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Original article

Nematode community structures in different deciduous tree fruits and grape in Colorado, USA and impact of organic peach and apple production practices

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ABSTRACT

Although free-living nematodes improve soil health and crop production, plant-parasitic nematodes (PPNs) damage crop plants and reduce crop yield. In this investigation, surveys were conducted to determine the PPNs associated with Colorado tree fruit orchards, and research blocks were evaluated to study the effects of crops and production practices on nematode community structure, a soil health indicator. During the surveys, a total of 16 PPN genera were observed: eight in grape, eight each in pear, cherry and peach, seven in apple and 11 each in prune, plum and apricot soils. *Xiphinema* sp. (dagger nematode), *Pratylenchus* sp. (root lesion nematode) and *Meloidogyne* sp. (root-knot nematode) were the predominant nematodes. Based on the results, *Xiphinema* sp. can be considered an important and other two potentially important PPNs. In the research blocks, nematode diversity, abundance, and structural indices were compared in organic and conventional peach and apple production systems as well as the apple cultivars 'Honeycrisp' and 'Cameo'. Both peach and apple organic production systems had higher nematode diversity and significantly higher total nematodes than their conventional counterparts. A significant interaction of variety and production system in apple was observed for *Ditylenchus* and total fungivores, *Helicotylenchus*, *Paratylenchus*, and *Discolaimium* populations. In peach, nematode indices such as percentage of fungivores, fungivore/bacterivore and fungivore/(fungivore + bacterivore) ratios, richness, diversity, and Maturity Index were significantly higher in organic soils. In apple, the Enrichment Index and Structure Index were significantly higher in the organic production systems, whereas the Basal Index was significantly lower in organic production systems as compared to the conventional ones. Subsets of other parameters studied were not significantly different between the organic and conventional production system in both apple and peach crops. These results indicate that crop type as well as management practices influence nematode communities and that organic apple and peach production may be better than conventional apple and peach production with respect to improved nematode community structure. However, soil improvement such as addition of organic matter, especially in conventional production systems and in apple orchards, might improve soil health and productivity.

1. Introduction

The association of nematodes of different trophic groups including plant-parasitic nematodes is important for crop production. Because plant-parasitic nematodes (PPNs) often cause economic damage, they are of special interest to many producers; in contrast, free-living nematodes (FLNs) are correlated with plant productivity [1] but are often neglected. The PPN-associated damage potential in a crop is mostly estimated by their numbers in the soil at planting time. The composition of nematode communities (plant-parasitic and free-living) and the genera, species, and abundance (number) of such nematodes may be used as bioindicators of soil health [2], an important aspect of sustainable crop production systems. The FLN to PPN ratio may be used as a crude estimate of soil health, as there is a negative correlation between PPNs and FLNs [3], and PPNs impact soil quality negatively.

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Western Colorado, which has a semiarid climate with 20 cm of annual rainfall and heavy calcareous clay soils with high pH and low organic matter content, has increasing organic fruit production acreage. Fruit cultivation, especially in deciduous tree production systems, differs from annual or biennial crop production. Deciduous fruit production adds debris to the soil as a source of organic matter, unless leaves and other materials are removed from the orchard. Organic production systems provide another variable in plant nutrient management because soil amendments such as compost, chicken manure or other materials added as a source of plant nutrients also add organic matter, a major food source for soil microbes including nematodes. Organisms in the soil food web depend on plant and other resources, including different types of added organic and green manures, and respond in characteristic ways to enrichment of their environment by organic matter [4]. Soil amendments increase fertility and organic matter and also may suppress root diseases by increasing the levels of competitive, predaceous, or antagonistic microorganisms present in the soil [5]. In general, a healthy soil with its high biological diversity harbors a diverse assemblage of organisms, including nematodes. Without high biological diversity, a soil ecosystem may be vulnerable to environmental change, disturbance, or other stresses [6].

Biologically, soil ecosystems support a diversity of soil organisms such as fungi, bacteria, algae, protozoa, arthropods, and nematodes [2]. Bacteria and fungi form the first level of the soil food web; protozoa, microbivorous nematodes and microarthropods form the second level. The top of the soil food web consists of higher level predators, including millipedes, centipedes and earthworms, which keep the protozoa and nematodes from overeating the bacteria and fungi. The benefit for plants is a slow, sustained release of nutrients which will not quickly leach away.

Because of the tremendous diversity of nematodes and their participation in many functions in the soil food web, several researchers have assessed soil quality by quantifying the nematodes in different families or trophic groups [7]. Being more stable in number than bacteria in response to changes in moisture and temperature, nematode populations may also be more useful ecological indicators [7].

Several ecological indices have been applied to soil nematode communities. For example, the Plant Parasite Index (PPI) and Maturity Index (MI) assign values on a colonizer–persister scale to indicate the degree of ecological succession in plant-feeding and other soil nematodes [8]. Nematode indices such as Structure Index (SI), Enrichment Index (EI), and fungivore to bacterivore (F/B) ratio can be used to indicate the soil food web condition [9], thereby reflecting soil health. The SI indicates the prevalence of trophic linkages in the soil food web [10]; BI and SI may be more suitable as general indicators for the health status of a soil; a high BI indicates poor ecosystem health, but a higher SI indicates a well-regulated, healthy ecosystem [11]. The F/B ratio may reflect decomposition and mineralization pathways [12]. Plotting EI versus SI provides a framework for nematode faunal analysis as an indicator of food web condition [9,13]. An inverse relationship exists between the nematode MI and PPI Index under enriched nutrient conditions [8]. For example, higher PPI values were observed in soils from perennial crops or pastures than in soils from tilled crop fields [14]. Additionally, nematode community composition varies with differences in soil types, such as sand, silt, or clay percentage, even with similar management practices [15], as well as with difference in soil pH [16].

Although the use of organic materials in perennial crop production can profoundly affect the structure of the soil food web [17], limited information exists about such effects in Colorado fruit orchards. Similarly, little information is available on nematode community, population, diversity, and ecological indices in Colorado fruit crop production systems, especially for peach crops and organic production systems. Information about these effects could be valuable for enhancing the productivity and sustainability of these systems.

Thus, these studies were carried out with the following objectives: 1) to study the importance of nematodes associated with different fruit crops in Colorado, 2) to estimate the soil health condition of the orchards, and 3) to understand the impact of fruit crop types (peach and apple) and production practices (organic and conventional) on soil health as indicated by nematode community indices.

