/2023	07/26/2023	07/26/2023
Units:		cells/g	cells/g	cells/g	cells/g	cells/g
Analyst/Reviewer:		AR/SK	AR/SK	AR/SK	AR/SK	AR/SK
Bacterivorous Nematodes						
Panagrolaimus spp.	PNGLN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Anaplectus spp.	ANPN	<3.33E+03	**6.22E+02 (J)**	<3.33E+03	**5.16E+03 (J)**	<3.33E+03
Plectus spp.	PLCN	<3.33E+03	**9.35E+02 (J)**	<3.33E+03	**7.38E+03**	<3.33E+03
Wilsonema, Tylocephalus spp.	WTCN	<3.33E+03	<3.33E+03	**3.32E+02 (J)**	**1.03E+04**	<3.33E+03
Mesorhabditis spp.	MHBN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Oscheius spp.	OSCN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Rhabditis spp.	RHBN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Acrobeles spp.	ACBLN	<3.33E+03	**9.12E+02 (J)**	**2.02E+04**	**2.48E+02 (J)**	**4.48E+03**
Eucephalobus spp.	ECPHN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Pseudacrobeles spp.	PABN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Acrobleoides/Cephalobus spp.	ACCBN	<3.33E+03	**6.39E+03**	**4.50E+04**	<6.67E+03	**2.14E+04**
Fungivorous Nematodes						
Ditylenchus spp.	BSFN	**7.55E+02 (J)**	**7.94E+03**	<3.33E+03	**2.47E+04**	**1.18E+02 (J)**
Aphelenchus spp.	APHN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Aphelenchoides spp.	ApFN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Aglenchus spp.	AGLN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Basiria spp.	BASN	**1.80E+03 (J)**	**2.87E+04**	**1.38E+04**	**1.48E+05**	**2.99E+03 (J)**
Filenchus spp.	FLCN	**3.03E+02 (J)**	**5.84E+02 (J)**	<3.33E+03	**2.57E+03 (J)**	<3.33E+03
Herbivorous Nematodes						
Stem nematodes (D. dipsaci)	DtDSP	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Sting nematodes (Belonolaimus)	BLMN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Ring nematodes (Mesocriconema)	McRN	**4.36E+03**	**7.57E+04**	**5.34E+04**	**3.95E+04**	**6.33E+02 (J)**
Cyst nematodes (Heterodera)	HdCN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Soybean cyst nematode	HdGLY	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Spiral nematodes	HtSN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Lance nematodes (Hoplolaimus)	HpLN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Needle nematodes (Longidorous)	LGDN	**1.66E+02 (J)**	**4.11E+03**	<3.33E+03	**7.65E+02 (J)**	<3.33E+03
Dagger nematodes (Xiphinema)	XpDN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Root-knot nematodes	MGRKN	**5.53E+03**	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Southern root-knot nematode	MgICG	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Northern root-knot nematode	MgHAP	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Pin nematodes (Paratylenchus)	PaTYN	**1.73E+03(J)**	<3.33E+03	<3.33E+03	**4.48E+02(J)**	<3.33E+03
Pin nematodes (Gracilacus)	GLPN	**2.28E+03(J)**	**1.10E+04**	**8.50E+03**	**4.16E+05**	**1.34E+03 (J)**
Root-lesion nematodes	PRLN	**6.01E+02 (J)**	**1.60E+03 (J)**	<3.33E+03	**3.72E+03 (J)**	<3.33E+03
Stunt nematodes	TYLRN	**8.39E+02(J)**	**1.62E+03(J)**	**3.31E+03 (J)**	**8.28E+03**	<3.33E+03
Stunt nematodes	QNSN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03

Herbivorous Nematodes

Stunt nematodes	MRLN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Stubby-root nematodes	TRDN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Stubby-root nematodes	PTDN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03

Nematodes (other)

