2023

Network analysis of nematodes with soil microbes on cool-season golf courses

Elisha Allen-Perkins

Daniel K. Manter

Robert Wick

Geunhwa Jung

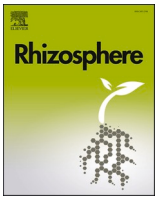
Allen-Perkins, Elisha; Manter, Daniel K.; Wick, Robert; and Jung, Geunhwa, "Network analysis of nematodes with soil microbes on cool-season golf courses" (2023). *Rhizosphere*. 45.
https://doi.org/10.1016/j.rhisph.2023.100798

Rhizosphere 28 (2023) 100798

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph



Network analysis of nematodes with soil microbes on cool-season golf courses

Elisha Allan-Perkins [a,1], Daniel K. Manter [b], Robert Wick [c], Geunhwa Jung [a,*]

[a] Stockbridge School of Agriculture, 206 Paige Laboratory, University of Massachusetts, Amherst, MA, USA 01003
[b] Soil Management and Sugar Beet Research, 2150 Cenre Avenue, Building D, Suite 100 USDA-Agricultural Research Service, Fort Collins, CO, USA 80526
[c] Stockbridge School of Agriculture, 270 Stockbridge Rd, Fernald Hall, University of Massachusetts, Amherst, MA, USA 01003

ARTICLE INFO

Keywords:
Nematode
Bacteria
Fungi
Network
Turf

ABSTRACT

Nematodes are an active part of complex soil food webs on golf courses, with some members promoting plant growth, while others are pathogenic or neutralists. The artificial, sand-based rootzone mixtures of putting greens, the most intensely managed areas of a golf course, are especially prone to nematode damage. A better understanding of the interactions of nematodes with soil microbes is key to developing improved turf management strategies. The coupling of amplicon sequencing with network analysis provides a way of better understanding which taxa may be closely associated, allowing hypothesis generation to learn more about how nematodes interact with soil microbes. We performed weighted gene correlation network analyses on bacteria, fungi, and bacteria with nematodes and fungi with nematodes collected from the soil of roughs, fairways, and putting greens of three cool-season turfgrass golf courses on Martha's Vineyard, Massachusetts. *Rhodoplanes* spp. were found in many bacterial modules, suggesting they may be a common species. Many nematodes formed positive correlations with known nematode antagonizing microbes. Among five nematode trophic groups, the carnivorous nematodes were most connected to both bacteria and fungi, suggesting these nematodes may have previously overlooked interactions with soil microbes. Consensus eigengene networks were highly preserved among management areas on each golf course for both the bacteria and fungi, showing conserved meta-modules despite management differences. The results of this work provide deeper insight into a unique, complex perennial ecosystem on golf courses that could be leveraged for future investigations on these relationships and eventually to improved turf health and disease management in the future. To our knowledge this study is the first use of network analysis to explore the relationship of the turf-associated bacterial and fungal phytobiomes with nematodes.

1. Introduction

The living soil biota play important roles in the overall ecological function of the soil and the plants that grow in it. Turfgrass microbes have been studied to learn more about communities in natural landscapes and those under intensive management. In native grasslands, the soil community provides carbon and nitrogen cycling and the removal of toxins (Dell et al., 2008, 2010; Shi et al., 2006). In managed systems, such as golf courses, the microbes are similar to those in native grasslands and provide additional benefits of degrading pesticides to prevent leaching, and promoting grass growth and health (Shi et al., 2007). Other microbes are more nefarious and cause turfgrass disease, such as the pathogenic bacteria *Xanthomonas campestris* pv. *graminis,* the pathogenic fungus, *Clarireedia jacksonii*, or herbivorous nematodes, such as *Hoplolaimus* spp. and *Longidorous* spp. Although there have been many studies using culture-based and phospholipid fatty acid (PLFA) analysis to characterize managed turf communities, there have been few studies so far that have used 16 S or 16 S and 18 S amplicon sequencing (Allan-Perkins et al., 2019; Beirn et al., 2017). Both studies analyzed the alpha and beta diversity of microbes but did not describe the interactions among organisms, including nematodes. Studies describing how microbes relate to one another are essential because microbes affect

* Corresponding author.
E-mail addresses: elisha.allanperkins@ncis.org (E. Allan-Perkins), daniel.manter@usda.gov (D.K. Manter), rlwick@umass.edu (R. Wick), jung@umass.edu (G. Jung).
[1] Present address: Independent Researcher, Brookline, NH USA 03033.

https://doi.org/10.1016/j.rhisph.2023.100798
Received 24 May 2023; Received in revised form 30 September 2023; Accepted 3 October 2023
Available online 11 October 2023

each other in positive and negative ways to maintain ecosystem functions (Shi et al., 2016).

Nematodes and microbes live in close relationships and influence one another within soil ecosystems. Many fungi and some bacteria feed on nematodes and many nematodes use microbes as a food source (LaMondia and Timper, 2016; Siddiqui and Mahmood, 1996). Chitin applications have been shown to reduce populations of certain herbivorous nematodes (Thoden et al., 2011), possibly due to increasing the population of chitinolytic microbes that degrade nematode eggs (Tian et al., 2000). Mycorrhizal fungi and herbivorous nematodes have an antagonistic relationship in which they inhibit one another (Elsen et al., 2008; LaMondia and Timper, 2016). *Bacillus* spp. suppress nematode populations through production of Cry proteins and antibiotics (Lian et al., 2007). *Pasteuria* spp. endospores adhere to nematode cuticles and eventually colonize within the nematode rendering it infertile (Chen and Dickson, 1998). In addition to the bacteria mentioned, numerous other microbe species have been studied for potential biocontrol of nematodes, including *Pseudomonas* spp., *Agrobacterium* spp., and *Streptomyces* spp. (Siddiqui and Mahmood, 1999).

Network analysis allows researchers to describe co-occurrence patterns, which can predict how connected microbes are and whether those relationships are positive or negative (Ding et al., 2015; Fuhrman, 2009; Shi et al., 2016). The network analysis groups taxa, termed nodes, that correlate with one another into clusters, termed modules (Barberán, et al., 2012; Langfelder and Horvath, 2008). It can be used to identify organisms central to modules or acting as connectors among modules that may represent keystone taxa (Dong and Horvath, 2007; Shi et al., 2016). Weighted gene correlation network analysis (WGCNA) was developed for integration in R to create either unsigned (absolute value of correlation) or signed (retaining positive versus negative correlations) networks based on adjacency (correlations) among genes or taxa (nodes) that are then assigned to modules using fuzzy membership to capture close neighbors or taxa that may group evenly with multiple modules (Langfelder and Horvath, 2008). Taxa within modules can be reduced to a best-representative sequence, referred to as the eigengene, using principle component analysis, reducing computation time for further downstream analyses and multiple testing (Langfelder and Horvath, 2007). Eigengene networks can further be compared among samples (e.g. different species, locations) using comparative WGCNA, by creating consensus eigengene networks (Langfelder and Horvath, 2007). The positive correlations of eigengenes of different modules are calculated and used to create an eigengene network consisting of meta-modules (groups of eigengenes that are highly correlated). These eigengenes networks can be compared among samples to determine which meta-modules are conserved using clustering dendrograms. The overall positive correlation of the eigengene networks, and each individual consensus eigengene module, can be compared using the preservation (D) statistic, which is calculated from correlations (or adjacencies) of the eigengene modules as described in (Langfelder and Horvath, 2007). The results of these studies provide data to help generate testable hypotheses to better understand the complex interactions among soil organisms, which might be biologically relevant in turfgrass ecosystems.

Castillo et al. (2017) used network analysis to understand the relationships among bacteria and two herbivorous nematodes, *Meloidogyne chitwoodi* and *Pratylenchus neglectus,* isolated from potato farms. They were able to identify modules associated with those two nematode species and determine the specific bacterial classes and genera correlated positively and negatively with them. Jiang et al. (2017) used network analysis to understand the relationship of bacterivorous nematodes with bacteria in different soil aggregate sizes. However, co-occurrence patterns of all five trophic nematode groups and bacteria and fungi on golf courses have not yet been studied using network analysis or by differential eigengene network analysis.

Determining the influences among microbes and nematodes is an important step to better understanding the turf soil ecosystem. Our goal was to determine how bacteria and fungi influence nematode communities. Our first hypothesis was total nematodes would be correlated negatively with modules containing nematode-inhibiting fungi and bacteria, as a result of direct predation and/or inhibition. Three golf courses that were studied in previous publications of turf nematodes (Allan-Perkins et al., 2017) and bacteria and fungi (Allan--Perkins et al., 2019) were used in these analyses to build on the understanding of how these organisms interact. Three management areas representing different management intensities (roughs as low input, fairways as intermediate, and putting greens as high input) were compared among three golf courses with different management types (conventional, organic, and hybrid course that used conventional practices with reduced inputs on fairways and roughs). The results of these studies showed the conventional and hybrid putting greens were dominated by herbivorous nematodes ($p = 0.017$ and $p = 0.001$, respectively), whereas the organic putting greens were dominated by bacterivorous nematodes ($p = 0.0146$) as compared using the generalized linear model analysis of variance (Allan-Perkins et al., 2017). We predicted a greater number of positive correlations of herbivorous nematodes with the conventional and hybrid putting green consensus eigengene modules and bacterivorous nematodes with the organic putting green. Bacterial abundance was not significantly different among management areas, however fungal abundance, diversity, and richness tended to be lower on putting greens compared to fairways and roughs for all three golf courses (Allan-Perkins et al., 2019). We hypothesized that differential eigengene networks would reveal similar networks among bacterial samples, but significantly different fungal networks on putting greens compared to fairways and roughs for all three golf courses. The results of our study will provide testable observations of how microbes and nematodes are associated with one another. Once we understand how these organisms interact, we will be able to develop integrated pest management practices that leverage these interactions to benefit turfgrass health, with potential extension to other perennial crop systems.

2. Materials and methods

2.1. Field collection

Three golf courses all located within 10 km of one another on Martha's Vineyard in Massachusetts were sampled in the Spring of 2013 and 2014 as described previously (Allan-Perkins et al., 2017, 2018, 2019). Briefly, soil samples were taken with a 2.5 cm diameter core at a depth of 10 cm on three holes (representing area of play from tee box to putting green) from three management areas (roughs, fairways, and putting greens) per course. The thatch was removed from each sample. Soil cores were taken at twelve locations on each fairway, four on each rough, and eight on each putting green for a total of 72 samples per sampling time. The three courses have been denoted as conventional, hybrid, and organic. The conventional course was managed with synthetic pesticides and fertilizers. The hybrid course also used synthetic chemicals but had reduced inputs on the fairways and roughs (only one fungicide application on the fairway in ten years) and applied a biological control bacterium, *Pseudomonas aureofaciens* TX-1 to the fairways. The organic course was managed without synthetic pesticides, herbicides, or fertilizers. All the courses had similar grass species composition (except the conventional and hybrid courses had encroachment of annual bluegrass (*Poa annua* L.) within the creeping bentgrass, *Agrostis stolonifera* L., putting greens) and cultural management practices, except for lightweight rolling on the organic putting greens. All samples were transferred back to the laboratory at the University of Massachusetts Amherst on ice.

2.2. Nematode identification and analysis

Nematode extraction and identification were performed as described previously (Allan-Perkins et al., 2017). Briefly, a portion of each soil

sample was taken for nematode identification and pooled within management area for a total of one sample per fairway, one per rough, and one per putting green for a total of 27 samples per sampling date. Nematodes were extracted in triplicate using the modified Cobb's sifting and gravitation method followed by centrifugation and sugar flotation (Neher, 1999; Neher and Campbell, 1994). They were identified to family level using the following keys (Bongers, 1988; Goodey, 1963; Mai and Lyon, 1975; Tarjan et al., 2014). They were counted under an inverted compound microscope and assigned to their appropriate trophic (feeding) group (Allan-Perkins et al., 2017; Okada et al., 2005; Yeates and Bongers, 1993). Total nematodes were calculated as average number of nematodes counted among the three replicate samples at each location per gram of soil.