2. Materials and methods

2.1. Description of research site

The survey site was located in a western region of Colorado called the Grand Valley, covering part of Mesa, Delta, and Montrose counties. This area has a semiarid climate with heavy, calcareous, clay to loamy soils with high pH; the irrigation water is from the Colorado River or Uncompahgre River and has pH near 8.0. The valley receives annual precipitation of about 20 cm, and fruit is one of the major agricultural commodities. Because of low pressure of pests and diseases and a long dry period of about 300 sunny days, the valley produces high quality fruit. Organic fruit production is an increasing trend.

2.2. Plant-parasitic and free-living nematodes in different fruit crops

Three grape vineyards and 15 orchards were sampled: three each of peach, apple and cherry; two each of prune and apricot; and one each of pear and plum. These represented different fruit-growing areas in Mesa, Delta, and Montrose counties in Western Colorado. Each orchard varied in number of fruit crops, but each crop with more than a hectare was selected to represent an orchard for each fruit type. An orchard with multiple crops with more than 1 ha in each fruit type was considered one orchard for each fruit type. In each orchard for each fruit type, ten fruit trees were randomly selected. Ten soil cores from each tree in each site were collected in a zigzag manner from just below the canopy by a soil auger at a 0–40 cm depth in July or August 2008 and 2010. The ten cores were mixed thoroughly, and a representative sample of 300–500 cm^3 was removed. These representative samples, collected in separate Ziploc bags, were brought to the laboratory in an ice cooler. In the laboratory, soil was mixed well, clods were broken, and a 100-cm^3 soil subsample was processed. Nematodes (both PPNs and FLNs) were extracted from each sample separately as per the method described below; PPNs were identified to genus level. Numbers of PPNs in each genus and total FLNs were counted to determine the ratio of PPN to FLN. Data from two years were combined.

2.3. Nematode community analysis in organic and conventional fruit production

The research site was located at Rogers Mesa, Delta County in the western part of Colorado at an altitude of 1654 m; the Mesa clay loam soil contained high pH and was irrigated by Uncompahgre River water. Research blocks that had been established in 2000 (peach) and 2001 (apple) were selected in order to compare the conventional with the organic production system in each crop separately. Peach (*Prunus persica*) cultivars selected were 'Cresthaven', 'Newhaven' and 'June Pride' grafted to seedling rootstock planted in 1.5 × 4.5 m rectangles; apple (*Malus domestica*) cvs.

'Cameo' and 'Honeycrisp' were grafted to M 26 and planted 2.1 × 4.5 m apart.

Different peach and apple organic blocks were maintained per Colorado organic fruit production standards in terms of management but were not officially registered as organic due to registration expense. In both apple and peach organic blocks, a 1.8 m wide band of composted cattle manure was applied pre-planting and mixed with soil by disking. Chicken manure was applied every fall at the rate of 1000 kg/ha, in addition to application of Nature Safe (12-0-0) at a 450 kg/ha rate each year. Soil sulfur was also applied annually in the fall beginning in 2003, at a rate of 250 kg/ha/year. For the first 3 years, weed control was accomplished using a weed fabric mat. Subsequently, weed control was done with a flamer and by mowing.

The conventional blocks were maintained following the conventional practices utilized by growers. In these blocks, ammonium nitrate (32-0-0) was applied in 2008 at a rate of 198 kg/ha each year. In 2010, each year ammonium sulfate (21-0-0) was applied at a rate of 169 kg/ha. To supply micronutrients a commercial grade multi-mineral (Albion Multi-mineral) was applied, containing 1% each of Ca, Mg, and Mo, plus 0.5% each of Cu, Fe, Mn, and Zn. All fertilizer and manure applications in all blocks involved banding material into the tree row. Weed control was effected with herbicides and mowing.

Nematode community analysis was compared in peach and apple crops separately where organic and conventional production systems were compared in each crop. In apple, production system (organic vs conventional) was considered as the main treatment and varieties ('Honeycrisp' and 'Cameo' in apple) and ('June Pride' and 'Cresthaven' in peach) were regarded as sub-treatments. In 2008 and 2009, soil samples for nematode analysis were collected in a factorial design with three replications where production system was considered as the main treatment and apple and peach varieties were the sub-treatments. Three random trees together in a row (considered as a replicate) were selected for sampling, with two 200-cm^3 soil cores collected from each tree in August–September 2008 and 2009 at a depth of 45 cm with a soil auger. All six soil cores near the three trees (two cores per tree) were mixed together to make a sample. Three such samples collected from different plots of 2.1 m × 14 m represented a treatment or variety. Each sample was mixed, clods were broken, pebbles were removed, and the composite sample was divided into four parts of about 600 cm^3 soil, from which 100 cm^3soil was processed for nematode extraction.

2.4. Extraction of nematodes from soil

The 100 cm^3soil was spread uniformly onto four layers of tissue paper nested on a milk filter paper on top of stainless steel wire screens (16.5 cm diameter), then placed in a stainless steel pie pan (17.5 cm diameter). Sufficient water was added to ensure the submergence of soils. Each assembly was then covered by an aluminum foil pie pan to minimize evaporation. After 72 h, the tissue, milk filter and soil were discarded. The remaining nematode suspension from the pie pan (clear water with nematodes) was collected in a beaker and put in a cold room until the nematodes were counted. After an hour, the nematode suspension was reduced to 250 cm^3 by careful pipetting at the upper surface of the water. A 5 cm^3 aliquot was transferred to a counting dish (6 cm diameter), and the nematode genera were identified and enumerated under an inverted compound microscope with a 10x ocular and 4x, 10x, and 20x objectives. An average was calculated for the ten samples per orchard per host species irrespective of orchard location.

2.5. Nematode trophic group and community analysis

Nematodes counted were categorized into one of the five trophic groups, bacterivores, fungivores, herbivores, omnivores or predators [18], and their percentages were calculated. The F/B ratio was calculated as a relative proportion as {fungivores/(fungivores + bacterivores)} [18]. The genera in Tylenchidae were classified as fungivores [19]. Nematode community indices including richness (number of species), Simpson's index of dominance, Simpson's index of diversity [20], fungivore to bacterivore (F/B) ratio [12], Maturity index (MI) [21], Enrichment Index (EI), Structure Index (SI) and Channel Index (CI) and Basal Index (BI) [9] were calculated. Due to lack of significant difference in nematode numbers in each genus and group between the two peach varieties, the comparison of peach varieties is not included in this paper.

