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SOIL MICROBIOLOGY

Bacterial Microbiome and Nematode Occurrence in Different Potato Agricultural Soils

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Abstract *Pratylenchus neglectus* and *Meloidogyne chitwoodi* are the main plant-parasitic nematodes in potato crops of the San Luis Valley, Colorado. Bacterial microbiome (16S rRNA copies per gram of soil) and nematode communities (nematodes per 200 g of soil) from five different potato farms were analyzed to determine negative and positive correlations between any bacterial genus and *P. neglectus* and *M. chitwoodi*. Farms showed differences in bacterial communities, percentage of bacterivorous and fungivorous nematodes, and numbers of *P. neglectus* and *M. chitwoodi*. The farm with the lowest population of *P. neglectus* and *M. chitwoodi* had higher abundances of the bacterial genera *Bacillus* spp., *Arthrobacter* spp., and *Lysobacter* spp., and the soil nematode community was composed of more than 30% of fungivorous nematodes. In contrast, the farm with higher numbers of *P. neglectus* and *M. chitwoodi* had a lower abundance of the abovementioned bacterial genera, higher abundance of *Burkholderia* spp., and less than 25% of fungivorous nematodes. The α-Proteobacteria *Rhodoplanes*, *Phenylobacterium*, and *Kaistobacter* positively correlated with *M. chitwoodi*, and the Bacteroidia and γ-Proteobacteria positively correlated with *P. neglectus*. Our results, based largely on co-occurrence analyses, suggest that the abundance of *Bacillus* spp., *Arthrobacter* spp., and *Lysobacter* spp. in Colorado potato soils is negatively correlated with *P. neglectus* and *M. chitwoodi* abundance. Further studies will isolate and identify bacterial strains of these genera, and evaluate their nematode-antagonistic activity.

Keywords *Pratylenchus neglectus* · *Meloidogyne chitwoodi* · Microbiome · *Bacillus* · *Arthrobacter* · *Lysobacter*

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Introduction

Currently, there is a growing interest in the study of soil and rhizosphere microbiome to understand the soil community composition and biodiversity, and the impact their interactions have on plants [1]. The soil ecosystem is a complex and diverse environment that contains millions of bacteria and fungi, nematodes, mites, earthworms, and arthropod species in a single gram of soil [2]. It has been demonstrated that the diversity of underground microorganisms significantly determines the aboveground biodiversity and ecosystem functioning [1, 2]. For the specific case of agricultural ecosystems, intensive agriculture is causing soil degradation, loss of biodiversity, reduction of soil-food trophic levels (predators), and decreasing functional groups with larger biomass (earthworms, enchytraeids, collembolans, mites) [3]. Hence, there is a need to understand soil microbial community, diversity and ecology, and beneficial or deleterious plant-microbe interactions in order to develop a more sustainable agriculture. The use of molecular biology techniques to study the soil and rhizosphere bacterial microbiome [4] and the use of soil free-living nematodes as soil health bioindicators can be used as tools to understand soil interactions within an agricultural system [5].

Genomic studies in recent years are allowing high-throughput analysis of cells, organisms, and populations, and are starting to reveal different types of mechanisms and

interactions between nematodes and bacteria [4]. Bacteria can be a food source for nematodes, can be pathogens of nematodes, or can develop symbiotic interactions [6–10]. Genome sequencing has allowed the study of transcriptional profiles of bacteria and nematodes, as well as the identification and quantification of proteins in complex mixtures. As an example of these genomic studies, it has been demonstrated that root-knot nematodes (*Meloidogyne* spp.) acquired most of the parasitism genes that encode for enzyme production from bacteria by horizontal gene transfer (HGT) [11, 12]. Studies on the bacterial microbiome of the soybean cyst nematode (*Heterodera glycines*) reported 290 bacteria associated with cysts of this nematode. Thirty of these bacterial isolates have been reported as nematode antagonists (*Lysobacter*, *Variovorax*), while other isolates produce polymer-hydrolyzing enzymes that decrease the rigidity of the cyst and facilitate the emergence of the nematode juveniles [13]. Furthermore, it has been demonstrated that the bacterial microbiome associated with the pinewood nematode (*Bursaphelenchus xylophilus*) helps the nematode to degrade α-pinene (the main compound in the pine resin that inhibits reproduction and development of pinewood nematodes) and successfully parasitize the pine tree [14].

Free-living or beneficial nematodes composed 60–80% of the soil nematode community, and due to the key role they play in the soil food web and soil-ecological processes (nutrient cycling, mineralization, dispersal of microbes), they can be used as soil health bioindicators to understand biological mechanisms and interactions in soil [15–17]. Soil nematodes are categorized into a 1–5 colonizer-persister (*c-p*) scale, which range from *r*- to *K*-strategists. At the lower end of the *c-p* scale are located the "colonizer" nematodes that are considered enrichment opportunists and therefore indicate resource availability. In contrast, at the high end of the *c-p* scale are located the "persister" nematodes that indicate system stability, food web complexity, and connectance [5, 16, 18, 19]. Furthermore, Ferris et al. [16] developed indices for nematode diversity, genera richness, structure, and functional guilds and based on them classified the nematode community in a graph composed by four quadrants: quadrant A for soils enriched but unstructured, quadrant B for enriched and structured, quadrant C for resource limited and unstructured, and quadrant D for resource-depleted and unstructured [16, 18].

Within the nematode soil community, the two main plant-parasitic nematodes in San Luis Valley of Colorado potato crops are the Columbia root-knot nematode (*Meloidogyne chitwoodi*) and root-lesion nematode (*Pratylenchus neglectus*). *Meloidogyne chitwoodi* is a major pest in commercial potato production in the northwestern USA, and has additional alternative hosts such as tomato, barley, oat, wheat, and rye [20, 21]. *Pratylenchus neglectus* is the most common plant-parasitic nematode in Colorado crops, has a wide host range, and can be a problem in potato, grape, wheat, and corn crops of Colorado [21].

Therefore, understanding the interaction(s) of bacteria with *P. neglectus* and *M. chitwoodi* in the soil will allow a better understanding about their ecology, and will provide new insights into possible biocontrol agents. The objective of this work was to study the soil bacterial microbiome, and the soil nematode community present in potato crops of the San Luis Valley, Colorado, and evaluate if there were any specific correlations between bacterial genera and the two main plant-parasitic nematodes present in potato crops of Colorado: *M. chitwoodi* and *P. neglectus*.