2.3. Microbial community analysis

A second portion of each soil sample was used for bacterial and fungal community analysis as described previously (Allan-Perkins et al., 2019). Briefly, DNA was extracted from 0.25 g of soil using the PowerSoil DNA Extraction kit (MoBio, Carlsbad, CA). Ten microliters of extracted DNA from each soil sample were sent to the USDA ARS Laboratory in Fort Collins, CO for quantitative PCR (qPCR) and amplicon sequencing as described previously (Allan-Perkins et al., 2019). Briefly, the abundance of bacteria (16 S) and fungi (18 S) was determined for each sample using qPCR. Bacterial abundance was estimated using the V1–V3 hypervariable region of 16 S amplified by the 27 F and 388 R primers (Lane et al., 1985; Marchesi et al., 1998) and genomic DNA *Pseudomonas putida* KT2440 as a DNA concentration standard. Fungal abundance was estimated using the nu-SSU-0817 and nu-SSU-1196 primers for 18 S rDNA (Borneman and Hartin, 2000) and *Aspergillus niger* genomic DNA was used as the concentration standard. Total abundances (copies g^{-1} soil FW) for each taxon within a sample were calculated as follows:

$$16S.CPS * DF * EV / SM * P.CPS = 16S.CPS_i$$

$16S.CPS_i$ = 16 S rRNA copies per g^{-1} soil for taxon i, in sample
$16S.CPS$ = 16 S rRNA copies for soil sample
DF = dilution factor
EV = extraction volume
SV = soil mass
P. CPS = concentration of *P. putida* 16 S copies pg^{-1}

Bacterial and fungal abundance was averaged from all DNA extracts taken from the same management area and hole for a total of 27 16 S abundance and 27 18 S abundance data points.

Amplicon sequencing was performed on a Roche GS Junior + cycler on three extracts from the fairway, three from the rough, and three from the putting green for a total of 9 samples per hole, 27 samples per course, and 81 samples per sampling date. Samples were processed through the default analysis pipeline in myPhyloDB v.1.2.0 (Manter et al., 2016), as previously described (Allan-Perkins et al., 2019). Briefly, samples were rarefied using Laplace smoothing (smoothing (λ = 0.01)) with sub-sampling with replacement at 100 iterations and any samples with less than 500 sequence reads for bacteria and 1000 sequence reads for fungi were removed from the dataset. The remaining 133 bacterial samples were rarefied to 1549 sequence reads and the 155 fungal samples were rarefied to 2930 sequence reads. All sequence reads were classified to the Green Genes reference database v. 13_5_99 (DeSantis et al., 2006) for bacteria or SILVA database v. 119 (Quast et al., 2013) for fungi and assigned to the closest operational taxonomic unit (OTU) at 99% sequence similarity using the knn nearest neighbor function within myPhyloDB. Reads mapping to chloroplasts, mitochondria, and unassigned/unmapped reads were removed before all down-stream analyses. The data set is available through the National Center for Biotechnology Information Sequence Read Archive under submission number SUB4681277, BioProject PRJNA511025.

2.4. Network analysis

Co-occurrence and network analysis was performed using total abundance of bacterial OTUs (as quantified by 16 S abundance) and total abundance of fungal OTUs (as quantified by 18 S abundance) to total nematodes and also total nematodes within each trophic group using the weighted gene correlation network analysis (WGCNA) package within myPhyloDB (Langfelder and Horvath, 2008; Manter et al., 2016). Separate networks were created for fungi and bacteria. Networks were generated with all golf course samples combined with nematodes to find relationships that were conserved among all areas and courses. Signed networks were constructed using default settings within myPhyloDB, specifically Pearson's correlation with gene reassignment at a threshold of 1 × 10-6, minCoreKME of 0.5 at a minimal size of 2 and a minKMEtoStay of 0.3. KME is the measure of eigengene-based connectivity, which uses fuzzy measurements to estimate how correlated a gene is to the eigengene for each module (Langfelder and Horvath, 2008). Trees were built with a deepSplit of 2, detection cut height of 0.995, a merge cut height of 0.15, and a minimal module size of 6. The grey and dark grey modules served as catchall groups within WGCNA for taxa that do not fit well within other modules, although these two module results are reported, specific interactions are not as they may be erroneous. Eigengenes were calculated for each module as a vector of the first principle component of the expression profile within the myPhyloDB default parameters (Langfelder and Horvath, 2008; Manter et al., 2016).

Comparisons among management areas (roughs, fairways, and putting greens) within each golf course type (conventional, hybrid, and organic) were performed with R using differential analysis to generate and compare consensus eigengenes among the management areas within each course (Langfelder and Horvath, 2007; R Core Team, 2020). Golf courses could not directly be compared because we could not replicate effect of management, since at time of study there was only one organic golf course within the United States. Networks between samples were compared by first creating consensus modules (those shared among the networks for each sample) and the correlation quantified using the preservation (D) value within the WGCNA package (Langfelder and Horvath, 2007). Module colors within a comparison (i.e., three management areas within a golf course for one kingdom) represent the same consensus eigengene module, but the same module colors among comparisons (e.g. the yellow consensus module for the bacterial analysis on the conventional golf course compared to the fungal analysis on the conventional golf course or bacterial analysis on the hybrid golf course) do not represent the same census eigengene modules.

3. Results

3.1. Bacteria networks

The bacteria network containing all samples formed 116 modules (Supplementary Table 1). The Acidobacteria, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteira, Solibacteres, and Thermoleophilia were found in the most bacteria modules (Supplementary Table 1). The genus, *Rhodoplanes*, was found in 57 modules representing the most frequently found genus of classified bacteria in the network (Supplementary Table 1).

3.2. Bacteria differential networks

Among management areas within the conventional course, bacterial networks were highly similar with preservation (D) values ranging from 0.80 to 0.82 (Fig. 1c, d, and g). All three meta-modules for the different management areas clustered with the yellow modules closely with the herbivores, but only the rough consensus network had the green meta-

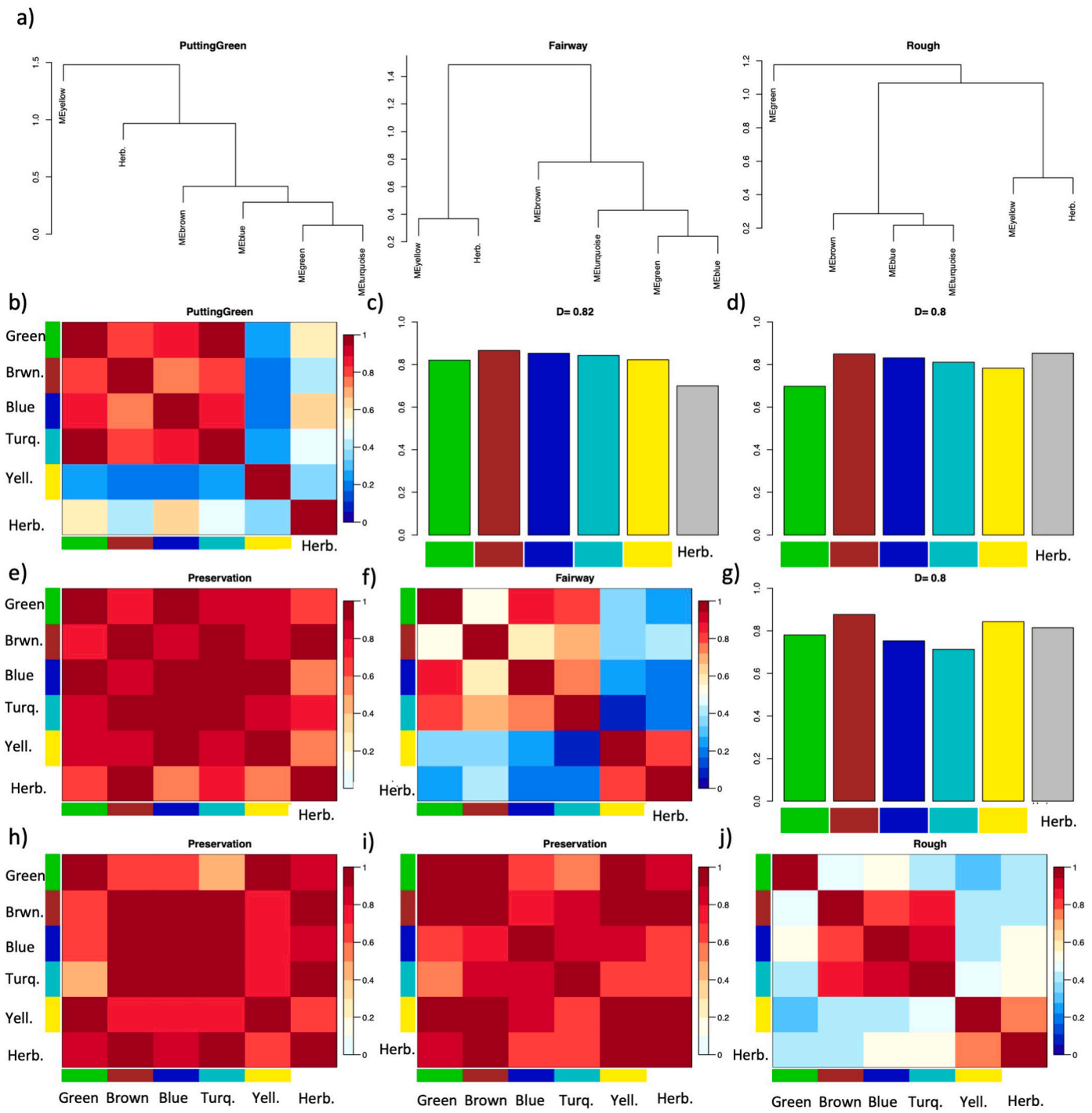


Fig. 1. Consensus bacterial eigengene network comparisons among management areas on the Conventional golf course a) Clustering dendrograms of consensus eigengene modules for each management area with herbivorous (herb.) nematode counts showing meta-modules. Heatmaps of eigengene adjacencies (measurement of how similar eigengenes are, using a transformed correlation coefficient (Langfelder and Horvath, 2008) showing strength of correlation among each module and the herbivore (herb.) nematodes with darker blue indicating lower adjacency and darker red higher adjacency for b) putting greens, f) fairways, and j) roughs. Preservation measures (D values) showing the strength of the positive correlations (darker red) for each consensus eigengene module between the c) putting green and fairway samples, d) the putting green and rough samples, and g) fairway and rough samples, the D value displayed above each plot is the mean preservation value among the consensus modules. Heatmap of preservation (D) values among the consensus modules of the e) putting green and fairway samples, h) putting green and rough samples, and i) putting green and rough samples with darker red indicating a stronger preservation value.

module separating away from the brown, blue and turquoise meta-modules (Fig. 1a). The hybrid course had similar high D values for the putting greens compared to roughs and the fairways to the roughs (0.80 in Fig. 2d and 0.85 in Fig. 2g, respectively), but only 0.66 for putting greens compared to fairways (Fig. 2c). The fairway and rough consensus network for the hybrid course had the herbivores clustering with the yellow meta-module and all other modules clustering together, whereas the putting green showed the herbivores branching with the brown meta-module (Fig. 2a). The organic course had lower network similarity among management areas than the other two courses. The putting greens and roughs were more similar at D = 0.77 (Fig. 3d) than the putting greens to fairways and fairways to roughs at D = 0.60 and 0.64,