2.6. Statistical analysis

Survey nematode data were subjected to simple calculation of mean and standard deviation of means using Microsoft Excel. Field experiment data were subjected to two-way analysis of variance (ANOVA) using the general linear model (GLM) procedure in Statistical Analysis System (SAS Institute, Cary, NC). Data were log-transformed [log $(x + 1)$] prior to ANOVA to normalize the data distribution.

Abnormally distributed nematode abundance data (except Rhabditidae, *Aphelenchus, Meloidogyne*, *Paratylenchus*, total nematodes, and total bacterivorous nematodes) were log-transformed [log $(x + 1)$] prior to analyzing the variance (ANOVA) to normalize the data distribution. Untransformed arithmetic means of all data are presented in Table 1. Data for all community indices were normally distributed and thus not transformed prior to analysis. Means for practices and varieties were separately compared at $P < 0.1$, and <0.05 levels for differed and highly differed results, respectively, based on *F*-test values. Additionally, interaction effects between practices and varieties were compared at $P < 0.1$ for differed, and <0.05 for highly differed results based on *F*-test values. Abnormally distributed (skewness >1) nematode abundance and nematode community index data were log $(x + 1)$- or arcsin (sqrt(x/100))-transformed, respectively, prior to ANOVA to normalize the data distribution. Untransformed arithmetic means of all data are presented. Data for all community indices were normally distributed and thus not transformed prior to analysis. Treatment means were separated by a Waller–Duncan *k*-ratio ($k = 100$) *t*-test wherever appropriate.

3. Results

3.1. Nematodes associated with different fruit crops

A total of 16 different genera of PPNs were associated with the different fruit crops (Table 1). The highest numbers of genera (11 genera per crop) of PPNs were observed with plum, prune and apricot. Eight different genera of PPN were associated with grape: *Aphelenchus, Criconemoides* including *Mesocriconema, Ditylenchus, Helicotylenchus, Meloidogyne, Pratylenchus, Trichodorus,* and *Xiphinema*. Seven genera–*Aphelenchus, Criconemoides, Ditylenchus, Paratylenchus, Pratylenchus, Tylenchulus,* and *Xiphinema*–were associated with apple. Similarly, we observed eight genera associated with peach soils (*Aphelenchus, Ditylenchus, Helicotylenchus, Hoplolaimus, Meloidogyne, Paratylenchus, Pratylenchus,* and *Xiphinema*); eight with pear (*Criconemoides, Ditylenchus, Helicotylenchus, Hoplolaimus, Longidorus, Meloidogyne, Pratylenchus,* and *Xiphinema*), and ten with cherry (*Aphelenchus*, *Criconemoides*, *Ditylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Paratrichodorus*,

Table 1
Abundance (Abun) ± standard deviation (Std) of the mean and Frequency (FQR) of occurrence of different plant parasitic nematodes per 100 cm^3 soil associated with different deciduous fruit trees and grapes in Colorado orchards in 2008–2010.

	Grape[3a]		Pear[1b]		Cherry[3c]		Peach[3d]		Apple[3e]		Plum[1f]		Prune[2g]		Apricot[2h]	
	Abun ± Std	FRQ	Abun ± Std	FRQ	Abun ± Std	FRQ	Abun ± Std	FRQ	Abun ± Std	FRQ	Abun ± Std	FRQ	Abun ± Std	FRQ	Abun ± Std	FRQ
Aphelenchus	6 ± 12	20	0 ± 0	0	1 ± 2	20	3 ± 5	55	18 ± 15	40	23 ± 16	20	45 ± 53	60	19 ± 23	20
Criconemoides	15 ± 27	40	5 ± 3	20	1 ± 2	20	0 ± 0	0	36 ± 77	40	20 ± 22	20	50 ± 66	40	9 ± 8	20
Ditylenchus	71 ± 34	60	3 ± 2	20	14 ± 18	20	3 ± 4	80	52 ± 36	60	192 ± 130	60	30 ± 35	40	4 ± 10	20
Helicotylenchus	98 ± 58	80	417 ± 320	80	34 ± 60	40	125 ± 120	90	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0
Hemicycliophora	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	37 ± 40	20	60 ± 110	50	0 ± 0	0
Hoplolaimus	0 ± 0	0	3 ± 6	20	3 ± 6	20	3 ± 4	40	0 ± 0	0	0 ± 0	0	0 ± 0	0	16 ± 24	40
Longidorus	0 ± 0	0	3 ± 5	20	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	4 ± 7	20
Meloidogyne	73 ± 56	20	44 ± 33	40	133 ± 266	40	47 ± 45	40	0 ± 0	0	369 ± 420	40	596 ± 708	40	230 ± 283	40
Paratrichodorus	0 ± 0	0	0 ± 0	0	16 ± 16	40	0 ± 0	0	0 ± 0	0	0 ± 0		0 ± 0	0	0 ± 0	0
Paratylenchus	6 ± 5	40	0 ± 0	0	1 ± 2	20	10 ± 13	80	14 ± 16	40	40 ± 49	20	0 ± 0	0	32 ± 34	60
Pratylenchus	160 ± 54	80	51 ± 42	80	47 ± 54	80	21 ± 24	80	4 ± 12	20	29 ± 19	60	45 ± 58	60	0 ± 0	0
Rotylenchus	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	20 ± 23	20	60 ± 63	80	0 ± 0	0
Trichodorus	100 ± 67	60	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	93 ± 163	60	0 ± 0	0
Tylenchorhynchus	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	55 ± 47	40	130 ± 212	60	43 ± 44	40
Tylenchulus	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	18 ± 15	40	29 ± 23	20	95 ± 222	40	21 ± 21	40
Xiphinema	36 ± 27	80	58 ± 41	80	34 ± 56	80	53 ± 44	90	36 ± 29	80	148 ± 93	80	39 ± 120	80	21 ± 36	80

[1,2,3] Number of orchards sampled. Ten soil samples were collected from each orchard from a fruit tree type; such as 10 samples from one, 20 from two, and 30 from three orchards in each fruit type. [a] *Vitis vinifera*, [b] *Pyrus* sp. [c] *Prunus avium*, [d] *Prunus persica*, [e] *Malus domestica*, [f] *Prunus americana*, [g] *Prunus domestica*, [h] *Prunus armeniaca*.