Pristionchus spp.	PSTCN	<3.33E+03	**1.30E+02 (J)**	<3.33E+03	<6.67E+03	<3.33E+03
Clarkus spp.	CLKN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Coomansus spp.	CMSN	<3.33E+03	<3.33E+03	<3.33E+03	**2.36E+04**	<3.33E+03
Mylonchulus spp.	MLCN	**1.73E+04**	**2.06E+05**	**6.03E+03**	**1.24E+06**	<3.33E+03
Prismatolaimus spp.	PRSMN	**3.46E+03**	**1.99E+04**	**2.11E+05**	**1.50E+05**	**3.40E+04**

Omnivorous Nematodes

Aporcelaimellus spp.	APCLN	**7.05E+02 (J)**	<3.33E+03	**9.11E+01 (J)**	<6.67E+03	<3.33E+03
Dorylaimellus spp.	DLMN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Mesodorylaimus spp.	MDLN	**1.10E+03 (J)**	**2.23E+03 (J)**	**8.63E+01 (J)**	<6.67E+03	<3.33E+03
Pungentus spp.	PNGN	**2.77E+03 (J)**	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Chrysonema spp.	CHRSN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Ecumenicus spp.	ECMCN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Microdorylaimus, Eudorylaimus spp.	MAEN	**3.01E+02 (J)**	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Labronema spp.	LBMN	**5.45E+04**	**1.91E+06**	**1.71E+06**	**2.31E+06**	**1.11E+04**
Thonus spp.	THNN	**5.21E+02 (J)**	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Tylencholaimus spp.	TYLMN	<3.33E+03	<3.33E+03	<3.33E+03	<6.67E+03	<3.33E+03
Tylencholaimellus spp.	TLMLN	**2.83E+03 (J)**	**1.53E+05**	**9.64E+04**	**8.82E+05**	**3.83E+03**

Legend:

NA = Not Analyzed NS = Not Sampled J = Estimated gene copies below PQL but above LQL I = Inhibited

< = Result not detected

Quality Assurance/Quality Control Data

Samples Received **8/9/2023**

Component	Date Prepared	Date Analyzed	Arrival Temperature	Positive Control	Extraction Blank	Negative Control
HdGLY	08/09/2023	10/09/2023	4 °C	95%	non-detect	non-detect
McRN	08/09/2023	10/09/2023	4 °C	101%	non-detect	non-detect
MgHAP	08/09/2023	10/09/2023	4 °C	99%	non-detect	non-detect
MgICG	08/09/2023	10/09/2023	4 °C	101%	non-detect	non-detect
MGRKN	08/09/2023	10/09/2023	4 °C	98%	non-detect	non-detect
ACBLN	08/09/2023	10/09/2023	4 °C	101%	non-detect	non-detect
ACCBN	08/09/2023	10/09/2023	4 °C	103%	non-detect	non-detect
AGLN	08/09/2023	10/09/2023	4 °C	103%	non-detect	non-detect
ANPN	08/09/2023	10/09/2023	4 °C	109%	non-detect	non-detect
APCLN	08/09/2023	10/09/2023	4 °C	105%	non-detect	non-detect
ApFN	08/09/2023	10/09/2023	4 °C	100%	non-detect	non-detect
APHN	08/09/2023	10/09/2023	4 °C	110%	non-detect	non-detect
BASN	08/09/2023	10/09/2023	4 °C	102%	non-detect	non-detect
BLMN	08/09/2023	10/09/2023	4 °C	101%	non-detect	non-detect
BSFN	08/09/2023	10/09/2023	4 °C	106%	non-detect	non-detect
CHRSN	08/09/2023	10/09/2023	4 °C	106%	non-detect	non-detect
CLKN	08/09/2023	10/09/2023	4 °C	98%	non-detect	non-detect
CMSN	08/09/2023	10/09/2023	4 °C	101%	non-detect	non-detect
DLMN	08/09/2023	10/09/2023	4 °C	99%	non-detect	non-detect
DtDSP	08/09/2023	10/09/2023	4 °C	100%	non-detect	non-detect
ECMCN	08/09/2023	10/09/2023	4 °C	96%	non-detect	non-detect
ECPHN	08/09/2023	10/09/2023	4 °C	106%	non-detect	non-detect
FLCN	08/09/2023	10/09/2023	4 °C	109%	non-detect	non-detect
GLPN	08/09/2023	10/09/2023	4 °C	108%	non-detect	non-detect
HdCN	08/09/2023	10/09/2023	4 °C	98%	non-detect	non-detect
HpLN	08/09/2023	10/09/2023	4 °C	101%	non-detect	non-detect
HtSN	08/09/2023	10/09/2023	4 °C	97%	non-detect	non-detect
LBMN	08/09/2023	10/09/2023	4 °C	102%	non-detect	non-detect
LGDN	08/09/2023	10/09/2023	4 °C	102%	non-detect	non-detect
MAEN	08/09/2023	10/09/2023	4 °C	97%	non-detect	non-detect