Materials and Methods

Soil Sampling and Nematode Extraction

Soil samples were collected from five different potato farms in the San Luis Valley, Colorado, with historically different levels of plant-parasitic nematodes (Agro Engineering Inc., *personal communication*). Soil conditions for farms sampled were Monte Vista (sandy soil; sand 88.8%, silt 6.2%, clay 5%; organic matter 0.9%; pH 7.4), Blanca (loamy sand soil; sand 82.5%, silt 10%, clay 7.5%; organic matter 0.4%; pH 8.0), Mosca I (loamy sand soil; sand 86%, silt 10%, clay 3.7%; organic matter 0.4%; pH 8.3), Mosca II (loamy sand soil; sand 81.3%, silt 8.7%, clay 10%; organic matter 0.5%; pH 8.6), and Sargent (loamy sand soil; sand 80%, silt 10%, clay 10%; organic matter 0.6%; pH 7.8).

Potato farms Monte Vista, Blanca, and Sargent were circular-shaped, while farms Mosca I and II were half-circular-shaped. Each circular crop was divided in quadrants, and in each quadrant two soil samples were taken for a total of four to eight soil samples per farm. Each soil sample consisted of a composite of rhizosphere soil from three randomly selected plants in the potato crop. Each plant was first removed using a spade, and then a single soil core (2″ dia. × 6″ deep) was collected and mixed by hand to create a pooled sample. All potato plants were approximately 60 days old at the time of sampling. Soil nematodes were extracted from each sample, taking 200 g of soil and using the modified gravity-sieving followed by the sucrose centrifugation-flotation method [22], and counted on an inverted microscope. Free-living and plant-parasitic nematodes were identified and counted, and the nematode community indices were estimated as described by Bongers and Ferris [5], Ferris and Bongers [23], and Neher and Darby [19].

Soil DNA Extraction and Amplification for Microbiome Analysis

DNA extraction from the soil (0.5 g) was conducted using a custom single-tube DNA extraction technique, which includes the initial lysis and cleanup steps of the PowerSoil DNA

Extraction Kit (MoBio) and MagNA Pure LC DNA Isolation Kit (Roche). Briefly, soils were weighted into garnet bead tubes (MoBio) and lysed in 750 μL of bead solution (MoBio, Cat. no. 12855-50-BS) and 60 μL C1 solution (MoBio, Cat. no. 1288-50-1) at 6000 rpm for 60 s using a MagNA Lyser (Roche). After lysis, the tube was cooled at 4C for 10 min, and then the supernatant (450 μL) was transferred to a new tube and combined with 250 μL of C2 solution (MoBio no. 1288-50-2). After a 10-min incubation at 4 °C, the sample was centrifuged at 10,000 rpm to 1 min. The supernatant was transferred to a new tube and mixed with 200 μL of C3 solution (MoBio, Cat. no. 1288-50-3) and incubated at 4 °C for 10 min. After a final centrifugation at 10,000 rpm for 60 s, 700 μL of the supernatant was transferred to a new tube and purified using a MagNA Pure LC DNA Isolation Kit (Roche) run on a MagNA Pure Compact robot (Roche). The final elution volume was 50 μL, and samples were stored at −20 °C until PCR analysis.

16S rRNA Quantitative PCR

Quantitative PCR (qPCR) amplification of the bacterial 16S ribosomal RNA (rRNA) genes (V1–V3 hypervariable region) was performed with the 27F and 388R primers [24, 25]. Each reaction contained 2 μL template DNA (diluted 1:20), 0.5 μM of each primer, and 1× Maxima SYBR Green Master Mix (Cat. no. K0242, Thermo Fisher Scientific). Amplification was performed as follows: (1) 95 °C for 8.5 min; (2) 95 °C × 15 s, 58 °C × 30 s, 72 °C × 60 s, repeated 35 times; and (3) 72 °C × 5 min. Genomic DNA isolated from *Pseudomonas putida* KT2440 was used as an external standard in order to calculate 16S rRNA copies per gram of soil FW extracted assuming a *P. putida* genome size of 3.174 fg and seven 16S rRNA copies per genome. qPCR efficiency was 93% and could detect as little as 100 *P. putida* genomes in a single PCR reaction.

Illumina 16S rRNA Library Preparation

The 515f-806r primers were used to amplify the v4 region of the bacterial 16S rRNA gene based on the methods outlined in Caporaso et al. [26]. PCR conditions were the following: initial denaturation for 2 min at 95 °C, 30 cycles of 20 s at 95 °C, annealing for 15 s at 55 °C, and extension of 50 s at 72 °C, and final extension of 15 min at 72 °C. The amplification product of the qPCR was confirmed as a single band of 400–500 bp in a 1% agarose gel. The DNA was cleaned by ethanol precipitation followed by a DNA quantification using Quant-iT™ dsDNA assay on a Qubit® fluorometer. All samples were pooled contributing exactly the same amount of DNA in the final library. Final DNA concentration in the library was 21.2 ng/μL. The final library was sent to the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign.

The raw Illumina sequence data was curated using the default sequencing pipeline contained within myPhyloDB v.1.2.0 [27] prior to statistical analyses. Briefly, sequence reads were (i) trimmed (bdiff = 0, pdiff = 0, qaverage = 25, minlength = 100, maxambig = 0, maxhomop = 10); (ii) aligned to the bacterial subset SILVA alignment available at the Mothur website (http://www.mothur.org); (iii) screened (optimize = minlength-end, criteria = 95) and filtered (vertical = T, trump = .) so that all sequences covered the same genetic space; (iv) pre-clustered (diff = 2) to remove potential sequencing noise and clustered (calc = onegap, coutends = F, method = average) into operational taxonomic units or OTUs [28]; (v) screened for chimeras with vsearch [29]; (vi) classified using the Greengenes reference database (gg_13_5_99) and the naïve Bayesian classifier [30] embedded in Mothur v.1.38 [31], after which all sequences identified as chloroplast or mitochondria (<1% of the total sequences) were removed; and finally (vii) assigned to unique phylotypes (i.e., taxonomic classifications). Data normalization was conducted with myPhyloDB using rarefaction (keep), sub-sample size = median (8633 reads), iterations = 100, and lambda = 0.1. Phylotype-specific abundances (16S rRNA copies per g soil) were calculated by multiplying each genus' relative abundance by the total community bacterial abundance (16S rRNA copies per g soil) obtained from the 27F-388R qPCR.

Statistical Analysis

Statistical analysis of the nematode community was conducted using SAS 9.2 (SAS Institute Inc.) using the PROC GLIMMIX software. Nematode counts and nematode community indices were fixed effects in the model statement, and random effects include replications. Normal distribution was evaluated with the student panel graphs. Nematode counts were transformed to log-normal to satisfy the normality assumption, and later were back-transformed to the original scale and then presented in the graphs. Nematode variables were analyzed at an alpha 0.05.

Statistical analysis of the microbiome was conducted using the following R packages embedded within myPhyloDB v.1.2.0 [27]. Genus-specific total abundances tested for differences between farms were analyzed by one-way ANOVA, and all pairwise comparisons were corrected using Tukey's HSD. Genus-specific total abundance profiles were analyzed by principal component analysis (PCA) performed using the R package FactoMineR [32], and weighted correlation network analysis (WGCNA) was performed using the R package WGCNA [33].