Paratylenchus, *Pratylenchus*, and *Xiphinema*). The highest numbers of PPNs were observed with prune and the fewest with apple. Among plant-parasitic nematodes, *Xiphinema* sp., *Pratylenchus* sp., and *Meloidogyne* sp. were commonly observed in almost all crops and locations. *Xiphinema* sp. frequently occurred irrespective of the crops and orchards surveyed, but *Pratylenchus* sp. and *Meloidogyne* sp. varied with orchard as well as crop. The frequency (numbers of samples with the nematode) of each nematode in each crop varied with crops and nematode species and ranged from 20 to 90% in a particular crop irrespective of location (Table 1); *Xiphinema* had the highest frequency in most of the crops. The populations of *Xiphinema* varied with crop and location, ranging from 21 (apricot) to 148 (plum) per 100 cm^3 soil. Low populations of *Criconemoides* were associated with all of the crop species except peach and occurred only in a few locations (Table 1). The ratio of phytoparasitic to free-living nematodes varied with crop, with apple soils having a particularly low PPN/FLN ratio (Fig. 1).

3.2. Nematode community analysis in organic and conventional peach orchards

Peach soils contained nine genera of bacterivores (*Acrobeles, Alaimus, Cephalobus, Eucephalobus, Geomonhystera, Monhystrella, Panagrolaimus, Plectus,* and *Prismatolaimus*), five fungivorous genera (*Diphtherophora, Ditylenchus, Filenchus, Tylencholaimellus,* and *Tylencholaimus*), four herbivores (*Helicotylenchus, Meloidogyne, Paratylenchus,* and *Pratylenchus*), three omnivores (*Eudorylaimus, Microdorylaimus,* and *Aporcelaimellus*), and three genera of predatory nematodes (*Clarkus, Thonus,* and *Tripyla*) (Table 2). The populations of *Alaimus, Cephalobus,* and *Eucephalobus* among bacterivores, *Meloidogyne* among herbivores, *Eudorylaimus* and *Aporcelaimellus* among omnivores, and *Clarkus* and *Tripyla* among predatory nematodes were significantly higher in the organic production system compared to the conventional production system (Table 2). The populations of all five groups except the bacterivores were significantly higher in the organic than the conventional system. Similarly, the percentage of bacterivores and fungivores, F/B, Diversity, F/(F + B), richness and Maturity Index (MI) were significantly higher in the organic peach production system but not the percentages of herbivores, omnivores and predators, as well as the Dominance Index, Channel Index (CI), Basal Index (BI), Enrichment Index (EI), and Structure Index (SI) (Table 3).

3.3. Effect of production system (organic versus conventional) and apple cultivar ('Cameo' versus 'Honeycrisp') on nematode community abundance and indices

A total of 32 nematode genera were found associated with apple; total nematode abundance was significantly higher in the organic apple production system than in the conventional system. Among these genera, 15 genera or groups of bacterivorous nematodes were observed without variation (i.e., non-significantly different) in the organic compared to the conventional system for 'Cameo' and 'Honeycrisp' varieties grafted to M26 rootstocks (Table 4). Among bacterivorous nematodes, only *Cephalobus* and *Panagrolaimus* populations were significantly higher in the organic apple production system as compared to the conventional apple production system. The populations of bacterivores were not significantly different between apple cvs. 'Honeycrisp' and 'Cameo'. A higher diversity of bacterivorous nematode genera was observed in 'Cameo' compared to 'Honeycrisp' apple soils.

Several genera of bacterivores were detected in the conventional apple soils: *Acrobeloides, Cephalobus, Cervidellus, Diploscapter, Eucephalobus, Geomonhystera, Panagrolaimus, Plectus,* and

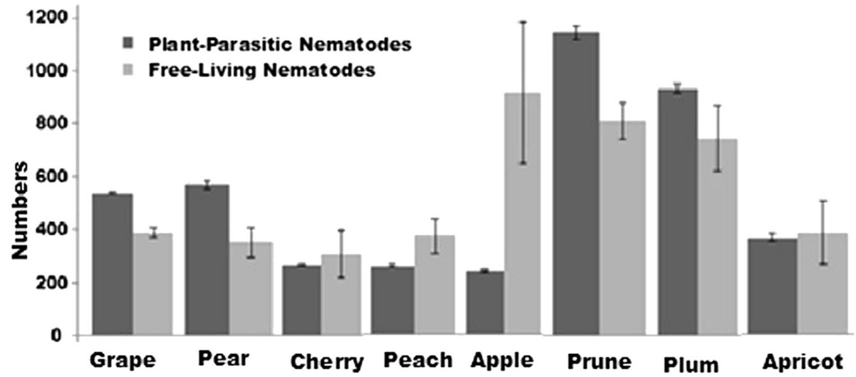

Fig. 1. Percentage of free-living and plant-parasitic nematodes and the standard error of the means associated with different fruit crop soils (per 100 cm^3) in western Colorado, average of 2008 and 2010.

Table 2
Nematode abundance ± standard error of the mean (average of three replications) in conventional and organic peach (*Prunus persica*) orchards at Rogers Mesa, Western Colorado Research Center in 2008 and 2009.

Nematode genus	c-p value	Peach orchard system	
		Conventional	Organic
Acrobeles	b2	0 ± 0	9 ± 4.48
Alaimus	b4	0 ± 0	6** ± 0.88
Cephalobus	b2	14 ± 8.41	25** ± 7.50
Eucephalobus	b2	1 ± 0.66	10** ± 2.60
Geomonhystera	b2	36 ± 23.72	12 ± 7.88
Monhystrella	b2	0 ± 0	5 ± 3.17
Panagrolaimus	b1	2 ± 0.88	0 ± 0
Plectus	b2	4 ± 2.08	6 ± 1.00
Prismatolaimus	b3	4 ± 1.52	8 ± 3.84
Total bacterivores		60 ± 31.20	82 ± 16.12
Fungivores			
Diphtherophora	f3	3 ± 1.73	15 ± 9.33
Ditylenchus	f2	3 ± 2.40	5 ± 2.90
Filenchus	f2	1 ± 1.00	6 ± 5.03
Tylencholaimellus	f4	2 ± 1.15	16 ± 8.18
Tylencholaimus	f4	0 ± 0	9 ± 5.54
Total		8 ± 3.48	44** ± 10.81
Herbivores			
Helicotylenchus	h3	1 ± 1.33	7 ± 6.66
Meloidogyne	h3	3 ± 1.52	36** ± 6.50
Paratylenchus	h2	5 ± 2.40	11 ± 5.81
Pratylenchus	h2	3 ± 3.33	10 ± 7.09
Total herbivores		12 ± 5.84	62** ± 12.06
Omnivores			
Eudorylaimus	o4	3 ± 0.88	15* ± 3.33
Microdorylaimus	o4	0 ± 0.33	27 ± 17.16
Aporcelaimellus	o5	0 ± 0	8* ± 2.18
Total omnivores		3 ± 1.15	51* ± 22.14
Predators			
Clarkus	P4	2 ± 0.57	12** ± 2.30
Thonus	P4	0 ± 0	3 ± 2.40
Tripyla	P3	0 ± 0	4* ± 0.88
Total predators		2 ± 0.57	19** ± 3.60
Total nematodes		86 ± 33.96	264** ± 47.75