Samples Received **8/9/2023**

Component	Date Prepared	Date Analyzed	Arrival Temperature	Positive Control	Extraction Blank	Negative Control
MDLN	08/09/2023	10/09/2023	4 °C	97%	non-detect	non-detect
MHBN	08/09/2023	10/09/2023	4 °C	97%	non-detect	non-detect
MLCN	08/09/2023	10/09/2023	4 °C	98%	non-detect	non-detect
MRLN	08/09/2023	10/09/2023	4 °C	99%	non-detect	non-detect
OSCN	08/09/2023	10/09/2023	4 °C	96%	non-detect	non-detect
PABN	08/09/2023	10/09/2023	4 °C	110%	non-detect	non-detect
PaTYN	08/09/2023	10/09/2023	4 °C	105%	non-detect	non-detect
PLCN	08/09/2023	10/09/2023	4 °C	96%	non-detect	non-detect
PNGLN	08/09/2023	10/09/2023	4 °C	93%	non-detect	non-detect
PNGN	08/09/2023	10/09/2023	4 °C	100%	non-detect	non-detect
PRLN	08/09/2023	10/09/2023	4 °C	102%	non-detect	non-detect
PRSMN	08/09/2023	10/09/2023	4 °C	107%	non-detect	non-detect
PSTCN	08/09/2023	10/09/2023	4 °C	118%	non-detect	non-detect
PTDN	08/09/2023	10/09/2023	4 °C	109%	non-detect	non-detect
QNSN	08/09/2023	10/09/2023	4 °C	86%	non-detect	non-detect
RHBN	08/09/2023	10/09/2023	4 °C	102%	non-detect	non-detect
THNN	08/09/2023	10/09/2023	4 °C	100%	non-detect	non-detect
TLMLN	08/09/2023	10/09/2023	4 °C	108%	non-detect	non-detect
TRDN	08/09/2023	10/09/2023	4 °C	98%	non-detect	non-detect
TYLMN	08/09/2023	10/09/2023	4 °C	96%	non-detect	non-detect
TYLRN	08/09/2023	10/09/2023	4 °C	104%	non-detect	non-detect
WTCN	08/09/2023	10/09/2023	4 °C	105%	non-detect	non-detect
XpDN	08/09/2023	10/09/2023	4 °C	105%	non-detect	non-detect

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Client: Will Kuhn
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1316 Cherokee Orchard Rd
Gatlinburg, TN 37738

Phone:

Fax:

Identifier: 032UL **Date Rec:** 12/08/2023 **Report Date:** 01/10/2024

Client Project #: **Client Project Name:** Sampling soil diversity in the Smokies

Purchase Order #:

Test results provided for: CENSUS

Reviewed By:

Results relate only to the items tested and the sample(s) as received by the laboratory.