Results

Nematode Community Assessment

There were statistical differences in the total number of nematodes in the farms (P = 0.001), number of bacterivores (P = 0.001), number of fungivores (P = 0.001), and number of plant-parasitic nematodes (P = 0.001). However, no statistical differences in nematode community variables were observed among the farms such as dominance (P = 0.449), diversity (P = 0.528), maturity index (P = 0.714), channel index (P = 0.979), and structure index (P = 0.770).

Bacterivorous nematodes composed at least half of the total nematode community in all the farms, and Sargent (P = 0.001) and Mosca II (P = 0.017) have higher populations than the rest of the farms. The Monte Vista farm has lower number of total nematodes (P = 0.026), the lowest population of fungivorous nematodes (P = 0.014), and the highest population of plant-parasitic nematodes (P = 0.002) compared to the other farms. In contrast, the Blanca farm has the lowest percentage of plant-parasitic nematodes (P = 0.001), and has the highest percentage of fungivorous nematodes compared to Mosca II and Monte Vista farms (P = 0.001) (Fig. 1). The nematode enrichment profile [18] showed that fungivorous nematodes composed 36% of the total nematode community in farms Blanca and Mosca I, compared to Mosca II, Sargent, and Monte Vista which have less than 25% of these nematodes (P < 0.001). The Monte Vista farm was the only farm where the percentage of herbivore nematodes (25%) was higher than the percentage of fungivorous nematodes (18%) (Fig. 2).

The composition of plant-parasitic nematodes showed that *P. neglectus* is present in all farms, while *M. chitwoodi* was absent in the Mosca I farm. There were differences in the number of *P. neglectus* (P = 0.024) and *M. chitwoodi* (P = 0.001) among farms. The Blanca farm has the lowest number of *P. neglectus* (P = 0.031) and *M. chitwoodi* (P = 0.001) compared to all the farms. No differences in the population of *Paratrichodorus* (P = 0.230) were detected for the three farms in which it was present: Blanca, Mosca II, and Sargent (Fig. 3).

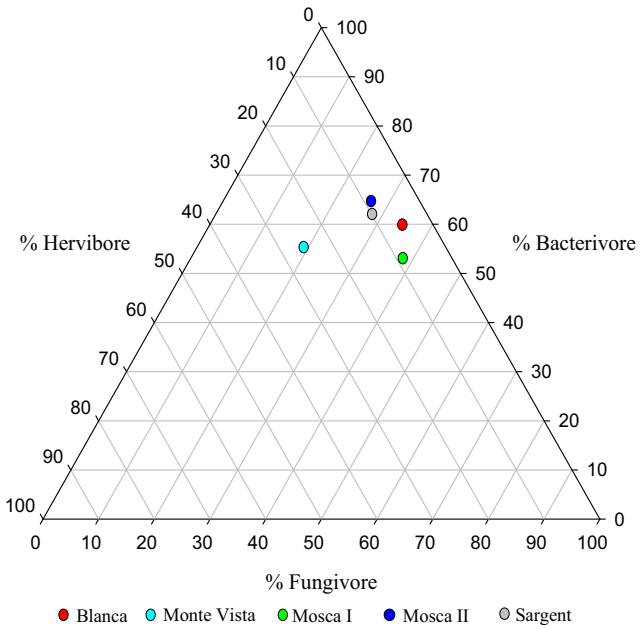

Fig. 2 Nematode enrichment profile of farms in San Luis Valley, CO

Microbiome Community Assessment

In order to get an understanding of the possible relationship between the presence of plant-parasitic nematodes and soil microbial communities, we conducted a soil microbiome analysis associated with each farm, using qPCR and a sequencing approach. Data collected was used to perform a targeted and non-targeted approach to see which bacterial genera correlate negatively or positively to *P. neglectus* and *M. chitwoodi*.

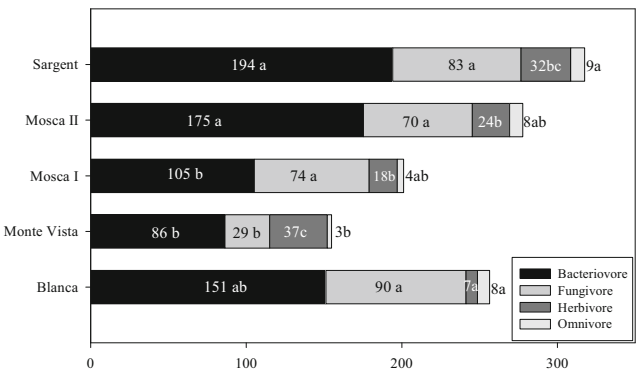

Fig. 1 Number of bacteriovore, fungivore, herbivore, and omnivore nematodes per 200 g of soil from SLV farms. Means followed by the same *letter* are not significantly different ($P \leq 0.05$)

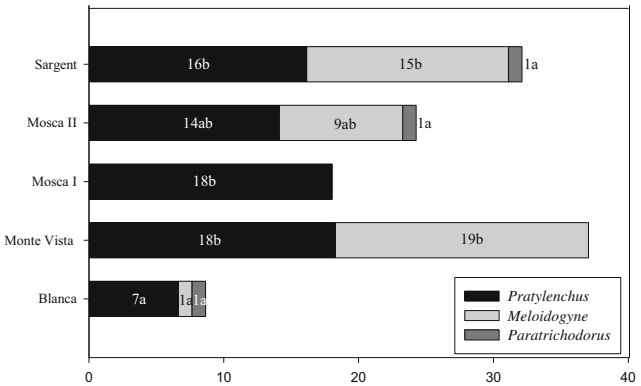

Fig. 3 Number of plant-parasitic nematodes per 200 g of soil in SLV farms. Means followed by the same *letter* are not significantly different ($P \leq 0.05$)

Targeted Microbiome Analysis—ANOVA

Total abundance of bacteria (16S rRNA copies g^{-1} soil), assessed by qPCR, was highest at the Sargent farm compared to the other farms ($P = 0.024$) (Fig. 4). Based on a literature search, we identified 35 genera that have previously been identified as potential biocontrol agents of plant-parasitic nematodes [34–36]. Differences in the total abundance (genus-specific 16S rRNA copies g^{-1} soil) between each farm were then tested using a simple one-way ANOVA. A total of 25 of the 36 potential biocontrol bacteria genera were present at each farm, 9 of which differed significantly between farms (Table 1). The genera *Agrobacterium*, *Arthrobacter*, *Bacillus*, and *Pseudomonas* were highest ($P < 0.05$) at the Blanca farm compared to all the other farms. No differences were observed in the abundance of *Pasteuria* spp., which has been regarded as an effective biocontrol of plant-parasitic nematodes, among the SLV soils ($P = 0.768$).