The c-p category [21] of soil nematodes (1–5) in a colonizer-persister series, ranging from extreme r-to extreme K-strategists.
*, ** - Differences in rows between conventional and organic treatments were at * = $P < 0.1$ and ** for $P < 0.05$ levels, respectively, based on *F*-test values.

Table 3
Nematode community indices in conventional and organic soils in peach (*Prunus persica*) blocks, average of three replications at Rogers Mesa, Western Colorado Research Center, 2008–2009.

Nematode index[a]	Conventional	Organic
Percentage bacterivores	62.85	30.90*
Percentage fungivores	9.07	16.19**
Percentage herbivores	18.86	25.61
Percentage omnivores	6.01	17.47
Percentage predators	2.53	8.01
F/B	0.15	0.53**
F/(F + B)	0.13	0.34**
Richness	11.83	18.33**
Simpson's Index of Dominance	0.21	00.08
Simpson's Index of Diversity	05.78	12.16**
Maturity index (MI)	2.47	03.19*
Enrichment index (EI)	12.24	11.34
Structure index (SI)	54.93	86.14
Channel index (CI)	33.91	100.00
Basal index (BI)	40.78	13.61

*, ** - Differences in rows between conventional and organic treatments were at * = $P < 0.1$ and ** for $P < 0.05$ levels, respectively, based on *F*-test values.
[a] Abnormally distributed (skewness >1) nematode abundance and nematode community index data were log ($x + 1$)- or arcsin(sqrt(x/100))-transformed, respectively, prior to ANOVA to normalize the data distribution.

Prismatolaimus, and unidentified members of the Rhabditidae. Genera occurring in organic apple soils were *Acrobeles*, *Acrobeloides*, *Alaimus*, *Cephalobus*, *Cervidellus*, *Geomonhystera*, *Heterocephalobus*, *Monhystrella*, *Panagrolaimus*, *Plectus*, *Wilsonema* and Rhabditidae (Table 4). Significantly higher numbers of *Cephalobus* and *Panagrolaimus* (bacterivores), *Aphelenchoides* and *Filenchus* (fungivores), *Helicotylenchus* (herbivores), *Mesodorylaimus* (omnivores) and *Discolaimium* (predators) were observed in the organic apple production system than in the conventional production system. Soils associated with apple cv. 'Cameo' had significantly higher total fungivore populations compared to 'Honeycrisp' apple (Table 2), even though the rootstocks were the same. However, no such differences in nematode numbers belonging to different genera and groups between 'Cresthaven' and 'June Pride' peach varieties were observed.

The abundance of six herbivore genera–*Helicotylenchus, Meloidogyne*, *Merlinius, Paratylenchus, Pratylenchus,* and *Xiphinema*–was not significantly different in organic compared to conventional production systems, as well as 'Honeycrisp' compared to 'Cameo' apple varieties. However, the interaction of variety with production system was significant for *Helicotylenchus* and *Paratylenchus* abundance. The numbers of four genera of omnivores were not significantly different between the organic and the conventional production systems and 'Cameo' versus 'Honeycrisp' varieties. Only one predatory nematode, *Discolaimium*, was observed in the apple soil samples, and it was significantly more abundant in the organic production system than in the conventional system and 'Honeycrisp' than in 'Cameo' apple. The interaction of production system

Table 4
Nematode abundance per 100 cm^3 soil (average of three replications) in conventional and organic ‘Cameo’ and ‘Honeycrisp’ apple (*Malus domestica*) at Rogers Mesa, Western Colorado Research Center, 2008–2009.

Nematode genus	c–p value	Practice		Variety		Interaction effect
		Conventional	Organic	‘Cameo’	Honeycrisp	
Bacterivores						
Acrobeles	2	0	1	1	0	
Acrobeloides	2	1	1	1	0	
Alaimus	4	0	1	1	2	
Cephalobus	2	8	1**	5	4	
Cervidellus	2	1	1	1	1	
Diploscapter	1	1	0	1	0	
Eucephalobus	2	1	0	1	0	
Geomonhystera	2	9	2	4	7	
Heterocephalobus	2	0	1	1	0	
Monhystrella	2	0	2	1	1	
Panagrolaimus	1	2	67**	38	30	
Plectus	2	3	3	3	3	
Prismatolaimus	3	1	0	1	0	
Rhabditidae	1	15	20	18	18	
Wilsonema	2	0	1	1	0	
Total		40	99	75	65	
Fungivores						
Aphelenchoides	2	0	15**	11	5	
Aphelenchus	2	8	6	10	4	
Ditylenchus	2	6	11	13	4	**
Filenchus	2	0	3**	1	2	
Psilenchus	2	1	0	0	1	
Tylencholaimus	4	1	1	1	0	
Total		16	32**	34	14**	**
Herbivores						
Helicotylenchus	3	2	6**	4	4	**
Meloidogyne	3	4	4	4	3	
Merlinius	3	1	0	1	0	
Paratylenchus	3	7	10	10	7	**
Pratylenchus	3	11	6	8	9	
Xiphinema	5	3	6	6	3	
Total		27	31	32	26	
Omnivores						
Aporcelaimellus	5	1	1	0	1	
Eudorylaimus	4	1	4	3	2	
Mesodorylaimus	4	0	2*	1	1	
Microdorylaimus	4	2	3	4	1	
Total		3	10	8	5	
Predators						
Discolaimium	5	1	3**	3	0**	**
Total nematodes		87	177**	154	111	

The c-p category [21] of soil nematodes (1–5) in a colonizer-persister series, ranging from extreme r-to extreme K-strategists.
*, ** - Differences in rows between conventional and organic treatments (left two columns) and between ‘Cameo’ and ‘Honeycrisp’ varieties (right two columns) were at * = $P < 0.1$ and ** for $P < 0.05$ levels, respectively, based on *F*-test values.

with variety for this predatory group was significant (Table 4).