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CENSUS

Client: **Discover Life in America**
Project: Sampling soil diversity in the Smokies

MI Project Number: **032UL**
Date Received: 12/08/2023

Sample Information

Client Sample ID:		GPAS - Goshen Prong ATBI Site	TGAS - Trillium Gap ATBI Site	MWAS - Mt LeConte West ATBI Site	BMAS - Brushy Mountain ATBI Site	KEPR - Kephart Prong Historic Site
Sample Date:		08/17/2023	08/17/2023	07/21/2023	08/07/2023	08/29/2023
Units:		cells/g	cells/g	cells/g	cells/g	cells/g
Analyst/Reviewer:		AR/SK	AR/SK	AR/SK	AR/SK	AR/SK
Bacterivorous Nematodes						
Panagrolaimus spp.	PNGLN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Anaplectus spp.	ANPN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	**2.73E+03 (J)**
Plectus spp.	PLCN	**6.00E+03 (J) (I)**	<2.00E+04	<2.00E+04	**1.56E+03 (J)**	**1.43E+04 (J)**
Wilsonema, Tylocephalus spp.	WTCN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Mesorhabditis spp.	MHBN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Oscheius spp.	OSCN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	**2.42E+06**
Rhabditis spp.	RHBN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Acrobeles spp.	ACBLN	**1.15E+05 (I)**	<2.00E+04	<2.00E+04	**2.94E+03 (J)**	**2.35E+04**
Eucephalobus spp.	ECPHN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	**2.49E+02 (J)**	**1.00E+03 (J)**
Pseudacrobeles spp.	PABN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Acrobleoides/Cephalobus spp.	ACCBN	**2.27E+06 (I)**	<2.00E+04	<2.00E+04	**5.04E+04**	**5.10E+05**
Fungivorous Nematodes						
Ditylenchus spp.	BSFN	<2.00E+04 (I)	<2.00E+04	**2.69E+04**	**2.08E+04**	<2.00E+04
Aphelenchus spp.	APHN	**3.10E+04 (I)**	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Aphelenchoides spp.	ApFN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Aglenchus spp.	AGLN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Basiria spp.	BASN	**1.19E+06 (I)**	**1.43E+04 (J)**	**1.28E+05**	**1.67E+05**	**1.30E+05**
Filenchus spp.	FLCN	**7.63E+08 (I)**	<2.00E+04	**4.45E+02 (J)**	**6.53E+02 (J)**	<2.00E+04
Herbivorous Nematodes						
Stem nematodes (D. dipsaci)	DtDSP	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Sting nematodes (Belonolaimus)	BLMN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Ring nematodes (Mesocriconema)	McRN	**1.41E+06 (I)**	**5.46E+04**	**6.22E+04**	**1.44E+05**	**2.22E+05**
Cyst nematodes (Heterodera)	HdCN	**4.86E+08 (I)**	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Soybean cyst nematode	HdGLY	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Spiral nematodes	HtSN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	**4.24E+03 (J)**	<2.00E+04
Lance nematodes (Hoplolaimus)	HpLN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Needle nematodes (Longidorous)	LGDN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	**6.41E+03 (J)**	**1.00E+05**
Dagger nematodes (Xiphinema)	XpDN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Root-knot nematodes	MgRKN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Southern root-knot nematode	MgICG	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Northern root-knot nematode	MgHAP	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Pin nematodes (Paratylenchus)	PaTYN	<2.00E+04 (I)	<2.00E+04	**1.20E+04 (J)**	**7.84E+04**	**1.15E+04 (J)**
Pin nematodes (Gracilacus)	GLPN	**1.05E+06 (I)**	**1.52E+04 (J)**	<2.00E+04	**7.91E+04**	**2.54E+04**
Root-lesion nematodes	PRLN	**9.46E+07 (I)**	**2.61E+03 (J)**	**3.19E+04**	**3.83E+04**	**6.63E+03 (J)**
Stunt nematodes	TYLRN	<2.00E+04 (I)	**3.41E+04**	**1.03E+04 (J)**	**3.39E+03 (J)**	**1.01E+04 (J)**
Stunt nematodes	QNSN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Stunt nematodes	MRLN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04