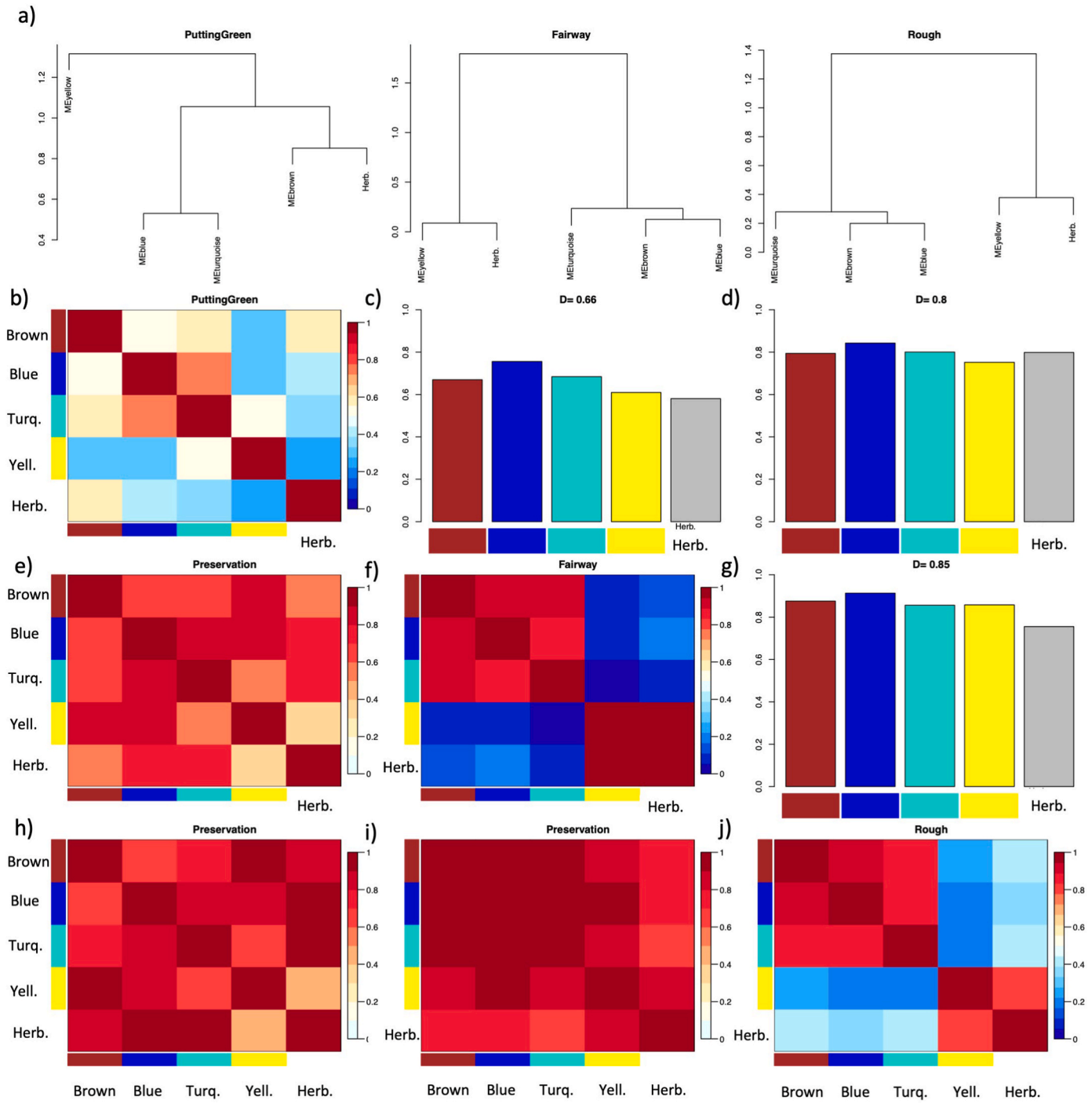


Fig. 2. Consensus bacterial eigengene network comparisons among management areas on the Hybrid golf course a) Clustering dendrograms of consensus eigengene modules for each management area with herbivorous (herb.) nematode counts showing meta-modules. Heatmaps of eigengene adjacencies (measurement of how similar eigengenes are, using a transformed correlation coefficient (Langfelder and Horvath, 2008) showing strength of correlation among each module and the herbivore (herb.) nematodes with darker blue indicating lower adjacency and darker red higher adjacency for b) putting greens, f) fairways, and j) roughs. Preservation measures (D values) showing the strength of the positive correlations (darker red) for each consensus eigengene module between the c) putting green and fairway samples, d) the putting green and rough samples, and g) fairway and rough samples, the D value displayed above each plot is the mean preservation value among the consensus modules. Heatmap of preservation (D) values among the consensus modules of the e) putting green and fairway samples, h) putting green and rough samples, and i) putting green and rough samples with darker red indicating a stronger preservation value.

respectively (Fig. 3c and g). The management area consensus networks were most different on the organic course with the herbivores clustering with the brown meta-module separating from the other meta-modules on the putting greens, where it clustered with the herbivores on the fairway, and was clustered away from the herbivores and with the other meta-modules on the roughs (Fig. 3a).

3.3. *Fungi networks*

The fungi grouped into 71 modules (Supplementary Table 2). *Aspergillus* spp., *Penicillium* spp., *Phoma* spp., *Cryptococcus* spp., and *Glomus* spp. were found in more than ten of the modules, representing the most common genera found in the network (Supplementary

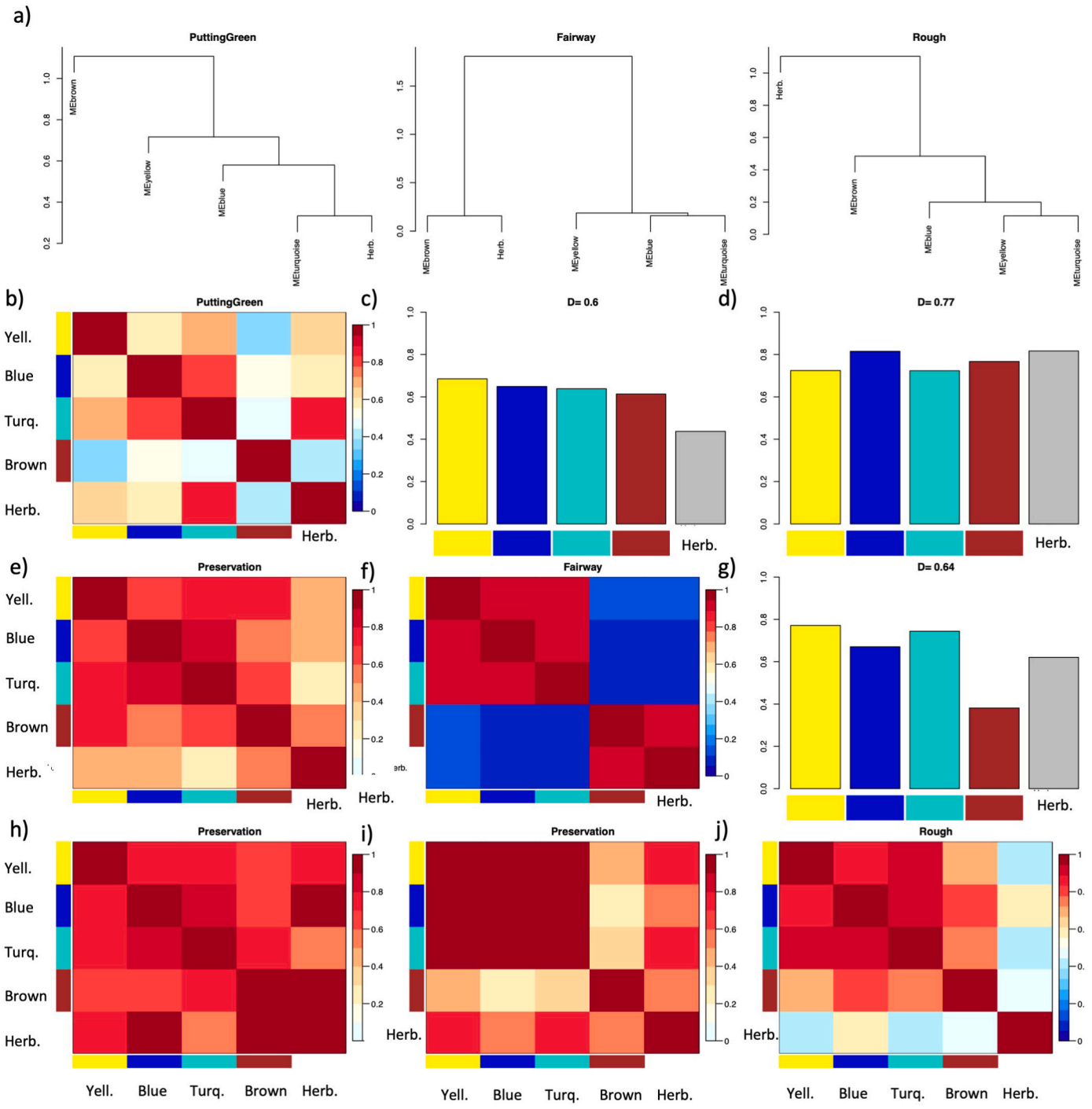


Fig. 3. Consensus bacterial eigengene network comparisons among management areas on the Organic golf course a) Clustering dendrograms of consensus eigengene modules for each management area with herbivorous (herb.) nematode counts showing meta-modules. Heatmaps of eigengene adjacencies (measurement of how similar eigengenes are, using a transformed correlation coefficient (Langfelder and Horvath, 2008) showing strength of correlation among each module and the herbivore (herb.) nematodes with darker blue indicating lower adjacency and darker red higher adjacency for b) putting greens, f) fairways, and j) roughs. Preservation measures (D values) showing the strength of the positive correlations (darker red) for each consensus eigengene module between the c) putting green and fairway samples, d) the putting green and rough samples, and g) fairway and rough samples, the D value displayed above each plot is the mean preservation value among the consensus modules. Heatmap of preservation (D) values among the consensus modules of the e) putting green and fairway samples, h) putting green and rough samples, and i) putting green and rough samples with darker red indicating a stronger preservation value.

Table 2).

3.4. *Fungi differential networks*

Among management areas within courses, fungal networks were highly similar for all three golf courses (Figs. 4–6). Putting greens to roughs and fairways to roughs had high preservation (D values) ranging from 0.79 to 0.85 on all three courses (Fig. 4d, g, 5d, 5g, 6d, and 6g) and slightly lower values among putting greens to fairways with D values of 0.75 for the conventional course (Figs. 4c), 0.77 for the hybrid course (Figs. 5c), and 0.66 for the organic course (Fig. 6c). Meta-modules comprised of the consensus eigengenes varied by management area