In apple soils, only the EI and SI parameters (Table 5) were significantly higher in the organic production system as compared to the conventional production system, but BI was significantly higher in the conventional system compared to organic system. The other parameters were not significantly different between the two systems. The predatory nematode populations were significantly higher in ‘Cameo’ compared to “Honeycrisp’ samples. However, the other parameters were not significantly different between ‘Honeycrisp’ and ‘Cameo’ apple varieties (Table 5). The EI in peach was almost half of that in apple, but opposite was the case of SI (Tables 3 and 5).

4. Discussion

Soil nematodes are ubiquitous and important soil inhabitants. Among these, the plant-parasitic nematode group causes economic damage to crop plants and the free-living group can indicate soil health and/or quality [9]. The importance of plant-parasitic nematode genera in a particular crop in an orchard can be indicated by their density (nematode numbers per unit soil, which is generally 100 cm^3) and frequency (the occurrence of a specific genus in the samples collected from each orchard). From an agricultural perspective, the PPN density is used to determine the economic damage potential and resulting need for nematode management strategies; thus density is more important than frequency. Nematode frequency, used to determine the distribution pattern, can help to establish the importance of the nematodes present in a location. In our investigation, nematode genera with frequency ≥80 were considered common for the locality and crop, but their importance was determined based on both frequency and abundance. We observed 16 genera of PPNs associated with different fruits in different western Colorado orchards and vineyards; *Xiphinema* sp., *Pratylenchus* sp., and *Meloidogyne* sp. were the most common nematodes in each fruit crop and had different densities in all examined locations. *Xiphinema* sp. was present in 80% of the samples examined in all crops; the second most frequent genus was *Pratylenchus* sp., which occurred in variable numbers with 0–80%

Table 5
Nematode community indices in conventional and organic treatments as well as 'Cameo' and 'Honeycrisp' apple (*Malus domestica*) varieties (average of three replications) at Rogers Mesa, Western Colorado Research Center, 2008–2009.

Nematode index[a]	Production system		Variety	
	Conventional	Organic	'Cameo'	'Honeycrisp'
% bacterivores	53.44	49.12	49.18	53.37
% fungivores	15.11	18.92	18.98	15.04
% herbivores	26.78	22.18	24.30	24.66
% omnivores	3.35	7.11	4.58	05.88
% predators	0.39	1.10	1.49	0.00**
Fungivores/bacterivores (F/B)	0.32	0.57	0.53	0.36
F/(F + B)	0.22	0.31	0.29	0.23
Richness	11.83	14.16	12.83	13.16
Dominance	0.13	0.21	0.17	0.17
Diversity	07.84	07.19	07.54	07.49
Maturity index (MI)	01.82	01.77	01.77	01.82
Enrichment index (EI)	66.13	82.52**	72.00	76.65
Structure index (SI)	25.93	57.38*	41.06	42.25
Channel index (CI)	14.02	17.53	16.31	15.24
Basal index (BI)	30.26	12.34*	23.50	19.10

*, * – Differences in rows between conventional and organic, and 'Honeycrisp' (HC) and 'Cameo' (C) under subheadings practices and varieties, and interaction between practice × variety are at $P < 0.1$, and <0.05 levels, respectively.

[a] Abnormally distributed (skewness >1) nematode abundance and nematode community index data were log ($x + 1$)- or arcsin(sqrt(x/100))-transformed, respectively, prior to ANOVA to normalize the data distribution.

frequency in different crops. *Meloidogyne* sp. was observed in high density when detected but at low frequency with a maximum of 40. This information, in combination with density, was utilized to determine the importance of a nematode genus in the locality.

The number of PPN of a specific genus associated with a plant beyond which economic damage occurs is called the economic threshold (ET); it depends upon several factors, especially crop and soil type. The ET of different nematodes has not been established for all fruit crops in all different soil types. Stone fruits, especially peach and cherry, are important crops in many states including Colorado. The ET for important nematodes in fruit crops is known for certain states and locations but not for Colorado. For example, the thresholds in peach in 100 cm^3 soil have been established as >39 for *Mesocriconema* sp., >99 for *Meloidogyne* sp. and >49 for *Xiphinema* sp. in clay loam to clay soils in South Carolina [22]; these ETs are higher than those in Virginia of 20 for *Mesocriconema* sp., 20 for *Meloidogyne* sp., 30 for *Pratylenchus* sp. and four for *Xiphinema* sp. [23]. Such threshold levels in different crops for the Midwestern United States in peach have not been established [24]. The potential damage threshold of *Criconemoides*/*Mesocriconema* in Colorado would be expected to be similar to that for *Mesocriconema* sp. observed elsewhere. In the absence of established economic damage thresholds in Colorado or similar locations, the importance of major nematode species must be estimated according to the available information on ET levels elsewhere.

Based on ETs on peach in South Carolina and Virginia, the observed populations of *Xiphinema* sp. in peach, plum and pear are suspected to be higher than the ET level. However, the ET of this nematode in plum and pear soils is not known. Although *Xiphinema* sp. may directly damage tree roots, the indirect impact of this nematode via transmitting plant viruses may be more important than direct impact because the observed numbers exceed the 10 nematodes per 100 m^3 soil sufficient to transmit Cherry Rasp Leaf Virus [25]. This virus, commonly observed in cherry and apple, is a potential threat to cherry production in Colorado [26]. In addition, *Xiphinema* sp. predisposes plants to infection by fungal or bacterial soilborne pathogens and to the effects of environmental stresses in Colorado, such as cold temperatures and adverse soil conditions such as high pH and micronutrient deficiencies. *Xiphinema* could be the most important PPN genus observed in our investigation.

High frequencies but low numbers of *Pratylenchus* were observed in all crop soils examined except apricot. Because the density of *Pratylenchus* was lower than the 30 nematodes per 100 cm^3 soil ET level in Virginia [23], this genus may not currently be important in Colorado peach production. However, the ET may be different for Colorado soils, climate and crops, as well as for replanted orchards. *Meloidogyne* sp. was observed in all orchards except apple. The density of this nematode was higher in cherry, prune, plum, and apricot soils than in the other orchards studied. When detected, the population of *Meloidogyne* sp. exceeded the ET level of >99 established in South Carolina and >20 in Virginia per 100 cm^3 soil for clay soil for peach crop. However, the low frequency of this nematode indicates that currently it is not widely distributed and may not be important except in localized situations.