Herbivorous Nematodes

Stubby-root nematodes	TRDN	**1.10E+05 (I)**	<2.00E+04	<2.00E+04	<6.67E+03	**2.75E+04**
Stubby-root nematodes	PTDN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04

Nematodes (other)

Pristionchus spp.	PSTCN	<2.00E+04 (I)	**1.62E+04 (J)**	<2.00E+04	<6.67E+03	<2.00E+04
Clarkus spp.	CLKN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Coomansus spp.	CMSN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	**7.50E+02 (J)**	<2.00E+04
Mylonchulus spp.	MLCN	**9.75E+05 (I)**	**2.39E+05**	**7.06E+05**	**2.30E+06**	**4.58E+06**
Prismatolaimus spp.	PRSMN	**1.42E+06 (I)**	**1.80E+05**	**2.77E+05**	**2.42E+04**	**1.80E+05**

Omnivorous Nematodes

Aporcelaimellus spp.	APCLN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Dorylaimellus spp.	DLMN	<2.00E+04 (I)	**7.33E+03 (J)**	<2.00E+04	**2.19E+04**	**2.05E+05**
Mesodorylaimus spp.	MDLN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	**1.45E+03 (J)**	**1.06E+05**
Pungentus spp.	PNGN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Chrysonema spp.	CHRSN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Ecumenicus spp.	ECMCN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Microdorylaimus, Eudorylaimus spp.	MAEN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Labronema spp.	LBMN	**7.64E+06 (I)**	**5.43E+05**	**2.59E+06**	**5.12E+06**	**1.98E+07**
Thonus spp.	THNN	<2.00E+04 (I)	<2.00E+04	<2.00E+04	<6.67E+03	<2.00E+04
Tylencholaimus spp.	TYLMN	<2.00E+04 (I)	**6.56E+03 (J)**	<2.00E+04	<6.67E+03	**2.91E+05**
Tylencholaimellus spp.	TLMLN	**1.82E+06 (I)**	**4.09E+04**	**3.59E+05**	**9.91E+05**	**2.00E+06**

Legend:

NA = Not Analyzed NS = Not Sampled J = Estimated gene copies below PQL but above LQL I = Inhibited