Non-targeted Microbiome Analysis

Principal component analysis of the bacterial microbiome showed distinct groupings with Monte Vista differentiating from the rest of the farms along the 2nd principal component axis (Fig. 5). Nine of the top 10 genera contributing to this axis were positively correlated with the abundance of *Meloidogyne*, whereas only one was positively correlated with the abundance of *Pratylenchus* (Table 2).

Weighted gene correlation network analysis (WGCNA) identified 31 modules of co-occurring genera, and 198 genera were not assigned to a module (identified as gray) (Fig. 6). Nine of the modules (yellow (4), magenta (8), cyan (13), midnight blue (14), light cyan (15), dark turquoise (22), orange (24), steel blue (28), and violet (30)) were positively correlated ($P < 0.05$) with *Meloidogyne* abundance, and two modules (red (6) and violet (30)) were positively correlated ($P < 0.05$)

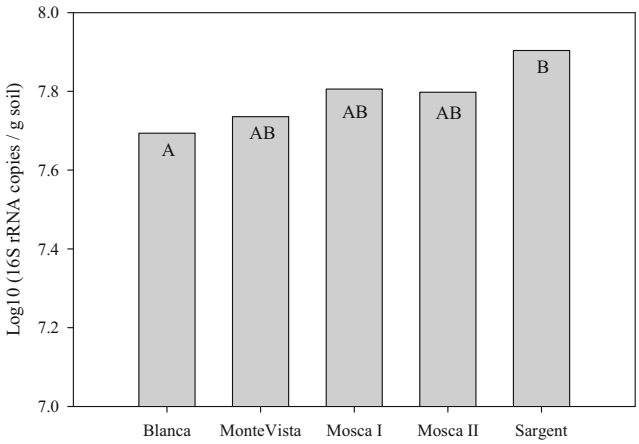

Fig. 4 Total abundance of bacterial 16S rRNA copies per gram of soil. Means followed by the same *letter* are not significantly different ($P \leq 0.05$)

with *Pratylenchus* (Table 3). These modules appear to be closely linked as they are all clustered on the right side of the network (Fig. 6). The largest module of this group was the yellow, which consisted of 25 genera, whereas the smallest was the steel blue, which consisted of 6 genera (Fig. 7).

Four of the modules (turquoise (1), light yellow (18), dark green (21), and dark gray (23)) were negatively correlated ($P < 0.05$) with *Meloidogyne* abundance, and one module (light yellow (18)) was negatively correlated ($P < 0.05$) with *Pratylenchus* (Table 4). The modules were also linked together and formed a cluster in the upper left of the network. The largest module of this group was the turquoise, which consisted of 97 genera, whereas the smallest was the dark grey, which consisted of 8 genera (Fig. 7). The module (dark green) with the highest negative correlation ($r = -0.663$) with *Meloidogyne* abundance contained the genus *Arthrobacter*, which was one of the a priori determined potential biocontrol agents of *Meloidogyne*.

Discussion

The nematode feeding group that predominates in the potato crops of SLV are the bacterivores (50–60%). This is common for agricultural soils where the continued chemical and physical disturbance reduces the flow through the fungal channel, favoring bacteria and bacterivorous nematodes with short-generation times, small body size, and rapid dispersal [38, 39]. Using nematodes as soil health bio-indicators to infer the condition of the soil food web of SLV farms [15, 16, 18, 19], farms Blanca and Mosca I fit in quadrant A of the nematode faunal profile proposed by Ferris et al. [16], in which food web was disturbed, there was a low C/N ratio, and the decomposition channel was mainly bacterial. In contrast, farms Mosca II, Sargent, and Monte Vista fit in quadrant D, where the food web was degraded, the C/N ratio was high, and the decomposition channel was fungal. A disturbed and degraded soil food web is common for conventional agricultural soils under continued disturbance [16]. Fungivorous nematodes affect food web status. In farms Blanca and Mosca I, where the food web was disturbed (based on nematode faunal profile), fungivorous nematodes composed 36% of the total nematode community compared to Mosca II, Sargent, and Monte Vista where the food web is degraded; these nematodes composed less than 25% of the total nematode community. Fungivorous nematodes spread fungi (including nematode biocontrol agents) on their bodies or on the gut within the soil, and grazing of nematodes on microbial populations stimulates microbial growth [39].

In all the farms, the main plant-parasitic nematode we found was *P. neglectus*, which agrees with Pokharel [21] who suggest that *Pratylenchus* is the most prevalent plant-parasitic nematode genus in Colorado. *M. chitwoodi* was only found in Sargent, Mosca II, and Monte Vista farms in equal

Table 1 ANOVA results comparing genus-specific total abundances (16S rRNA copies/g soil) of potential nematode biocontrol agents between farms in the San Luis Valley

Mode of action	Genus	Farm					*P* value	Ref.[1]
		Blanca	Monte Vista	Mosca I	Mosca II	Sargent		
Parasitic	*Pasteuria*	2.865	2.902	2.729	2.886	3.019	0.768	[a]
Opportunistic parasitic	*Brevibacillus*	3.112	3.185	2.914	3.198	3.343	0.312	[a]
	Bacillus	5.331a	4.893b	4.781bc	4.330c	4.880bc	<0.001	[a,b]
	Pseudomonas	4.888a	4.204b	4.948ab	4.537ab	4.447ab	0.046	[a]
Rhizobacteria	*Actinomyces*	–	–	–	–	–	–	[a]
	Agrobacterium	4.363a	3.787ab	3.740ab	3.462ab	3.568b	0.038	[a]
	Arthrobacter	6.292a	5.597c	6.131ab	5.892bc	5.791bc	<0.001	[a,b]
	Alcaligenes[d,e]	3.439	3.280	2.892	3.061	3.353	0.530	[a]
	Aureobacterium	–	–	–	–	–	–	[a]
	Azotobacter	–	–	–	–	–	–	[a]
	Beijerinckia[d,f]	3.722b	3.492b	4.379a	4.623a	4.513a	0.001	[a]
	Burkholderia	3.724b	4.733a	3.141b	3.441b	3.681b	<0.001	[a]
	Brevundimonas	3.249b	2.821b	4.325a	2.876b	3.160b	<0.001	[b]
	Chromobacterium	–	–	–	–	–	–	[a]
	Clavibacter	–	–	–	–	–	–	[a]
	Clostridium	3.703	3.814	3.629	3.718	3.876	0.600	[a]
	Comamonas[d,g]	5.653	5.752	5.640	5.788	5.917	0.093	[a]
	Corynebacterium	2.952	2.824	2.622	2.894	3.200	0.509	[a]
	Curtobacterium	–	–	–	–	–	–	[a]
	Desulfovibrio	–	–	–	–	–	–	[a]
	Enterobacter[d,h]	3.530	3.413	2.834	3.248	3.541	0.785	[a]
	Flavobacterium	4.256	4.682	4.357	4.111	4.509	0.376	[a]
	Gluconobacter	–	–	–	–	–	–	[a]
	Hydrogenophaga	2.700	2.854	2.905	3.325	3.081	0.228	[a]
	Klebsiella	–	–	–	–	–	–	[a]
	Lysinibacillus	–	–	–	–	–	–	[b]
	Lysobacter	4.865a	4.053b	5.182a	5.116a	4.892ab	0.002	[c]
	Methylobacterium	3.236a	3.821a	3.117a	3.164a	3.539a	0.036	[a]
	Mycoplana	4.958	4.699	4.755	4.511	5.014	0.221	[b]
	Phyllobacterium	3.369	3.362	3.751	4.173	3.550	0.288	[a]
	Rhizobium	4.391a	4.173ab	3.244c	4.471a	3.926bc	0.005	[a]
	Serratia	–	–	–	–	–	–	[a,b]
	Sphingobacterium	3.536	3.387	3.613	3.376	3.602	0.751	[a]
	Stenotrophomonas	4.331	3.985	3.907	4.030	4.172	0.706	[a,c]
	Streptomyces	5.320	5.233	5.146	5.043	5.542	0.149	[b]
	Variovorax	3.767	3.190	3.722	3.360	3.085	0.254	[a]