One important issue in stone and pome fruit production in Colorado and elsewhere is the replant problem; one of the causes may be phytoparasitic nematodes, specifically *Pratylenchus*, *Mesocriconema, Meloidogyne* and *Xiphinema* [27]. The importance of nematodes in replant problems may vary with several factors such as nematode species and densities, crop plants, soil types, and environmental conditions. The nematode genera with potential to cause replant problems are present in Western Colorado, although their exact role in causing replant problems in Colorado is not well known. Indeed, we discovered growers treating replant soil with a nematicide without assessing nematode populations present, under the assumption that nematodes might be playing a role in replant problem. This prophylactic application wastes chemical resources and negatively impacts the soil environment.

Among ectoparasites, *Helicotylenchus* had high populations in grape, pear, cherry, and peach soils. Its exact role in fruit production is not well known. *Criconemoides* sp., commonly observed in prune, plum and apple soil samples, occurred in low numbers, indicating that they may not be important. This genus causes peach decline in the Eastern United States including North and South Carolina and was present in the Colorado soil samples from all crops in the present study except peach. *Criconemoides* (which we did not distinguish from *Mesocriconema*) was absent in heavy soil and from the peach soils in Colorado. Consequently, peach tree decline due to this nematode in Colorado peach orchards is unlikely to occur in the near future, as most of the fruit trees in Colorado are grown in heavy soil (high clay and low organic matter, <1.5%) and this group of nematodes favors light soils. At present, *Xiphinema* seems important and *Meloidogyne* and *Pratylenchus* potentially important plant-parasitic nematodes in Colorado fruit orchards.

The nematode community composition (plant-parasitic and free-living) may be used as a soil health bio-indicator because the composition correlates well with nitrogen cycling and decomposition, two critical ecological processes in soils [2]. The ratios of FLNs to PPNs were lower in grape, pear, plum and prune soils indicating poor soil health [3] compared to cherry, peach, and apple soils, that may be due to difference in soil management practices utilized by different growers in different orchards and fruit crops. This was in contrast to our assumption that returning plant materials in the soil in all fruit types as organic matter enhances microbial populations thereby increasing FLN populations. Perhaps the high quantity of applied fertilizers had contributed to the change in nematode communities, as nematode community structure is also affected by soil nutrients [13]. Additionally, the different grasses and crop types affected nematode communities as occurs with other cropping systems and to some extent crop types [28]. Our observed differences in nematode community structure (at taxonomic and trophic levels) may also have been influenced by soil pH, as previously reported [3].

Significantly higher numbers of total and most individual genera occurred in the soils of organic apples and peaches than conventionally produced orchards. In this study, 32 and 24 genera or groups of nematodes belonging to different feeding types were observed in apple and peach, respectively. Significantly higher numbers of total nematodes were observed in organic peach (264) compared to conventional peach (86), as well as in organic apple (177) compared to conventional apple (87). This indicated a difference in diversity and density of nematodes in different crops and production systems, which may be due to difference in plant types and or soil management practices. Several plant species in different functional groups were found to affect nematode community composition, where plant species identity was often more important than plant diversity for nematode community composition [29]. The influence of rootstock genotypes on soil microbial communities in apple orchards has also been reported [30]. Soil amendments with composted manure were associated with increased numbers of free-living nematodes [31]. In our studies, nematode populations might have increased in organic blocks but decreased in conventional blocks during more than 10 years of plant growth and soil management causing lower nematode populations in conventional systems. The lower nematode numbers in conventional blocks may possibly have resulted from agricultural management practices such as soil amendment, fertilization, tillage, and pesticide application that might have caused disturbance in the soil ecosystem [8], thereby affecting the soil nematode community structure [10]. Compost application in organic blocks and inorganic fertilizers in conventional blocks might have changed food sources for soil microbes including nematodes, as soil microbe communities including nematodes are sensitive to changes in tillage or food supply [32] and pesticide and fertilizer applications [33]. Under the organic production system, peach soils had higher numbers of nematodes than apple soils; this difference did not occur in conventional production systems. These higher nematode populations could have resulted from difference in response of plants and or soil management practices especially addition of chicken compost in organic blocks.

Fifteen and nine bacterivorous nematode genera were observed in soils of apple and peach, respectively. Bacterivores such as *Acrobeloides, Cervidellus, Diploscapter,* and *Heterocephalobus* and unidentified members of the family Rhabditidae were present only in apple, whereas *Acrobeles, Alaimus, Cephalobus, Geomonhystera, Eucephalobus, Monhystrella, Panagrolaimus, Plectus,* and *Prismatolaimus* were present in both crops. In both peach and apple, higher numbers of bacterivorous genera and individuals occurred in comparison to the other trophic groups. *Ditylenchus, Filenchus,* and *Tylencholaimus* were common to both crops, *Diphtherophora* and *Tylencholaimus* were present only in peach, and *Aphelenchoides, Aphelenchus* and *Psilenchus* were absent from peach. The difference in genera of different nematode groups and their numbers in peach and apple soils indicate that the nematode populations are influenced by crop (Table 1).

Generally, bacterivores with short lifecycles and high reproductive potential most closely mirror the bloom of bacteria; longer-lived, hardier fungivores are indicators of fungal abundance [34]. Fewer fungivorous genera than bacterivore genera were found in both peach and apple soils. Bacterivore populations in organic production systems in our study did not significantly differ from those in the conventional production system. Among bacterivores, three genera (*Acrobeles, Alaimus,* and *Heterocephalobus*) in apple soils and three genera (*Acrobeles, Alaimus,* and *Monhystrella*) in peach soils were depleted in the conventional systems as compared to the organic production systems. In our current study, the lack of significant difference in bacterivore populations between organically and conventionally managed soils was similar to previous results [35,36]. In another investigation, adverse effects of insecticide applications on the abundance of bacterivores and fungivores were found in association with an increase in plant parasites compared to an untreated control [33]. No impact of conventional practices on bacterivore populations was observed in our current study, despite the use of chemicals including herbicides and insecticides for fruit production. The higher diversity and number of bacterivorous nematode genera as compared to the other groups of nematodes were observed irrespective of crop and production system. To provide a healthy soil ecosystem with adequate soil fertility, enrichment-opportunists (bacterivorous nematodes) should be maintained at a high level [34].