< = Result not detected

Quality Assurance/Quality Control Data

Samples Received **12/8/2023**

Component	Date Prepared	Date Analyzed	Arrival Temperature	Positive Control	Extraction Blank	Negative Control
ACBLN	12/08/2023	01/10/2024	0 °C	91%	non-detect	non-detect
ACCBN	12/08/2023	01/10/2024	0 °C	106%	non-detect	non-detect
AGLN	12/08/2023	01/10/2024	0 °C	101%	non-detect	non-detect
ANPN	12/08/2023	01/10/2024	0 °C	101%	non-detect	non-detect
APCLN	12/08/2023	01/10/2024	0 °C	100%	non-detect	non-detect
ApFN	12/08/2023	01/10/2024	0 °C	98%	non-detect	non-detect
APHN	12/08/2023	01/10/2024	0 °C	105%	non-detect	non-detect
BASN	12/08/2023	01/10/2024	0 °C	105%	non-detect	non-detect
BLMN	12/08/2023	01/10/2024	0 °C	101%	non-detect	non-detect
BSFN	12/08/2023	01/10/2024	0 °C	99%	non-detect	non-detect
CHRSN	12/08/2023	01/10/2024	0 °C	98%	non-detect	non-detect
CLKN	12/08/2023	01/10/2024	0 °C	102%	non-detect	non-detect
CMSN	12/08/2023	01/10/2024	0 °C	112%	non-detect	non-detect
DLMN	12/08/2023	01/10/2024	0 °C	98%	non-detect	non-detect
DtDSP	12/08/2023	01/10/2024	0 °C	94%	non-detect	non-detect
ECMCN	12/08/2023	01/10/2024	0 °C	86%	non-detect	non-detect
ECPHN	12/08/2023	01/10/2024	0 °C	105%	non-detect	non-detect
FLCN	12/08/2023	01/10/2024	0 °C	103%	non-detect	non-detect
GLPN	12/08/2023	01/10/2024	0 °C	102%	non-detect	non-detect
HdCN	12/08/2023	01/10/2024	0 °C	93%	non-detect	non-detect
HdGLY	12/08/2023	01/10/2024	0 °C	94%	non-detect	non-detect
HpLN	12/08/2023	01/10/2024	0 °C	107%	non-detect	non-detect
HtSN	12/08/2023	01/10/2024	0 °C	99%	non-detect	non-detect
LBMN	12/08/2023	01/10/2024	0 °C	103%	non-detect	non-detect
LGDN	12/08/2023	01/10/2024	0 °C	104%	non-detect	non-detect
MAEN	12/08/2023	01/10/2024	0 °C	92%	non-detect	non-detect
McRN	12/08/2023	01/10/2024	0 °C	103%	non-detect	non-detect
MDLN	12/08/2023	01/10/2024	0 °C	98%	non-detect	non-detect
MgHAP	12/08/2023	01/10/2024	0 °C	92%	non-detect	non-detect
MgICG	12/08/2023	01/10/2024	0 °C	91%	non-detect	non-detect

Samples Received **12/8/2023**

Component	Date Prepared	Date Analyzed	Arrival Temperature	Positive Control	Extraction Blank	Negative Control
MgRKN	12/08/2023	01/10/2024	0 °C	95%	non-detect	non-detect
MHBN	12/08/2023	01/10/2024	0 °C	98%	non-detect	non-detect
MLCN	12/08/2023	01/10/2024	0 °C	105%	non-detect	non-detect
MRLN	12/08/2023	01/10/2024	0 °C	100%	non-detect	non-detect
OSCN	12/08/2023	01/10/2024	0 °C	100%	non-detect	non-detect
PABN	12/08/2023	01/10/2024	0 °C	99%	non-detect	non-detect
PaTYN	12/08/2023	01/10/2024	0 °C	101%	non-detect	non-detect
PLCN	12/08/2023	01/10/2024	0 °C	103%	non-detect	non-detect
PNGLN	12/08/2023	01/10/2024	0 °C	90%	non-detect	non-detect
PNGN	12/08/2023	01/10/2024	0 °C	101%	non-detect	non-detect
PRLN	12/08/2023	01/10/2024	0 °C	100%	non-detect	non-detect
PRSMN	12/08/2023	01/10/2024	0 °C	111%	non-detect	non-detect
PSTCN	12/08/2023	01/10/2024	0 °C	115%	non-detect	non-detect
PTDN	12/08/2023	01/10/2024	0 °C	106%	non-detect	non-detect
QNSN	12/08/2023	01/10/2024	0 °C	88%	non-detect	non-detect
RHBN	12/08/2023	01/10/2024	0 °C	104%	non-detect	non-detect
THNN	12/08/2023	01/10/2024	0 °C	102%	non-detect	non-detect
TLMLN	12/08/2023	01/10/2024	0 °C	103%	non-detect	non-detect
TRDN	12/08/2023	01/10/2024	0 °C	100%	non-detect	non-detect
TYLMN	12/08/2023	01/10/2024	0 °C	103%	non-detect	non-detect
TYLRN	12/08/2023	01/10/2024	0 °C	115%	non-detect	non-detect
WTCN	12/08/2023	01/10/2024	0 °C	105%	non-detect	non-detect
XpDN	12/08/2023	01/10/2024	0 °C	97%	non-detect	non-detect