Means with different lowercase letters are significantly different (Tukey HSD, $P < 0.05$). All data were log10 transformed

[a] Reference: [37]

[b] Reference: [35]

[c] Reference: [34]

[d] A unique phylotype at the genus level was not identified. The phylotype used to perform the ANOVA analysis includes all parent taxonomic level (i.e., family) sequences that could not be assigned to a genus

[e] Parent taxonomic level: Alcaligenaceae

[f] Parent taxonomic level: Beijerinckiaceae

[g] Parent taxonomic level: Comamonadaceae

[h] Parent taxonomic level: Enterobacteriaceae

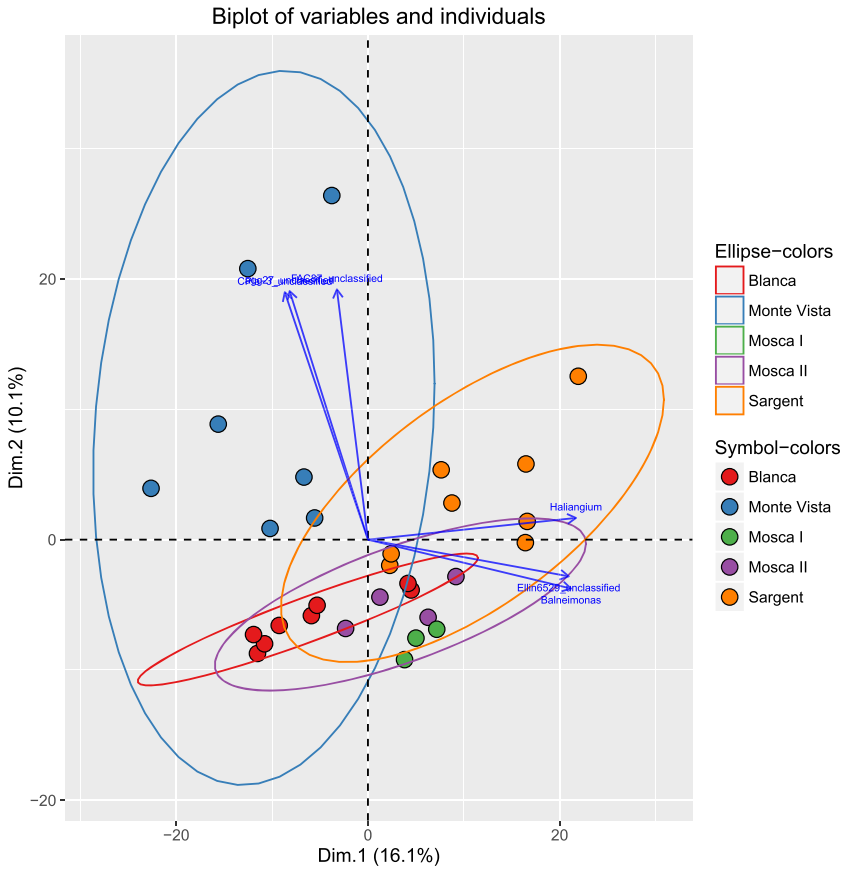

Fig. 5 Principal component analysis (PCA) analysis of the farms in SLV. Additional biplot details can be found in Table 2

proportions than *P. neglectus*. This nematode has been reported as a pest in potato crops of Colorado [20, 40].

Blanca farm has the lowest plant-parasitic nematode population, and the highest presence of the bacteria *Bacillus* spp. and *Arthrobacter* spp. of all the farms. The presence of these bacteria may explain the lower populations of *M. chitwoodi* and *P. neglectus*, because both genera have been reported as nematode antagonists. *Bacillus megaterium*, *B. thuringiensis*,

Table 2 Regression analysis relating the abundance of *Meloidogyne* or *Pratylenchus* abundance and the genus-specific abundance of the top 10 contributors to the 2nd PCA axis (see Fig. 5)

Phylum	Class	Order	Family	Genus	PCA[a] Contrib.	ANcOVA			
						Pratylenchus		*Meloidogyne*	
						Slope	*P* value	Slope	*P* value
Proteobacteria	α-Proteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	0.999	–	0.287	11,155	0.009
Actinobacteria	Acidimicrobiia	Acidimicrobiales	EB1017	Unclassified[b]	0.985	–	0.921	3915	<0.001
Proteobacteria	α-Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Unclassified[b]	0.969	–	0.598	6085	0.008
Acidobacteria	Solibacteres	Solibacterales	Unclassified[b]	–	0.963	–	0.881	7928	0.002
Proteobacteria	α-Proteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	0.958	–	0.301	4698	0.028
Actinobacteria	Actinbacteria	Actinomycetales	Frankiaceae	Unclassified[b]	0.940	–	0.226	1618	0.055
Proteobacteria	α-Proteobacteria	Ellin329	Unclassified[b]	–	0.901	–	0.366	7809	0.003
Proteobacteria	β-Proteobacteria	SC-I-84	Unclassified[b]	–	0.883	–	0.407	1353	0.009
Proteobacteria	α-Proteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	0.878	–	0.749	-	0.130
Verrucomicrobia	Pedosphaerae	Pedospaerales	Ellin515	Unclassified[b]	0.856	531	0.042	930	0.002

[a] Variable contribution to axis ($PC_i^2/\Sigma PC_i^2$ * 100, where PC_i is the axis score for each phylotype)