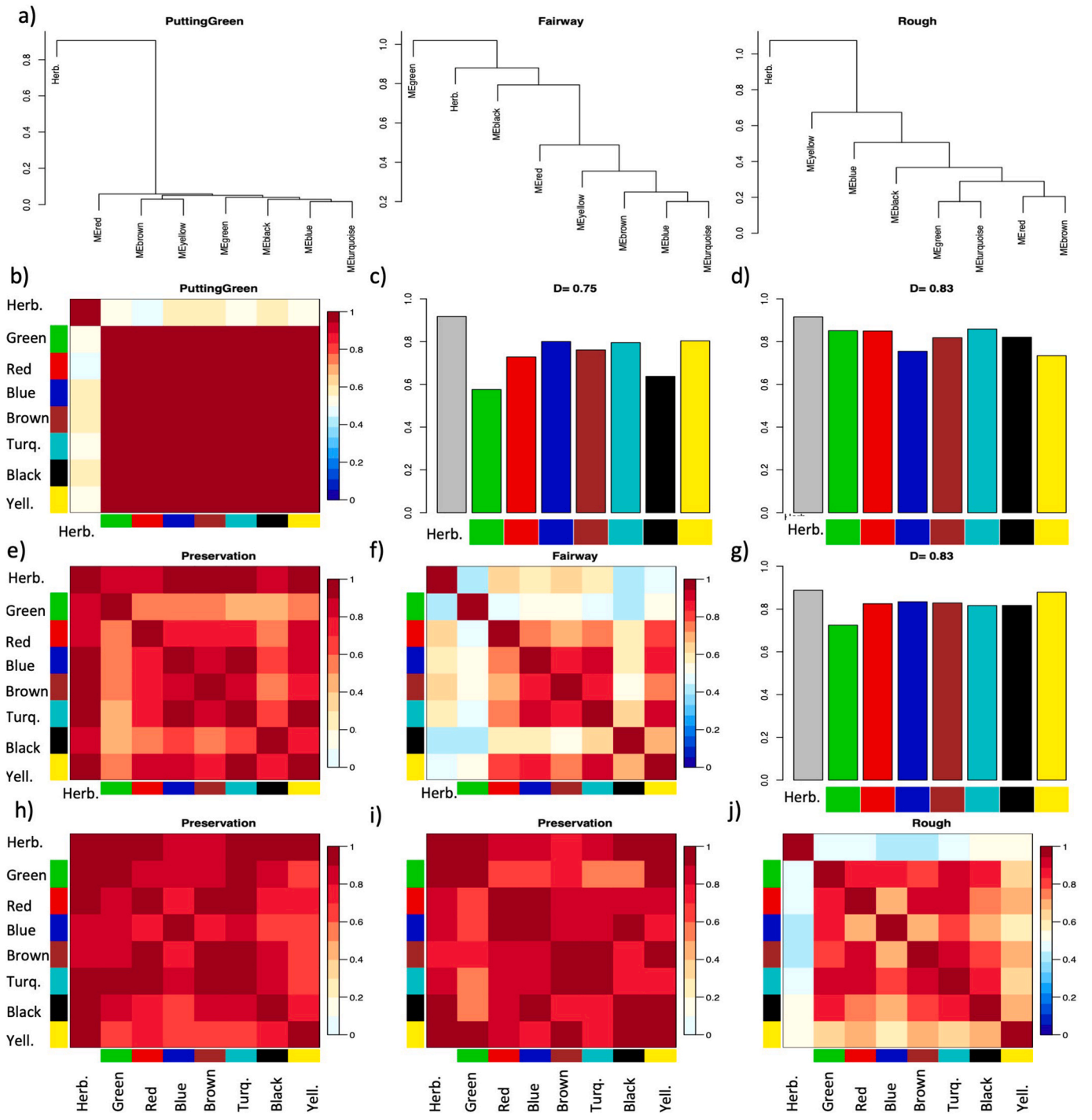


Fig. 4. Consensus fungal eigengene network comparisons among management areas on the Conventional golf course a) Clustering dendrograms of consensus eigengene modules for each management area with herbivorous (herb.) nematode counts showing meta-modules. Heatmaps of eigengene adjacencies (measurement of how similar eigengenes are, using a transformed correlation coefficient (Langfelder and Horvath, 2008) showing strength of correlation among each module and the herbivore (herb.) nematodes with darker blue indicating lower adjacency and darker red higher adjacency for b) putting greens, f) fairways, and j) roughs. Preservation measures (D values) showing the strength of the positive correlations (darker red) for each consensus eigengene module between the c) putting green and fairway samples, d) the putting green and rough samples, and g) fairway and rough samples, the D value displayed above each plot is the mean preservation value among the consensus modules. Heatmap of preservation (D) values among the consensus modules of the e) putting green and fairway samples, h) putting green and rough samples, and i) putting green and rough samples with darker red indicating a stronger preservation value.

within each course. On both the conventional (Fig. 4a) and the organic course (Fig. 6a), putting greens and roughs had one meta-module separated from the herbivorous nematodes, whereas the fairways had two meta-modules: one containing only the green eigengene module and the second containing the herbivorous nematodes and all other eigengene modules (Fig. 6a). On the hybrid course, the putting greens formed two meta-modules, one consisting solely of the pink eigengene module, the fairways consisted of one meta-module, and the roughs were split into three meta-modules (Fig. 6a).

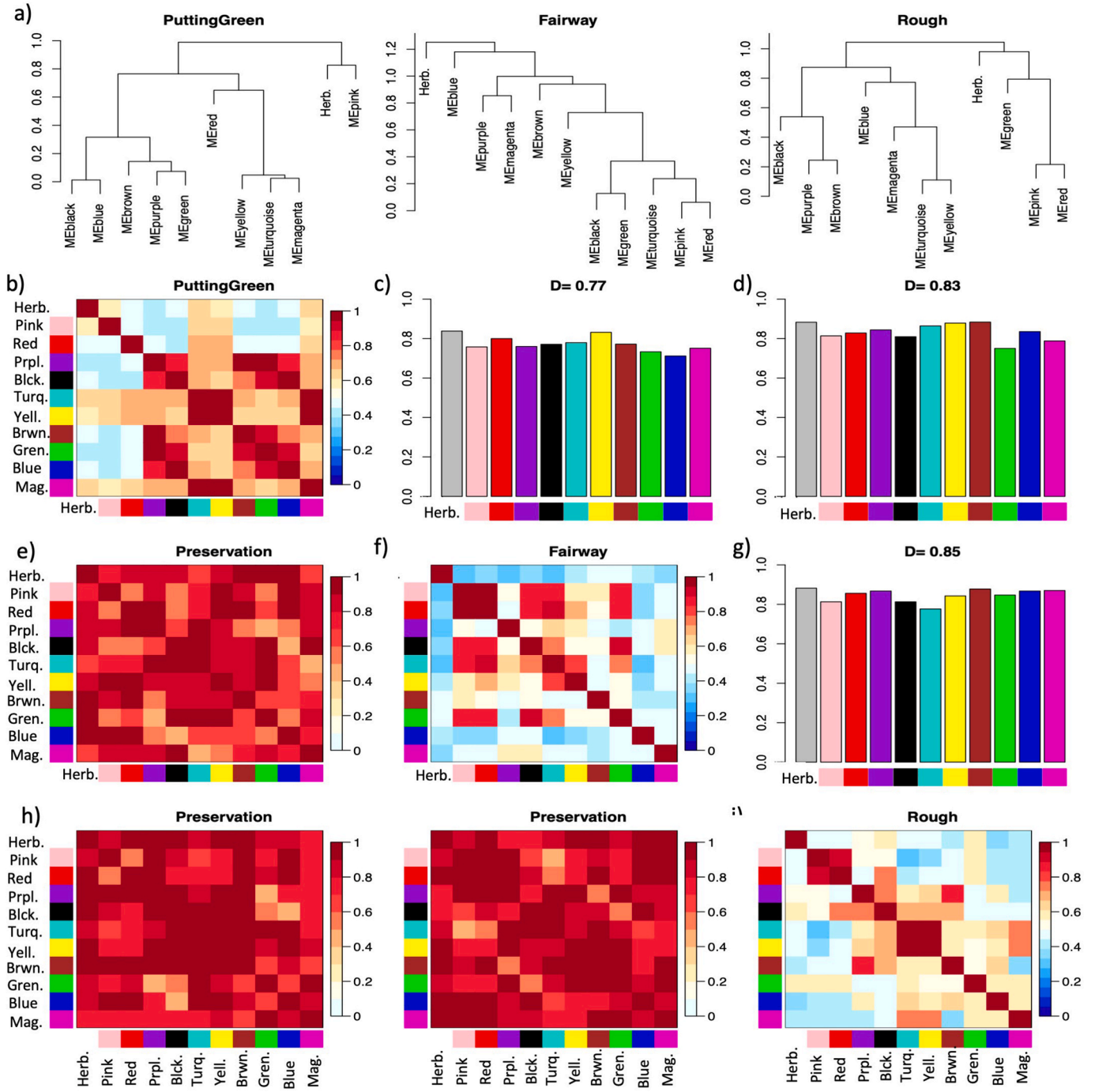


Fig. 5. Consensus fungal eigengene network comparisons among management areas on the Hybrid golf course a) Clustering dendrograms of consensus eigengene modules for each management area with herbivorous (herb.) nematode counts showing meta-modules. Heatmaps of eigengene adjacencies (measurement of how similar eigengenes are, using a transformed correlation coefficient (Langfelder and Horvath, 2008) showing strength of correlation among each module and the herbivore (herb.) nematodes with darker blue indicating lower adjacency and darker red higher adjacency for b) putting greens, f) fairways, and j) roughs. Preservation measures (D values) showing the strength of the positive correlations (darker red) for each consensus eigengene module between the c) putting green and fairway samples, d) the putting green and rough samples, and g) fairway and rough samples, the D value displayed above each plot is the mean preservation value among the consensus modules. Heatmap of preservation (D) values among the consensus modules of the e) putting green and fairway samples, h) putting green and rough samples, and i) putting green and rough samples with darker red indicating a stronger preservation value.

3.5. Bacteria and nematode networks

Thirty-four of the modules correlated significantly with nematode trophic groups and ten of those correlated with total nematodes (Table 1). Most of the correlations were positive. The only negative correlation was total nematodes with the lemonchiffon1 module, which contained no known nematode-suppressing bacteria (Table 1). Many of the modules with positive correlations to nematodes did include bacteria previously reported to reduce total nematode or specifically herbivorous nematode populations (Siddiqui and Mahmood, 1996) (Table 1). The bacterivorous nematodes were associated positively with six of the modules (Table 1). The carnivorous nematodes were correlated positively with 13 bacterial modules (Table 1).

On all three golf courses, the bacterial networks had similar

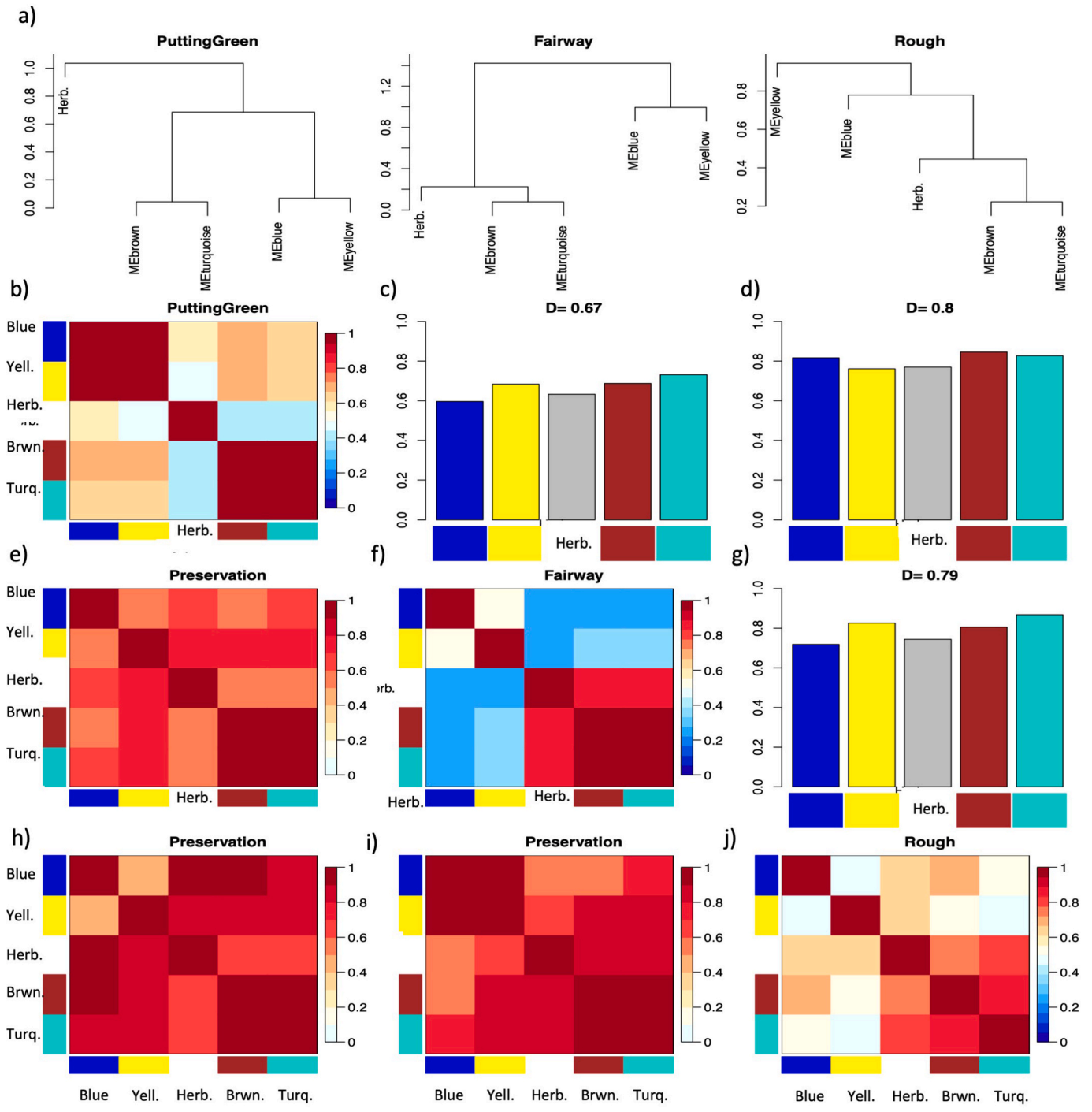


Fig. 6. Consensus fungal eigengene network comparisons among management areas on the Organic golf course a) Clustering dendrograms of consensus eigengene modules for each management area with herbivorous (herb.) nematode counts showing meta-modules. Heatmaps of eigengene adjacencies (measurement of how similar eigengenes are, using a transformed correlation coefficient (Langfelder and Horvath, 2008) showing strength of correlation among each module and the herbivore (herb.) nematodes with darker blue indicating lower adjacency and darker red higher adjacency for b) putting greens, f) fairways, and j) roughs. Preservation measures (D values) showing the strength of the positive correlations (darker red) for each consensus eigengene module between the c) putting green and fairway samples, d) the putting green and rough samples, and g) fairway and rough samples, the D value displayed above each plot is the mean preservation value among the consensus modules. Heatmap of preservation (D) values among the consensus modules of the e) putting green and fairway samples, h) putting green and rough samples, and i) putting green and rough samples with darker red indicating a stronger preservation value.

correlation trends with nematodes on the fairways (Supplementary Figs. 2, 5, & 8). The bacterivores and herbivores showed the same positive or negative correlations with each meta-module. The other management areas on each of the golf courses did not show the same trends. The bacterivorous nematodes on the conventional putting green had weak positive correlations with the meta-modules, whereas the herbivores had strong negative correlations to all but the yellow meta-module (Supplementary Fig. 1). The herbivores were positively correlated at 91% with meta-module yellow whereas the bacterivores had a neutral response (−3.9%). On the roughs, both bacterivores and herbivores showed similar trends, being correlated strongly and positively to meta-module yellow, and correlated weakly with the rest of the meta-

Table 1
Bacterial modules significantly correlated with nematode trophic groups.