In apple soils, fungivore populations were lower in the conventional system than in the organic production system; the remaining trophic groups did not differ between organic and conventional apple. These results clearly indicated that higher diversity and densities of total nematodes were observed in the organic system as compared to the conventional system irrespective of the crop. Significantly higher numbers of fungivorous but not bacterivorous nematodes occurred in the organic production system than in the conventional system, regardless of whether apple or peach was grown. We had expected an increase in numbers and genera of fungivores and bacterivores because of the addition of organic matter. Higher fungivore populations in organic peach and apple soils were observed than in their conventional counterparts. A higher {fungivores/(fungivores + bacterivores)} ratio was observed in organic peach as compared to conventional peach soils but not in organic versus conventional apple soils It is expected that higher soil fungal populations harbor higher numbers of fungivorous nematodes as longer-lived and hardier fungivorous species are indicators of fungal abundance [4].

Thus, the higher number of fungivores in the organic soils observed in the current study, reflecting higher soil fungi populations, is consistent with the previously reported higher fungal populations in a replanted organic apple orchard in Italy than in a conventionally managed orchard [36]. The significantly higher populations of fungi but not culturable bacteria under organic conditions in the Italian study and an organically associated stimulation of fungivorous nematodes in our study provides preliminary evidence that an organic production system favors fungal populations over bacterial populations irrespective of fruit crop. However, additional research under various conditions and with other crops is warranted to make such a conclusion.

In our current study, total herbivore populations associated with organic peach were significantly higher than in conventional peach, unlike apple. Similarly, soil populations of *Helicotylenchus*, an ectoparasite in organic apple, and *Meloidogyne*, an endoparasite in organic peach soils, were significantly higher than in the conventional system of the same crop. These results agree with those of Neher [37], who found greater numbers of herbivorous and bacterivorous nematodes in soils managed organically compared to conventionally managed soils. A significantly higher number of predatory nematodes in peach and organic production blocks than in conventional production blocks in both peach and apple might be due to higher soil disturbance in conventional than organic blocks. Similarly, the occurrence of significantly higher omnivorous nematode populations in organic peach soils compared to conventional peach soils but not in organic apple compared to conventional apple may have resulted from a higher disturbance in soil ecology in peach than apple. Both peach and apple conventional blocks received fertilizers and tillage, either which could have disturbed soil ecology and affected omnivore populations, which are presumably sensitive to soil management practices, like many nematodes [38]. Similarly, significantly fewer predatory nematodes occurred in the conventional production system than in the organic system regardless of crop, again possibly because omnivore and predatory trophic groups are very sensitive to soil disturbance [39]. Most of the genera differing in the organic vs the conventional system were lower in the conventional production system, perhaps because such nematodes were impacted directly or indirectly by management practices [37].

The lower total nematode populations associated with conventional production might have resulted from a reduction in amount or source of organic matter, extensive tillage, or chemical inputs. Similarly, Yeates and King [15] observed higher nematode populations in organic versus conventional grasslands (another perennial crop production system). Inputs in conventional agricultural systems such as fertilizers, pesticides, and herbicides are important, but their use can impact nematode community composition by different mechanisms, both directly and indirectly. Available ammonium, pH, electrical conductivity (EC), and soil texture have been correlated with specific nematode families, even with similar management practices [18]. In one comparison of conventionally and organically managed sites, the effects of different management practices were less important than the effects of soil properties [37].

The high EI and low CI values observed in apple consistently regardless of production system and cultivar did not occur in peach despite similar organic management practices utilized in both crops. These high CI and EI values indicated a greater dominance of the bacterial decomposition pathway [40]. However, a high CI is possible in highly disturbed agroecosystems as well as in undisturbed natural ecosystems [11]. The high CI in apple soils may indicate that apple production systems have a greater abundance of bacterial decomposition with associated bacterial-feeding nematodes. The significantly higher enrichment index (EI) observed in organic apple as compared to conventional apple soils indicates that the former ecosystem is nutrient enriched and contains higher numbers of primary consumers of organic matter [9]. The significantly higher SI observed in organic than conventional apple soils in our current study indicates a difference in the functional diversity of the soil food web in these two systems [17]. This SI difference indicates that the organic apple production system is a better regulated, healthy ecosystem compared to conventional apple production [11]. The CIs in organic peach and apple were higher than in conventional peach and apple, respectively; this index reflects the dominant decomposition pathways, whether by bacterial or fungal decomposition, and the level of stress in the soil community.

A significant interaction of variety and production system (organic vs conventional) was observed on herbivores (PPN), *Paratylenchus*, *Helicotylenchus*, total fungivores, *Ditylenchus*, and the predator *Discolaimium*. This interaction indicates that both factors play a combined role on the abundance of these nematodes. This study indicated that higher diversity in total nematode abundance was observed in apple as compared to peach. Similarly, higher diversity of all trophic groups in apple soils was observed as compared to peach soils, except for predatory nematodes, which were more diverse and numerous in peach than apple.

Colorado fruit orchards in this location seem to have fewer plant-parasitic nematode problems than other crops and states. Currently, only *Xiphinema* sp. appears important enough to potentially damage crops as well as transmit some plant viruses; currently there is a high prevalence of such viruses in Colorado fruit orchards (Pokharel, unpublished). In addition, *Meloidogyne* and *Pratylenchus* could be potentially important depending upon crop, soil, and management practice. To the best of our knowledge, many nematode genera, especially the *Merlinius* found in this investigation, have not been reported before from Colorado tree fruit orchards.

Addition of organic matter, not practiced in conventional production systems at present, may help to increase soil health and system productivity while enhancing soil organic matter decomposition and shifting nematode community structure [41]. Tillage combined with soil organic amendments may increase the activity of beneficial soil inhabitants even in deeper layers; otherwise microbial activity is concentrated within the topsoil [41]. Based on nematode community structure, peach may be better suited for organic production in Colorado than apple; additionally, growers consider peach better than apple because of market and price considerations. The demand for and the prices of organic fruits and vegetables have been increasing [42], thereby providing another inducement for adoption or organic production methodologies. Studies of nematode communities and impacts of fruit production practices over time are needed to understand the changes in soil microbial processes, thereby suggesting soil health management practices useful in semiarid regions with high soil pH for promoting the sustainability of fruit production systems.

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