[b] Phylotype includes all parent taxonomic level sequences that could not be assigned to a unique taxon for the designated taxonomic level (i.e., unclassified)

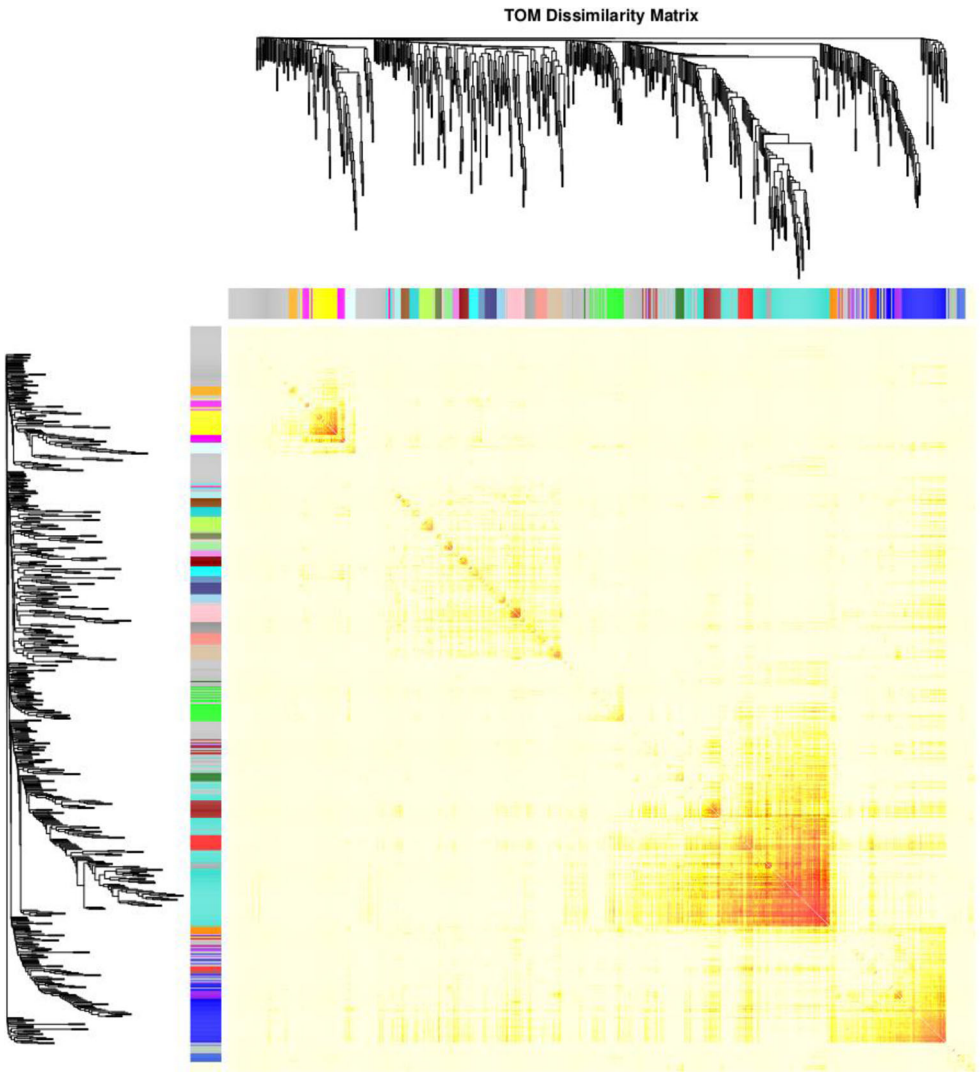

Fig. 6 Weighted correlation network analysis (WGCNA) of soil bacteria communities from five farms (n = 3–8) in the San Luis Valley. Bacterial phylotypes (genera) were clustered by total abundance (16S rRNA copies/g soil) as shown by the dendrogram and correlation heat map. Clusters of co-occurring phylotypes or modules are indicated by the color bars. The intensity of red coloring (heat map) indicates the strength of the correlation between pairs of phylotypes

B. idriensis, and *B. altitudinis* have been reported as efficient biocontrol agents for *M. incognita* by producing nematode-toxic volatiles in the soil [35, 41]. A commercial strain of *B. firmus* produces a biosurfactant compound toxic to *M. incognita*, *Radopholus similis*, *Ditylenchus dipsaci*, and *Rotylenchulus reniformis*, and reduced *M. incognita* and *R. reniformis* in tomato and cotton field crops, respectively [36, 42–44]. *Arthrobacter globiformis*, *A. humicola*, *A. mysorens*, *A. scleromae*, *A. tumbae*, and *A. nicotianae* produce VOCs that are toxic to *M. incognita* [45, 46]. The S-methyl thiobutyrate was the VOC from *A. nicotianae* that showed high nematicidal activity against *Caenorhabditis elegans* and *M. incognita*, even stronger nematicidal activity than the commercial standard dimethyl disulfide [46]. Results obtained by Gu et al. [47] show that VOCs from bacteria usually have more than one kind of nematicidal compounds. The mixture of nematicidal compounds produced by soil bacteria is more effective to control nematodes than a synthetic nematicide composed by one single compound [47]. The VOC identification with nematicidal activities can provide a basic chemical structure for novel nematicidal compounds. Therefore, the mix of VOCs and metabolites produced by species of the genus *Bacillus* and *Arthrobacter* present in the Blanca farm may have an antagonistic effect against *M. chitwoodi* and *P. neglectus*.

In contrast, farm Monte Vista has the highest plant-parasitic nematode populations and the highest population of *Burkholderia* spp. of all the farms. This positive correlation has been previously reported between both *Pratylenchus* sp. and *Helicotylenchus* sp. and *B. tropica*, and between *Helicotylenchus* sp. and *Burkholderia cepacia* in sugar cane crops [48]. Typically, most of the species of *Burkholderia* spp. have an intimate association with plant roots, and have been reported as biocontrol agents of soil-borne pathogens, nitrogen fixers, and nematode antagonists [48–50]. However, the species within this genus of bacteria present in Monte Vista farm are positively correlated with *M. chitwoodi* and *P. neglectus*.