Module	Total Nematodes[a]	Herbivores	Bacterivores	Fungivores	Omnivores	Carnivores	Bacterial Taxa [b, c]
Aquamarine4				0.197 (0.0208)			Acidobacteria, Chloracidobacteria, Polyangiaceae, *SBR1093,* Thermoleophilia, Thermomicrobia
Cyan	0.178 (0.037)	0.283 (0.0008)					Acidobacteria, Acidobacteriaceae, Actinobacteria, Betaproteobacteria, *Burkholderia, Candidatus_Koribacter, Candidatus_Solibacter,* Caulobacteraceae, Deltaproteobacteria, *Devosia,* Koribacteraceae, Ktedonobacteraceae, Methylocystaceae, *Pseudonocardia, Rhodoplanes,* Rhodospirillaceae, Sinobacteraceae, Solibacteres, Streptomycetaceae, Thermogemmatisporaceae, *WPS-2*
DarkGoldenrod3						0.264 (0.0017)	Acetobacteraceae, Acidimicrobiales, Acidobacteriaceae, Alphaproteobacteria, Betaproteobacteria, *Bradyrhizobium,* Gaiellaceae, *Methylibium, Mycobacterium,* Myxococcaceae, Solibacteres, Thermogemmatisporaceae
DarkGreen			0.256 (0.0025)				Alphaproteobacteria, *Anaerolineae,* Betaproteobacteria, *Bradyrhizobium, Candidatus_Solibacter,* Chloroflexi, *Devosia,* Gammaproteobacteria, *Hyphomicrobium,* Hyphomonadaceae, Koribacteraceae, Kouleothrixaceae, *Labrys, Nitrospira,* Nitrospirales, Nocardioidaceae, Piscirickettsiaceae, *Pleomorphomonas,* Rhizobiaceae, Rhodospirillaceae, Sinobacteraceae, Solibacteres, *Steroidobacter,* Syntrophobacteraceae
DarkGrey			0.204 (0.0164)				Acidimicrobiales, Acidobacteria, *Afifella,* Alphaproteobacteria, *Anaerolineae,* Betaproteobacteria, *Bradyrhizobium,* Chloracidobacteria, Chloroflexi, Conexibacteraceae, *Devosia,* Gemmatimonadetes, Hyphomicrobiaceae, Hyphomonadaceae, *Kaistobacter,* Koribacteraceae, *OD1, Pedomicrobium, Phenylobacterium,* ***Pseudomonas****, Ramlibacter, Rhodoplanes,* Rhodospirillaceae, Saprospiraceae, Sphingomonadaceae, *Thiobacillus*
DarkMagenta					0.187 (0.0289)		Acidobacteria, ***Agrobacterium,*** Alphaproteobacteria, Betaproteobacteria, *Candidatus_Solibacter,* Caulobacteraceae, Chloroflexi, Deltaproteobacteria, *Flavobacterium,* Gaiellaceae, Gemmatimonadetes, Koribacteraceae, Methylocystaceae, Phycisphaerae, *Rhodoplanes,* Rhodospirillaceae, Sinobacteraceae, *WPS-2*
DarkOliveGreen1					0.388 (<0.0001)		Acetobacteraceae, Acidimicrobiia, Actinobacteria, *Candidatus_Solibacter, Chloracidobacterium,* Pelagibacteraceae, *Rhizobium*
DarkOlive Green2				0.213 (0.0123)			*Afipia,* Caulobacteraceae, Gaiellaceae, Koribacteraceae, Ktedonobacteraceae, *Rhizobium, Rhodoplanes, Thermogemmatispora,* Xanthomonadaceae
DarkOrange	0.198 (0.0201)					0.228 (0.0073)	Acidobacteria, Acidobacteriaceae, Actinobacteria, *AD3,* Alphaproteobacteria, Betaproteobacteria, *Conexibacter,* Conexibacteraceae, *Fibrobacter,* Koribacteraceae, *Mycobacterium, Rhodoplanes,* Sinobacteraceae, Streptomycetaceae, *Thermogemmatispora,* Thermogemmatisporaceae, *WPS-2*
Green2				0.241 (0.0044)			Acidobacteria, Acidobacteriaceae, Actinobacteria, Sinobacteraceae
Greenyellow			0.293 (0.0005)				Acetobacteraceae, Acidimicrobiales, Acidimicrobiia, Acidobacteria, Acidobacteriaceae, Actinobacteria, Alphaproteobacteria, *Anaerolineae, Asteroleplasma,* Betaproteobacteria, *Candidatus_Koribacter, Candidatus_Solibacter, Catellatospora,* Chitinophagaceae, Deltaproteobacteria, Gaiellaceae, Gemmatimonadetes, Haliangiaceae, Hyphomicrobiaceae, Intrasporangiaceae, Koribacteraceae, *Nitrospira, OD1, OP11, Paenibacillus, Pedomicrobium,* Phycisphaerae, *Pilimelia,* Piscirickettsiaceae, *Rhodoplanes,* Rhodospirillaceae, *SBR1093,* Solibacteres, Thermoleophilia, Thermomicrobia, *TM6, WS4*
Grey			−0.211 (0.013)				Acidimicrobiales, Acidobacteria, Actinobacteria, *AD3,* ***Agrobacterium,*** Alphaproteobacteria, *Aquicella,* Betaproteobacteria, *Bradyrhizobium, Burkholderia,* Chloroflexi, Chthonomonadaceae, Deltaproteobacteria, Frankiaceae, Gaiellaceae, Gammaproteobacteria, *Geobacter,* Holophagaceae, Hyphomicrobiaceae, *Hyphomicrobium, Janthinobacterium,* Koribacteraceae, *Kouleothrix,* Microbacteriaceae, *Mycobacterium, Pedomicrobium, Phenylobacterium,* Piscirickettsiaceae, *Pseudonocardia, Rhodoplanes,* Rhodospirillaceae, Sinobacteraceae, *Steroidobacter,* ***Streptomyces,*** Thermogemmatisporaceae, *TM7, WPS-2,* Xanthomonadaceae

(*continued on next page*)

Table 1 (*continued*)

Module	Total Nematodes[a]	Herbivores	Bacterivores	Fungivores	Omnivores	Carnivores	Bacterial Taxa [b, c]
Grey60	0.263 (0.0018)	0.199 (0.0198)			0.276 (0.0011)	0.187 (0.0287)	Acidimicrobiales, Acidimicrobiia, Acidobacteria, Acidobacteriaceae, **Alcaligenaceae**, Betaproteobacteria, Bradyrhizobiaceae, *Bradyrhizobium*, *Candidatus_Solibacter*, *Chloracidobacterium*, Chloroflexi, Chthonomonadetes, Cytophagaceae, Deltaproteobacteria, Gemmatimonadetes, Hyphomicrobiaceae, Koribacteraceae, Kouleothrixaceae, Methylocystaceae, *Mycobacterium*, *Nitrospira*, *Rhodoplanes*, Rhodospirillaceae, *Sphingomonas*, *TM7*, Xanthomonadacea
Khaki1					0.387 (<0.0001)		Acidobacteria, *Burkholderia*, *Chloracidobacterium*, Hyphomicrobiaceae, ***Pseudomonas***
LemonChiffon1	−0.178 (0.0368)						Acidobacteria, *Burkholderia*, Chloracidobacteria, Hyphomicrobiaceae, ***Pseudomonas***, Acidobacteria, Acidobacteriaceae, Betaproteobacteria, *Candidatus_Koribacter*, Koribacteraceae, *Rhodoplanes*, Rhodospirillaceae, Solibacteres
LemonChiffon4						0.293 (0.0005)	Acidimicrobiales, Acidimicrobiia, Acidobacteria, Betaproteobacteria, *Candidatus_Solibacter*, Chthonomonadaceae, *Devosia*, Koribacteraceae, Methylocystaceae, Planctomycetes, *Pseudonocardia*, *Steroidobacter*, Thermoleophilia
LightCoral						0.18 (0.0355)	Acidobacteria, Actinobacteria, Koribacteraceae, Rhodospirillaceae, Solibacteres, Thermoleophilia
LightGreen						0.292 (0.0005)	Acidimicrobiales, Actinobacteria, *Anaerolineae*, *Aquicella*, Armatimonadia, *Azospirillum*, Betaproteobacteria, Bradyrhizobiaceae, *Bradyrhizobium*, *Burkholderia*, *Candidatus_Solibacter*, Caulobacteraceae, Chloroflexi, *Devosia*, Gemmatimonadetes, Koribacteraceae, Ktedonobacteraceae, Nocardioidaceae, *Rhodoplanes*, Rhodospirillaceae, Thermoleophilia, *TM7*, Xanthomonadaceae
LightSteelBlue1					0.383 (<0.0001)		Acetobacteraceae, Armatimonadaceae, Betaproteobacteria, *Hyphomicrobium*, Koribacteraceae, Ktedonobacteria, Methylocystaceae, Phycisphaerae
Magenta3				0.263 (0.0018)			Acidobacteria, Anaerolineae, Betaproteobacteria, Gemmatimonadetes, *Geobacter*, *GN02*, *Ktedonobacteria*, Sinobacteraceae
Orchid2	0.177 (0.038)					0.183 (0.0322)	Acidobacteria, Alphaproteobacteria, Bradyrhizobiaceae, *Candidatus_Solibacter*, Caulobacteraceae, Chloracidobacteria
PaleTurquoise	0.19 (0.026)	0.309 (0.0002)					Acidobacteria, Actinobacteria, Alphaproteobacteria, *Candidatus_Koribacter*, *Candidatus_Solibacter*, Frankiaceae, Gaiellaceae, Gemmatimonadetes, Hyphomicrobiaceae, *Mycobacterium*, *Pedomicrobium*, *Rhodoplanes*, Rhodospirillaceae, Sinobacteraceae, Thermogemmatisporaceae, Thermoleophilia
Papayawhip				0.213 (0.0123)			Acidobacteria, Alphaproteobacteria, Anaerolineae, Chloroflexi, Deltaproteobacteria, Gaiellaceae, Gemmatimonadetes, *GN04*, Hyphomicrobiaceae, Rhodospirillaceae, Spirochaetaceae, Syntrophobacteraceae
Purple						0.172 (0.0441)	Acidimicrobiia, Acidobacteria, Acidobacteriaceae, Actinobacteria, Alphaproteobacteria, *Amycolatopsis*, *Asteroleplasma*, Betaproteobacteria, *Burkholderia*, *Candidatus_Koribacter*, *Candidatus_Solibacter*, Chitinophagaceae, Chloroflexi, *Conexibacter*, Conexibacteraceae, *Dechloromonas*, Deltaproteobacteria, *Flavobacterium*, Gaiellaceae, Koribacteraceae, *Limnohabitans*, *Methylibium*, Methylocystaceae, *Mycobacterium*, *Rhodanobacter*, *Rhodoplanes*, Rhodospirillaceae, Sinobacteraceae, Solibacteres, *Solirubrobacter*, ***Streptomyces***, Thermoleophilia, Xanthomonadaceae
RosyBrown1	0.182 (0.033)					0.189 (0.0267)	Acidimicrobiales, Acidimicrobiia, Acidobacteria, Betaproteobacteria, *Candidatus_Koribacter*, *Candidatus_Solibacter*, Caulobacteraceae, Gemmatimonadetes, Haliangiaceae
SeaGreen4	0.198 (0.0202)	0.284 (0.0007)					Anaerolineae, *Bradyrhizobium*, *Candidatus_Koribacter*, *Candidatus_Solibacter*, Chloroflexi, Koribacteraceae, *OP11*, *Rhodoplanes*, Rhodospirillaceae, Thermoleophilia, *Thiobacillus*
Snow2			0.287 (0.0006)				Alphaproteobacteria, Fimbriimonadaceae, Gaiellaceae, *GN02*, Hyphomicrobiaceae, *Kaistobacter*, *Kitasatospora*, Microbacteriaceae, *Plesiocystis*, Solibacteres, Syntrophobacteraceae, *TM7*
Snow3	0.23 (0.0066)	0.178 (0.0374)			0.286 (0.0007)	0.194 (0.0228)	Acidimicrobiia, Acidobacteria, Acidobacteriaceae, *AD3*, **Alcaligenaceae**, *Candidatus_Solibacter*, Chloroflexi, Chthoniobacteraceae, Koribacteraceae, *Mycobacterium*, *Rhodoplanes*, Rhodospirillaceae, Sinobacteraceae
Steelblue	0.261 (0.0020)	0.198 (0.0203)			0.267 (0.0016)	0.192 (0.0244)	Acetobacteraceae, Acidimicrobiales, Acidobacteria, *AD3*, Alphaproteobacteria, Betaproteobacteria, Caulobacteraceae, *Kaistobacter*, Koribacteraceae, Ktedonobacteraceae,