Table 3 WGCNA modules positively correlated with *Meloidogyne* or *Pratylenchus* abundance and the three top phylotypes associated with each module

Module	*Pratylenchus*		*Meloidogyne*		Taxonomy					kME
	r	*P*	*r*	*P*	Phyla	Class	Order	Family	Genus	
Yellow (4)	–	0.912	0.626	<0.001	Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae	Unclassified[a]	0.928
					Chloroflexi	Thermomicrobia	Ellin6537	Unclassified[a]	–	0.922
					Proteobacteria	β-Proteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia*	0.900
Red (6)	0.391	0.033	–	0.404	Acidobacteria	Acidobacteria-6	iii1–15	Unclassified[a]	–	0.969
					Acidobacteria	Chloroacidobacteria	RB41	Ellin6075	Unclassified[a]	0.939
					Acidobacteria	Sva0725	Sva0725	Unclassified[a]	–	0.927
Magenta (8)	–	0.255	0.592	<0.001	Chloroflexi	Ktedonobacteria	B12-WMSP1	Unclassified[a]	–	0.917
					Armatimonadetes	Armatimonadia	FW68	Unclassified[a]	–	0.916
					Actinobacteria	Actinobacteria	Actinomycetales	Dermacoccaceae	Unclassified[a]	0.908
Cyan (13)	–	0.661	0.393	0.031	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Caloramator*	0.905
					Proteobacteria	α-Proteobacteria	Rhodospirillales	Rhodospirillaceae	*Azospirillum*	0.897
					FCPU426	Unclassified[a]	–	–	–	0.856
Midnight blue (14)	0.350	0.057	0.362	0.049	Armatimonadetes	Chithonomonadetes	Unclassified[a]	–	–	0.890
					Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Winogradskyella*	0.875
					Proteobacteria	γ-Proteobacteria	34P16	Unclassified[a]	–	0.846
Light cyan (15)	–	0.262	0.558	0.001	Acidobacteria	Solibacteres	Solibacterales	Unclassified[a]	–	0.903
					Proteobacteria	α-Proteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	0.865
					Proteobacteria	α-Proteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	0.863
Dark turquoise (22)	–	0.909	0.374	0.041	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	*Garciella*	0.912
					Chlamydiae	Chlamydia	Chlamydiales	Rhabdochlamydiaceae	*Rhabdochlamydia*	0.860
					Acidobacteria	iii1–8	Unclassified[a]	–	–	0.822
Orange (24)	–	0.824	0.501	0.004	Chloroflexi	TK10	B07_WMSP1	FFCH4570	Unclassified[a]	0.900
					Proteobacteria	β-Proteobacteria	SC-I-84	Unclassified[a]	–	0.850
					Chloroflexi	TK10	B07_WMSP1	Unclassified[a]	–	0.846
Steel blue (28)	–	0.976	0.499	0.004	Planctomycetes	C6	Unclassified[a]	–	–	0.939
					Dlusimicrobia	Unclassified[a]	–	–	–	0.904
					Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae	*Dyella*	0.817
Violet (30)	0.391	0.032	0.455	0.011	WS2	Kazan-3B-09	Unclassified[a]	–	–	0.925
					Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unclassified[a]	0.846
					Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae	*Luteibacter*	0.843

[a] Phylotype includes all parent taxonomic level sequences that could not be assigned to a unique taxon within the designated taxonomic level (i.e., unclassified)

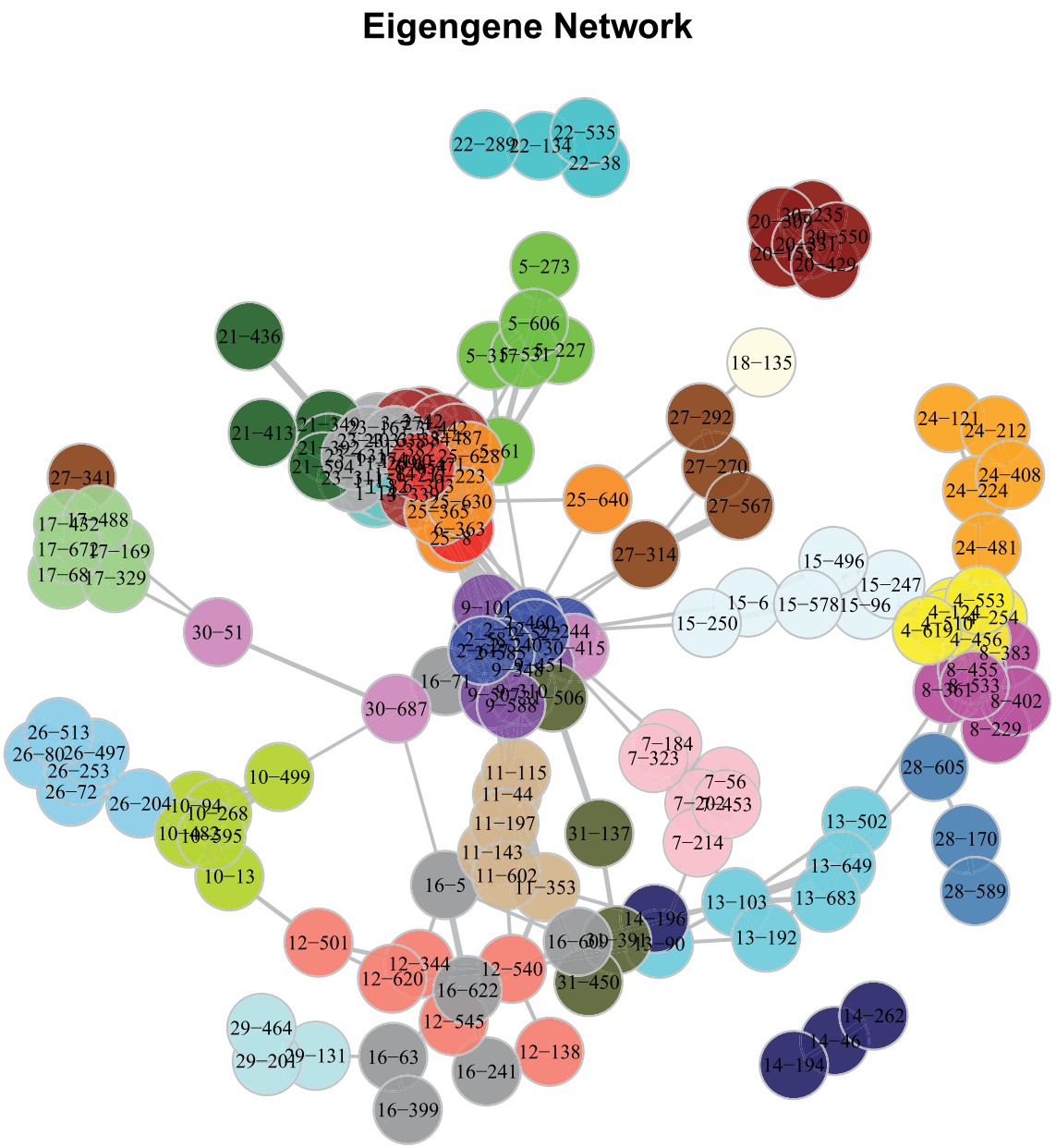

Fig. 7 Neural network depicting the weighted correlation network analysis (WGCNA) of soil bacteria communities from five farms (n = 3–8) in the San Luis Valley. Only the top six phylotypes for each module (*color*) are shown. The first number of each node's label refers to the assigned module, and the second number is a unique identifier for each phylotype (see Table S1). Connection strength is represented by edge width (edges <0.05 omitted)