(*continued on next page*)

Table 1 (*continued*)

Module	Total Nematodes[a]	Herbivores	Bacterivores	Fungivores	Omnivores	Carnivores	Bacterial Taxa [b, c]
							Ktedonobacteria, *Mycobacterium,* Nitrospirales, *OD1, Rhodoplanes,* Rhodospirillaceae, Sinobacteraceae, *Sphingomonas,* Thermogemmatisporaceae
Turquoise1			0.315 (0.0002)				Actinobacteria, Alphaproteobacteria, Anaerolineae, Deltaproteobacteria, Gammaproteobacteria, Gemmatimonadetes, *GN02,* Hyphomicrobiaceae, Hyphomonadaceae, *Nitrospira,* Rhodospirillaceae, Thermoleophilia, *TM6*
Turquoise2		0.193 (0.0233)				0.208 (0.0145)	Acidobacteriaceae, *Candidatus_Solibacter,* Gemmatimonadetes, Holophagaceae, Koribacteraceae, *Mycobacterium, Rhodanobacter, Rhodoplanes,* ***Streptomyces***
Violet				0.251 (0.003)			Acidimicrobiales, Acidobacteria, Alphaproteobacteria, Anaerolineae, *Candidatus_Solibacter,* Chloracidobacteria, Chthonomonadaceae, Deltaproteobacteria, Gaiellaceae, Gemmatimonadetes, Koribacteraceae, Planctomycetes, *Rhodoplanes,* Solibacteres, Thermoleophilia, *WS3*
VioletRed4		0.195 (0.0224)				0.209 (0.0141)	Actinobacteria, Alphaproteobacteria, Chloroflexi, Cyanobacteria, *Edaphobacter,* Gaiellaceae, Koribacteraceae, *Rhodoplanes*, Sinobacteraceae, Thermoleophilia, Thermomicrobia
Yellow			0.305 (0.0003)				Acidimicrobia, Acidobacteria, *Alicyclobacillus,* Alphaproteobacteria, *Anaerolineae,* Betaproteobacteria, Bradyrhizobiaceae, *Candidatus_Koribacter, Candidatus_Solibacter, Chloracidobacteria, Chlorobi,* Chloroflexi, Chthoniobacteraceae, Comamonadaceae, Deltaproteobacteria, ***Desulfovibrio,*** Desulfuromonadaceae, Gaiellaceae, *Gallionella,* Gemmatimonadetes, *GN02,* Hyphomicrobiaceae, *Hyphomicrobium,* Hyphomonadaceae, Kouleothrixaceae, Ktedonobacteria, Nitrospiraceae, Nitrospirales, Nocardioidaceae, *OD1, Oxobacter, Pedomicrobium, Phyllobacterium, Rhodoplanes,* Rhodospirillaceae, Saprospiraceae, *SBR1093,* Sinobacteraceae, Sphingomonadaceae, Syntrophobacteraceae

[a] Correlation coefficient, expressed as the eigengene significance, and in parenthesis the p value.
[b] Taxa presented at genus level or, if unclassified, at lowest taxonomic resolution possible.
[c] Genus with reported antagonism against nematodes shown with bold (Siddiqui and Mahmood, 1996).

modules (Supplementary Fig. 3).

On the hybrid course putting greens, bacterivore and herbivorous nematodes were correlated positively with two meta-modules (only herbivores were correlated strongly at 64% with meta-module yellow), correlated weakly negatively with one meta-module, and bacterivores were neutral while herbivores correlated weakly negatively with meta-module brown (Supplementary Fig. 4). On the roughs, the bacterivores and herbivores showed the same correlations to the different meta-modules, being strongly positively correlated with meta-module yellow and negatively correlated with all other modules (Supplementary Fig. 6). On the organic course, the bacterivore and herbivore had a similar weak positive response to two meta-modules (Supplementary Fig. 7). The bacterivores had strong correlations with meta-module brown (0.83%) and the catch-all meta-module grey (−82%), whereas the herbivores had a weak positive correlation to meta-module brown and a weak negative correlation to the grey meta-module. On the roughs, the bacterivore and herbivore showed an opposite relationship for all meta-modules, with bacterivores having positive correlations with all modules and herbivores negative correlations, except for the grey meta-module which showed the reverse trend (Supplementary Fig. 9).

3.6. Fungi and nematode networks

There were nineteen fungal modules that correlated significantly with nematodes (Table 2). As with the bacterial modules, most of the correlations were positive. The herbivores were correlated negatively with the steelblue module, which contained *Arthrobotrys* spp., *Colletotrichum* spp., *Dactylella* spp., *Glomerella* spp., *Monacrosporium* spp., *Penicillium* spp., and *Phoma* spp. all of which contain species known to inhibit nematodes (Siddiqui and Mahmood, 1996). The bacterivores were correlated negatively with two modules: the darkred and mistyrose. Members of the Glomeraceae and *Aspergillus* spp. were found in only two modules. Fungivorous nematodes were correlated positively with the gold and tomato1 modules, but they did not share any fungal genera (Table 2). The carnivorous nematodes were associated positively with nine fungal modules with no negative associations found (Table 2).

Fungal network consensus eigengene modules on the conventional putting greens had strong negative correlations with herbivorous nematodes and weak positive correlations with bacterivores (Supplementary Fig. 10), whereas on fairways there were weak positive correlations (Supplementary Fig. 11) and on roughs were weak negative correlations (Supplementary Fig. 12), with the exception of the grey catchall module in both instances. The consensus eigengene networks among the three management areas all had low correlations with herbivorous nematodes (Supplementary Figs. 10–12).

Generally, on the hybrid course putting greens, bacterivore and herbivorous nematodes had weak negative correlations with all of the consensus eigengene modules, except for four modules that had strong negative correlations to herbivores (Supplementary Fig. 13). The fairways had weak negative correlations with bacterivorous nematodes and mostly weakly positive (with 4 weakly negative) correlations to herbivores (Supplementary Fig. 14). The rough also showed weak negative and positive correlations to bacterivore and herbivorous nematodes (Supplementary Fig. 15).

On the organic course, bacterivorous nematodes had strong negative correlations with fungal modules on the putting greens, and herbivorous nematodes showed no strong correlations to any of the eigengene modules (Supplementary Fig. 16). On fairways, bacterivore and herbivorous nematodes had similar patterns, with both having strong positive correlations with the turquoise and brown modules and weak negative correlations with the other modules (Supplementary Fig. 17).

Table 2
Fungal modules significantly correlated with nematode trophic groups.

Module	Total Nematodes[a]	Herbivores	Bacterivores	Fungivores	Omnivores	Carnivores	Fungal Taxa[b,c]
Chartreuse2	0.264 (0.0008)	0.184 (0.0217)	0.166 (0.0385)		0.26 (0.001)	0.192 (0.0165)	***Aspergillus***, *Asteroma, Ctenodrilus,* Eukaryota, *Hyponectria,* ***Metarhizium***, *Ophiocordyceps*
DarkGrey			0.16 (0.0462)	−0.159 (0.0485)			*Ambispora, Coniosporium, Cryptococcus,* ***Glomus***, *Lacrymaria, Ophiostoma,* Orbiliales, *Otidea, Sporobolomyces, Suillus,* Tremellaceae, *Tuber*
DarkMargenta		0.258 (0.0011)					Ascomycota, ***Aspergillus***, *Callistosporium, Lecythophora, Orbilia, Repetobasidium, Talaromyces, Trichocoma,* Trichocomaceae
DarkOlive Green					0.223 (0.0053)		*Archaeospora,* ***Aspergillus***, *Coccomyces, Cryptococcus, Didymocrea,* Eukaryota, ***Penicillium***, *Termitomyces, Trechispora,* Tremellales, *Tretopileus, Umbilicaria*
DarkOrchid1						0.273 (0.0006)	*Cenococcum,* **Glomeraceae**, *Isaria, Neocudoniella, Paraglomus, Thermoascus*
DarkRed			−0.181 (0.0239)				*Acaulospora,* Ascomycota, Boletales, *Byssoascus, Chlorencoelia, Cryptococcus, Cystofilobasidium,* Eukaryota, **Glomeraceae**, ***Glomus***, Leotiomycetes, *Lipomyces, Phaeoacremonium, Phaeomoniella*
DarkTurquoise			0.179 (0.0256)				Acaulosporaceae, *Catenomyces, Ceriporia,* Chytridiomycota, Eukaryota, ***Glomus***, ***Paecilomyces***, *Phyllobaeis, Scolecobasidium, Waitea*
Gold				0.212 (0.008)			***Colletotrichum***, *Endosporium, Exidia, Hyphozyma, Hypocrea, Letharia*
Grey			−0.296 (0.0002)		0.2 (0.0125)	0.219 (0.0061)	*Ambispora, Arachnomyces, Arthonia, Ascobolus,* Ascomycota, ***Aspergillus***, Basidiomycota, *Blumeria,* Boletales, *Bombardia, Bullera, Candida, Capronia, Chalciporus,* Chytridiomycota, *Cladophialophora, Claviceps, Coccidioides, Coniophora, Coniosporium, Cryptococcus,* ***Dactylella***, *Dactylellina, Davidiella, Dicellomyces, Donadinia, Elaphocordyceps, Entorrhiza,* Eukaryota, ***Exophiala***, ***Fusarium***, *Geosiphon, Geosmithia,* **Glomeraceae**, *Graphium, Gymnascella, Gymnoascus, Haloguignardia,* Halosphaeriaceae, *Helvella,* ***Hirsutella***, *Hydnotrya, Hygrophorus, Hymenostilbe, Hyphodiscus,* Hypocreales, *Jaminaea, Kionochaeta, Koerberia, Kurtzmanomyces, Lachnellula, Lecania, Lobulomyces, Lopharia, Madurella, Magnisphaera, Malassezia, Malbranchea, Mallocybe, Marchandiomyces, Mariannaea, Melastiza, Moniliophthora, Mycosphaerella, Myriangium, Myrothecium, Myxotrichum, Myxozyma, Naohidea, Neophyllis, Neotestudina,* Ochromonadaceae, *Ophioceras, Ophiostoma, Orbilia,* ***Paecilomyces***, *Panaeolus, Panorbis, Paraglomus,* ***Penicillium***, *Pertusaria, Pesotum, Pestalotiopsis,* Pezizaceae, *Phaeoacremonium,* ***Phoma***, *Physalospora, Physoderma, Placopsis, Pluteus, Podosphaera, Pseudoplectania, Psilolechia, Raffaelea, Ramaria, Rhexocercosporidium, Rhizidium, Rhytisma, Rogersella, Rozella, Saccharicola, Sagenomella, Scutellospora, Scytinostroma, Sebacina, Sirococcus, Sphaerobolus, Sporobolomyces, Stachybotrys, Stylodothis, Taphrina, Teratosphaeria, Thelephora, Thysanophora, Tilletiopsis, Tolypocladium, Torrubiella,* Tremellomycetes, ***Trichoderma***, *Tricholoma, Trimorphomyces, Tubeufia,* Ustilaginoidea, *Verrucospora, Xanthoparmelia, Xeromyces*
Khaki4						0.261 (0.001)	*Abortiporus,* ***Aspergillus***, Candida, Eukaryota, **Glomeraceae**, Leotiomycetes, *Neurospora, Oidiodendron,* Thraustochytriidae
MistyRose			−0.161 (0.454)				Ascomycota, ***Aspergillus***, *Dimorphospora,* Eukaryota, Eurotiomycetes, *Lasallia, Phacopsis, Rhodocybe, Spathularia*
Plum						0.18 (0.0248)	*Amylostereum, Cladophialophora, Cymatoderma, Hericium, Laxitextum, Pectinotrichum, Russula, Sistotrema, Thanatephorus*
RosyBrown					0.159 (0.0475)		Ascomycota, *Dioszegia,* Eukaryota, **Glomeraceae**, ***Penicillium***, ***Phoma***, *Physcia, Scutellospora, Sphaerographium, Umbelopsis*
SaddleBrown	0.201 (0.0121)	0.159 (0.0479)				0.212 (0.008)	Boletales, *Capnobotryella, Crucibulum, Cryptococcus,* Eukaryota, ***Exophiala***, *Galerina,* **Glomeraceae**, **Lasiosphaeriaceae**, *Mycena, Mycosphaerella,* ***Pleurotus***, *Serpula*
SkyBlue						0.165 (0.0402)	*Arthroderma, Basidioradulum, Cordyceps,* Eukaryota, ***Fusarium***, *Marasmius, Marchandiomyces, Paraglomus, Paulia, Pseudohydnum, Spiromastix*
SteelBlue		−0.164 (0.0408)	0.22 (0.0059)				*Anguillospora,* ***Arthrobotrys***, ***Colletotrichum***, ***Dactylella***, Eukaryota, *Geosmithia,* ***Glomerella***, ***Monacrosporium***, *Nais,* ***Penicillium***, ***Phoma***