Furthermore, α-Proteobacteria *Rhodoplanes*, *Phenylobacterium*, and *Kaistobacter* are all positively correlated with *M. chitwoodi*; however, there are no previous reports of such correlations in the literature. *Rhodoplanes* are photosynthetic bacteria with denitrification properties. The ecological significance of this genus remains unclear [51]. *Phenylobacterium* is an inhabitant of the upper aerobic part of the soil, and is a chloridazon-degrading bacterium. Chloridazon is the active ingredient of an herbicide used for broadleaf weeds [52]. Our results suggest that these genera are favoring *M. chitwoodi* directly or indirectly. Symbiotic associations are common between plant-parasitic nematodes and bacteria. The bacterial endosymbiont '*Candidatus* Paenicardinium endonii' has been found in *H. glycines* in reproductive organs of the females and hypodermal chords of males [9]. *Wolbachia*-like bacteria have been reported in the reproductive tract of female adults of *R. similis* [8]. These two endosymbionts could be playing an important role in reproduction and nematode development similar to the α-Proteobacteria *Wolbachia* spp. due to filarial nematodes, where the bacteria manipulate the reproduction of the nematode (i.e., killing or feminization of genetic males, induction of

Table 4 WGCNA modules negatively correlated with *Meloidogyne* or *Pratylenchus* abundance and the three top phylotypes associated with each module

Module	*Pratylenchus*		*Meloidogyne*		Taxonomy					
	r	*P*	*r*	*P*	Phyla	Class	Order	Family	Genus	kME
Turquoise (1)	–	0.141	−0.472	0.007	Acidobacteria	Acidobacteria-6	CCU21	Unclassified[a]	–	0.964
					Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Unclassified[a]	0.953
					Acidobacteria	iii1–8	DS-18	Unclassified[a]	-	0.935
Light yellow (18)	−0.386	0.035	−0.391	0.032	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	*Parapedobacter*	0.838
					Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	0.82
					Proteobacteria	δ-Proteobacteria	Myxococcales	Myxococcaceae	*Corallococcus*	0.807
Dark green (21)	–	0.234	−0.663	<0.001	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	*Arthrobacter*	0.959
					Firmicutes	Bacilli	Bacillales	Planococcaceae	*Planomicrobium*	0.838
					Proteobacteria	δ-Proteobacteria	Myxococcales	Cystobacteraceae	*Cystobacter*	0.825
Dark grey (23)	–	0.626	−0.575	<0.001	Actinobacteria	Rubrobacteria	Rubrobacterales	Rubrobacteraceae	*Rubrobacter*	0.938
					Chloroflexi	Chloroflexi	Chloroflexales	Chloroflexaceae	*Chloronema*	0.933
					Chloroflexi	Anaerolineae	A31	S47	Unclassified[a]	0.908

[a] Phylotype includes all parent taxonomic level sequences that could not be assigned to a unique taxon within the designated taxonomic level (i.e., unclassified)

parthenogenetic reproduction, and induction of reproductive incompatibility) [53]. Furthermore, bacteria associated with the pinewood nematode (*B. xylophilus*) allow the nematode to successfully parasitize the pine tree by degrading α-pinene, which is the main compound in the pine resin that inhibits reproduction and development of pinewood nematodes [14].

Bacteria from the class γ-Proteobacteria (*Luteibacter* spp.) and Bacteroidia (unclassified genus) correlated positively with *M. chitwoodi* and *P. neglectus*, thus suggesting possible symbiosis/mutualism between these bacteria and the two main plant-parasitic nematodes in potato crops of Colorado. Bacteria from these classes have been documented as nematode endosymbionts. The γ-Proteobacteria *Xenorhabdus* and *Photorhabdus* have symbiotic associations with entomopathogenic nematodes (EPNs) *Steinernema* and *Heterorhabditis*, respectively [54]. The infective juveniles of EPNs carry the bacteria and search for their insect hosts. Once the nematode penetrates its host, it releases the bacteria into the insect hemolymph. The bacteria will kill the insect, and degrade its contents to make nutrients available to the nematode. Finally, bacteria re-associate with new generations of nematode-infective juveniles [54]. Furthermore, *H. glycines* endosymbiont 'Candidatus *Paenicardinium endonii*' is related to the class Bacteroidia [9].

Lysobacter is present in higher proportions in the Blanca farm (lowest plant-parasitic nematodes), and in lower proportions in the Monte Vista farm (highest plant-parasitic nematodes). This suggests a possible antagonistic effect of strains of *Lysobacter* present in the Blanca farm and absent in Monte Vista. *Lysobacter* spp. are soil inhabitants that produce a wide variety of lytic enzymes and antimicrobial compounds with biocontrol potential against nematodes [55]. *Lysobacter antibioticus* inhibits hatch and survival of second-stage juveniles of *M. incognita* under greenhouse conditions [45], and *L. enzymogenes* has toxins and enzymes that are active against, *Meloidogyne javanica*, *Pratylenchus penetrans*, and *Heterodera schachtii* [56].

The genus *Beijerinckia* is present in higher populations in farms Mosca I, Mosca II, and Sargent, and in lower proportions in Blanca and Monte Vista, which suggests that it does not have any beneficial or deleterious effect against plant-parasitic nematodes. *Beijerinckia* are characterized as free-living, aerobic, chemoheterotrophic bacteria with the ability to fix nitrogen [57]. There is a report on *Beijerinckia* as a nematode antagonist; however, its mode of action against nematodes is not clear [37]. The strains present in *Beijerinckia* do not have any effect on plant-parasitic nematodes in Colorado.

In summary, the lower number of plant-parasitic nematodes in the Blanca farm may be partially explained by the higher abundance of *Bacillus* spp., *Arthrobacter* spp., and *Lysobacter* spp. These three genera have been reported as antagonists to plant-parasitic nematodes by the production of VOCs, metabolites, toxins, and enzymes. Furthermore, the presence of fungivorous nematodes (36% of the total nematode community) may be contributing indirectly to the lower populations of *M. chitwoodi* and *P. neglectus* by spreading antagonistic fungi within the soil profile; however, we did not quantify the fungal communities of these soils. Due to the correlative nature of the work reported in this manuscript, additional studies will be required to determine if the identified genera (e.g., *Bacillus*, *Arthrobacter*, and *Lysobacter*) truly act as plant-parasitic nematode antagonists in potato soils. Therefore, we

are conducting further studies with isolates of these genera from the Blanca farm soil to evaluate their effectiveness as biocontrol potential to *M. chitwoodi* and *P. neglectus*.

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