(*continued on next page*)

Table 2 (*continued*)

Module	Total Nematodes[a]	Herbivores	Bacterivores	Fungivores	Omnivores	Carnivores	Fungal Taxa[b,c]
Tomato1				0.239 (0.0027)			*Bullera, Cladophialophora, Combea, Laetisaria,* ***Metarhizium***
Tomato2						0.162 (0.0441)	*Athelia,* Basidiomycota, *Bionectria, Byssothecium, Ceratobasidium, Dendryphion,* ***Glomus****, Kirschsteiniothelia, Myrothecium, Phlebia, Pleospora, Schizosaccharomyces*
Violetred2						0.203 (0.0003)	*Aquaphila,* Ascomycota, **Glomeraceae**, ***Glomus****, Isaria, Lasiosphaeria, Lunulospora, Neurospora, Phaeoacremonium*

[a] Correlation coefficient, expressed as the eigengene significance, and in parenthesis the p value.
[b] Taxa presented at genus level or, if unclassified, at lowest taxonomic resolution possible.
[c] Genus with reported antagonism against nematodes shown with bold (Siddiqui and Mahmood, 1996).

On roughs, both bacterivore and herbivorous nematodes had weak positive correlations to the fungal modules, with the herbivores having a stronger correlation (0.51 and 0.055) to the turquoise and brown modules, respectively (Supplementary Fig. 18).

4. Discussion

We hypothesized that total nematodes would correlate negatively with modules containing known nematode-inhibiting bacteria and fungi. There were often positive correlations with nematodes and modules containing nematode-inhibiting microbes. If nematode populations are in high enough abundance, they could be supporting pathogenic bacteria and fungi at high levels without their own population collapsing, a classic example of carrying capacity with pathogens. Potentially the application of biocontrol products aimed at inhibiting nematode populations would need to be applied in high enough densities to affect the nematodes. The bacterivores were affected negatively by bacterial and fungal populations more so than herbivores, the latter being the target of nematode biocontrol. Nematode biocontrol agents may affect bacterivores more than herbivores, although to our knowledge, there have been no reports of this, possibly due to lack of testing on non-target species. Some bacterial species have adapted defense mechanisms against bacterivores, such as *Pseudomonas fluorescens* that produces toxic metabolites in the presence of bacterivorous nematodes (Neidig et al., 2011). Potentially, modules associated negatively with bacterivores contain members with currently unknown defense mechanisms. In addition to known nematode-inhibiting taxa, we investigated four additional taxa that have been linked with nematode populations in previous studies.

4.1. Taxon-specific nematode interactions

Rhodoplanes spp. were the most represented bacteria in the modules and were correlated positively with total nematodes (cyan, dark orange, seagreen4, snow3, steelblue, turquoise2, and pale turquoise modules), herbivores (cyan, seagreen4, snow3, steelblue, pale turquoise, and violetred4 modules), bacterivores (green yellow and yellow modules), fungivores (darkolivegreen2 and violet modules), omnivores (dark magenta, snow3, and steelblue modules), and carnivores (dark orange, light green, snow3, steelblue, turquoise2, purple, and violetred4 modules) and correlated negatively with total nematodes in the lemonchiffon1 module. *Rhodoplanes* spp. significantly correlated with *M. chitwoodi* in potato fields (Castillo et al., 2017). *Rhodoplanes* spp. are photoheterotrophic in anoxic conditions with potential for chemotrophy in oxygen rich or denitrification in oxygen-limited environments. They are involved in nitrogen cycling, degradation of the herbicide atrazine, and potentially other biological processes like degradation of plant compounds (Fan et al., 2021; Lin et al., 2018; Oren and Xu, 2014). Lin et al. (2018) found earthworm presence in bulk soil increased the abundance of *Rhodoplanes* spp. Although considerably smaller in size than earthworms, nematodes may potentially have a similar effect on *Rhodoplanes* spp., resulting in the high number of positive correlations of this bacteria genus with the different nematode trophic groups. The mostly positive correlations of *Rhodoplanes* spp. with nematodes in this study and Castillo et al. (2017) may warrant further investigation to understand if and what this relationship means biologically.

Castillo et al. (2017) also found a significant positive correlation of *Phenylobacterium* spp. and *Kaistobacter* spp. with *M. chitwoodi* in potato fields. In this study, *Kaistobacter* spp. were correlated positively with herbivorous and total nematodes in one module (steelblue). It also correlated positively with bacterivorous nematodes in the snow2 module. Lin et al. found that *Kaistobacter* spp. and *Rhodoplanes* spp. were found in increased abundance in the presence of earthworms, and perhaps respond in a similar manner to the presence of herbivore nematodes (Lin et al., 2018). *Phenylobacterium* spp. were only identified in the grey module as having a positive correlation to bacterivores, however because the grey module serves as a catchall within the WGCNA it cannot be used to imply relationships among nematodes and bacteria. The relationship with *Phenylobacterium* spp. and *Kaistobacter* spp. with nematodes in potatoes and turfgrass and these potential interactions should be further investigated. Jiang et al. (2017) found a positive relationship between *Mesorhizobium* spp. and the bacterivore nematode, *Protorhabditis* spp. in maize. However, we did not find *Mesorhizobium* spp. in any of the modules that were correlated positively or negatively with nematodes in this study; however that does not preclude that there could be a relationship among these organisms in other turfgrass environments.

4.2. Positive association between bacteria and fungi with nematodes

In addition to specific taxa, bacteria and fungi in general were associated positively with nematodes in most instances. This was consistent with our second hypothesis that bacterivore and fungivorous nematodes would increase along with higher populations of their food sources. The same positive correlation of bacteria and bacterivores was shown in red soil of maize (Jiang et al., 2017). This positive association with bacteria was expected because nematodes are known to increase bacterial populations in the soil by moving them through the soil increasing their colonization rate (Knox et al., 2003). Additionally, the food incompletely digested by nematodes becomes a food source for bacteria (Ferris and Venette, 1998). Nematodes, especially bacterivores and omnivores, ingest more nitrogen than they can use and excrete the excess ammonium in readily available form to the surrounding plant roots and microbes (Anderson et al., 1983; Ekschmitt et al., 1999; Knox et al., 2003). Bacterivorous nematodes have experimentally been found to have a positive feedback on their bacterial prey (Marchesi et al., 1998). Although it has not been studied, they could also be increasing fungal populations through the same mechanisms.

4.3. Association of bacterial and fungal modules with carnivorous nematodes

One surprising result was how many bacterial and fungal modules were associated with carnivorous nematodes. This group represents a small fraction of the nematode community (Neher, 2001) and little is known about their interactions with microbes. Potentially they increase food sources for bacteria and fungi by leaving behind detritus as they feed which becomes food for the microbes. As with bacterivorous nematodes, they secrete nitrogen back into the soil which may potentially benefit certain microbial species (Neher, 2001). Wardle and Yeates (1993) determined in both asparagus, and a lesser extent in maize, that carnivorous nematodes had strong positive correlations with bacterial and fungal biomass. They proposed this interaction showed carnivorous nematodes rely on resources at the bottom of the food web (bacteria and fungi) as the latter is direct resource for intermediate nematodes, and that carnivorous nematodes are limited by competition amongst themselves and not with other nematode trophic groups (Wardle and Yeates, 1993). It is also important to consider the results of our study could be erroneous as the relationship may have occurred due to the low abundance of carnivores collected, being absent from many of the samples and in low total counts when observed, and with a larger population of these nematodes the high correlation may not be present.

4.4. Comparison of networks among management areas

We predicted, based on the results presented in a previous publication on turf microbes (Allan-Perkins et al., 2019) that the consensus eigengene networks would be similar among management areas for all three golf courses for bacteria, but would be significantly different for fungi on putting greens compared to fairways and roughs. We did not see this effect in the bacterial or fungal consensus eigengene networks. All preservation (D) values were similar among bacteria and fungi (ranging from 0.6 to 0.85) and were slightly lower for the consensus bacterial networks than fungal networks in some instances. This is different from the results for fungal abundance, diversity, and richness which tended to be lowest on putting greens in the previous publication (Allan-Perkins et al., 2019). The differential eigengene network analysis uses eigengenes to represent a specific module and creates consensus modules across the samples being compared. Potentially, even if overall diversity and richness is different among areas, the eigengenes are conserved and the relationships among modules, displaying a conserved functional role of fungi within the different management areas of the golf courses that is conserved among the different areas. This would show a robustness in bacterial and fungal functions regardless of different management inputs. The bacterial networks may have shown slightly more differences among areas compared with richness and diversity estimates as potentially a few taxa altered network interactions more significantly in this group compared to the fungi.

Lastly, we hypothesized that herbivorous nematodes would have more positive correlations with the putting greens on the conventional and hybrid courses, based on the high relative abundance of herbivores on conventional and hybrid putting greens in a previous publication (Allan-Perkins et al., 2017). We also predicted that the bacterivorous nematodes would have more positive correlations with the putting greens on the organic course, since they had high relative abundance on the organic putting greens in the previous publication. Herbivorous nematodes on the conventional putting green had a strong (91%) positive correlation with the yellow meta-module, so potentially this module interacted with herbivores to increase populations while having a neutral effect on bacterivores. The hybrid course had a less dramatic response, but still one module, the yellow meta-module, had strong positive correlation (64%) with herbivores and a slightly positive correlation (23%) with bacterivores. On the organic course we did see a very strong positive correlation of bacterivores with meta-module brown. Potentially, as with the conventional and hybrid course, there are interactions within this one meta-module that are increasing specific nematode trophic groups. For the fungal networks, the herbivorous nematodes had a strong negative correlation with all fungal modules for the conventional course and a weak negative correlation with fungal modules on the hybrid course. Bacterivores had a strong negative correlation with the fungal modules on the organic putting green. Although these specific fungal modules do not affect nematodes in the way we predicted, it may be that other organisms within the community (such as the bacterial community or healthy turf increasing food supply for herbivorous nematodes) has a stronger effect on nematode trophic groups. For the organic putting green, the fungal modules may directly compete with the bacteria on the course that are a food source for the bacterivorous nematodes, explaining why they are related negatively.

5. Conclusions

The results of our co-occurrence and network analyses provide new insight into the soil ecosystems on golf courses. Additionally, the biological functional role of *Rhodoplanes* in microbial communities and with nematodes should be further investigated. Lastly, as this study is based on correlations and limited to genus level, the interactions of specific bacteria and fungi identified in our networks associated with bacterivores, herbivores, and carnivores should be assessed to understand relationships and develop better management strategies to control herbivorous nematodes and increase potentially beneficial species.

Declaration of competing interest

The authors declare no financial interests or personal relationships which may be considered as potential competing interests.

Data availability

The data are available through myPhyloDB (https://myphylodb.scinet.usda.gov/myPhyloDB/home) and the National Center for Biotechnology Information Sequence Read Archive under BioProject ID PRJNA868599.

Acknowledgements

We thank James Popko, Jr. and Dr. Hyunkyu Sang for their assistance with sample collection and processing and Sarah Barnett for running the amplicon sequencing and qPCR reactions. This research was partially funded by United States Golf Association, the New England Regional Turfgrass Foundation, and the University of Massachusetts Plant Biology Gilgut Fellowship and University Fellowship. This material is based upon work supported by the National Institute of Food and Agriculture (NIFA), U.S. Department of Agriculture (USDA), the Massachusetts Agricultural Experiment Station and the Stockbridge School of Agriculture at the University of Massachusetts Amherst, under project number MAS00436. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA or NIFA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2023.100798.

References

Allan-Perkins, E., Manter, D.K., Wick, R., Ebdon, S., Jung, G., 2017. Nematode communities on putting greens, fairways, and roughs of organic and conventional cool-season golf courses. Appl. Soil Ecol. 121, 161–171. https://doi.org/10.1016/j.apsoil.2017.09.014.

Allan-Perkins, E., Manter, D., Jung, G., 2018. Abundance of bacteria, fungi, and *Sclerotinia homoeocarpa* in the thatch and soil of golf courses. Phytobiomes J. 2, 71–81. https://doi.org/10.1094/PBIOMES-09-17-0036-R.

Allan-Perkins, E., Manter, D.K., Jung, G., 2019. Soil microbial communities on roughs, fairways, and putting greens of cool-season golf courses. Crop Sci. 59, 1753–1767. https://doi.org/10.2135/cropsci2018.04.0220.

Anderson, R.V., Gould, W.D., Woods, L.E., Cambardella, C., Ingham, R.E., Coleman, D.C., 1983. Organic and inorganic nitrogenous losses by microbivorous nematodes in soil. Oikos 40, 75. https://doi.org/10.2307/3544201.

Barberán, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. ISME J. 6, 343–351.

Beirn, L.A., Hempfling, J.W., Schmid, C.J., Murphy, J.A., Clarke, B.B., Crouch, J.A., 2017. Differences among soil-inhabiting microbial communities in *Poa annua* turf throughout the growing season. Crop Sci. 57 https://doi.org/10.2135/cropsci2016.06.0463.

Bongers, T., 1988. De Nematoden Van Nederland. Stichting Uitgeverij van de Koninklijke Nederlandse Natuurhistorische Vereniging.

Borneman, J., Hartin, R.J., 2000. PCR primers that amplify fungal rRNA genes from environmental samples. Appl. Environ. Microbiol. 66, 4356–4360.

Castillo, J.D., Vivanco, J.M., Manter, D.K., 2017. Bacterial microbiome and nematode occurrence in different potato agricultural soils. Microb. Ecol. 74, 888–900. https://doi.org/10.1007/s00248-017-0990-2.

Chen, Z., Dickson, D., 1998. Review of *Pasteuria penetrans*: biology, ecology, and biological control potential. J. Nematol. 30, 313–340.

Dell, E.A., Bowman, D., Rufty, T., Shi, W., 2010. The community composition of soil-denitrifying bacteria from a turfgrass environment. Res. Microbiol. 161, 315–325. https://doi.org/10.1016/j.resmic.2010.03.010.

Dell, E.A., Bowman, D., Rufty, T., Shi, W., 2008. Intensive management affects composition of betaproteobacterial ammonia oxidizers in turfgrass systems. Microb. Ecol. 56, 178–190.

DeSantis, Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E., Keller, K., Dalevi, D., Hu, P., Andersen, G., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72, 5069–5072. https://doi.org/10.1128/AEM.03006-05.

Ding, J., Zhang, Y., Deng, Y., Cong, J., Lu, H., Yang, C., Yuan, T., Van Nostrand, J.D., Li, D., Zhou, J., Yang, Y., 2015. Integrated metagenomics and network analysis of soil microbial community of the forest timberline. Sci. Rep. 5, 7994. https://doi.org/10.1038/srep07994.

Dong, J., Horvath, S., 2007. Understanding network concepts in modules. BMC Syst. Biol. 1, 24. https://doi.org/10.1186/1752-0509-1-24.

Ekschmitt, K., Bakonyi, G., Bongers, M., Bongers, T., Boström, S., Dogan, H., Harrison, A., Kallimanis, A., Nagy, P., O'Donnell, A.G., Sohlenius, B., Stamou, P., Wolters, V., 1999. Effects of nematofauna on microbial energy and matter transformation rates in European grassland soils. Plant Soil 212, 45–61.

Elsen, A., Gervacio, D., Swennen, R., De Waele, D., 2008. AMF-induced biocontrol against plant parasitic nematodes in Musa sp.: a systemic effect. Mycorrhiza 18, 251–256. https://doi.org/10.1007/s00572-008-0173-6.

Fan, K., Delgado-Baquerizo, M., Guo, X., Wang, D., Zhu, Y., Chu, H., 2021. Biodiversity of key-stone phylotypes determines crop production in a 4-decade fertilization experiment. ISME J. 15, 550–561. https://doi.org/10.1038/s41396-020-00796-8.

Ferris, H., Venette, R.C., 1998. Nitrogen mineralization by bacterial-feeding nematodes: verification and measurement. Plant Soil 203, 159–171.

Fuhrman, J.A., 2009. Microbial community structure and its functional implications. Nature 459, 193–199. https://doi.org/10.1038/nature08058.

Goodey, J.B., 1963. Soil and Freshwater Nematodes, second ed. John Wiley and Sons, Inc., London, UK.

Jiang, Y., Liu, M., Zhang, J., Chen, Y., Chen, X., Chen, L., Li, H., Zhang, X.-X., Sun, B., 2017. Nematode grazing promotes bacterial community dynamics in soil at the aggregate level. ISME J. 11, 2705–2717. https://doi.org/10.1038/ismej.2017.120.

Knox, O., Killhaim, K., Mullins, C., Wilson, M., 2003. Nematode-enhanced microbial colonization of the wheat rhizosphere. FEMS Microbiol 225, 227–233.

LaMondia, J., Timper, P., 2016. Interactions of microfungi and plant-parasitic nematodes. In: Li, D.W. (Ed.), Biology of Microfungi, Fungal Biology. Springer International Publishing, Switzerland, pp. 573–614.

Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L., Pace, N.R., 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci. U.S.A. 82, 6955–6959.

Langfelder, P., Horvath, S., 2008. WGCNA: an R package for weighted correlation network analysis. BMC Bioinf. 9, 559. https://doi.org/10.1186/1471-2105-9-559.

Langfelder, P., Horvath, S., 2007. Eigengene networks for studying the relationships between co-expression modules. BMC Syst. Biol. 1, 54. https://doi.org/10.1186/1752-0509-1-54.

Lian, L.H., Tian, B.Y., Xiong, R., Zhu, M.Z., Xu, J., Zhang, K.Q., 2007. Proteases from Bacillus: a new insight into the mechanism of action for rhizobacterial suppression of nematode populations. Lett. Appl. Microbiol. 45, 262–269. https://doi.org/10.1111/j.1472-765X.2007.02184.x.

Lin, Z., Zhen, Z., Ren, L., Yang, J., Luo, C., Zhong, L., Hu, H., Liang, Y., Li, Y., Zhang, D., 2018. Effects of two ecological earthworm species on atrazine degradation performance and bacterial community structure in red soil. Chemosphere 196, 467–475. https://doi.org/10.1016/j.chemosphere.2017.12.177.

Mai, E., Lyon, H., 1975. Pictorial Key to Genera of Plant-Pathogenic Nematodes. Cornell University Press, Ithaca, NY.

Manter, D., Korsa, M., Tebbe, C., Delgado, J., 2016. myPhyloDB: a local web server for the analysis of metagenomics data. Database baw037, 2016.

Marchesi, J., Takuichi, S., Weightman, A., Martin, T., Fry, J., Hiom, S., Wade, G., 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl. Environ. Microbiol. 64, 795–799.

Neher, D., 2001. Role of nematodes in soil health and their use as indicators. J. Nematol. 33, 161–168.

Neher, D., 1999. Nematode communities in organically and conventionally managed agricultural soils. J. Nematol. 31, 142–154.

Neher, D., Campbell, C., 1994. Nematode communities and microbial biomass in soils with annual and perennial crops. Appl. Soil Ecol. 1, 17–28.

Neidig, N., Paul, R.J., Scheu, S., Jousset, A., 2011. Secondary metabolites of *Pseudomonas fluorescens* CHA0 drive complex non-trophic interactions with bacterivorous nematodes. Microb. Ecol. 61, 853–859. https://doi.org/10.1007/s00248-011-9821-z.

Okada, H., Harada, H., Kadota, I., 2005. Fungal-feeding habits of six nematode isolates in the genus *Filenchus*. Soil Biol. Biochem. 37, 1113–1120. https://doi.org/10.1016/j.soilbio.2004.11.010.

Oren, A., Xu, X., 2014. The family Hyphomicrobiaceae. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes. Springer, Berlin, Heidelberg, pp. 247–281.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. D590–D596.

R Core Team, 2020. R: A Language and Environment for Statistical Computing.

Shi, S., Nuccio, E.E., Shi, Z.J., He, Z., Zhou, J., Firestone, M.K., 2016. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. Ecol. Lett. 19, 926–936. https://doi.org/10.1111/ele.12630.

Shi, W., Bowman, D., Rufty, T., 2007. Soil microbial community composition and function in turfgrass ecosystems. Bioremediation, Biodivers. Bioavailab. 1, 72–77.

Shi, W., Yao, H., Bowman, D., 2006. Soil microbial biomass, activity and nitrogen transformations in a turfgrass chronosequence. Soil Biol. Biochem. 38, 311–319. https://doi.org/10.1016/j.soilbio.2005.05.008.

Siddiqui, Z.A., Mahmood, I., 1999. Role of bacteria in the management of plant parasitic nematodes: a review. Bioresour. Technol. 69, 167–179. https://doi.org/10.1016/S0960-8524(98)00122-9.

Siddiqui, Z.A., Mahmood, I., 1996. Biological control of plant parasitic nematodes by fungi: a review. Bioresour. Technol. 58, 229–239. https://doi.org/10.1016/S0960-8524(96)00122-8.

Tarjan, A., Esser, R., Chang, S., 2014. Interactive Diagnostic Key to Plant Parasitic, Freeliving, and Predaceous Nematodes. University of Nebraska Lincoln Nematology Laboratory. http://nematode.unl.edu/key/nemakey.htm. accessed 13.05.25.

Thoden, T.C., Korthals, G.W., Termorshuizen, A.J., 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management? Nematology 13, 133–153. https://doi.org/10.1163/138855410X541834.

Tian, Honglin, Riggs, Robert, D., Crippen, Devany, L., 2000. Control of soybean cyst nematode by chitinolytic bacteria with chitin substrate. J. Nematol. 32, 370–376.

Wardle, D.A., Yeates, G.W., 1993. The dual importance of competition and predation as regulatory forces in terrestrial ecosystems: evidence from decomposer food-webs. Oecologia 93, 303–306. https://doi.org/10.1007/BF00317685.

Yeates, G.W., Bongers, T., 1993. Feeding habits in soil nematode families and genera-an outline for soil ecologists. J. Nematol. 25, 315–331.