Brassicaceous seed meal, root removal, and chemical fumigation vary in their effects on soil quality parameters and *Pratylenchus penetrans* in a replanted floricine raspberry production system

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**A R T I C L E   I N F O**

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- Plant-parasitic nematodes
- Biofumigation
- Soilborne disease management
- Soil health
- Microbial community

**A B S T R A C T**

A decline in red raspberry (*Rubus idaeus* L.) crop longevity has been partially attributed to parasitism by the plant-parasitic nematode *Pratylenchus penetrans*. Soil fumigation with 1,3-dichloropropene (1,3-D) and chloropicrin is the most common management practice for *P. penetrans* in this production system, but the effects are variable and there are strict regulations regarding application; alternatives are needed. A three-year study was conducted in a replanted commercial red raspberry field with a history of *P. penetrans* to evaluate brassicaceous seed meal (BSM) soil amendment combined with root inoculum removal (RR) as preplant alternatives to traditional soil fumigation with 1,3-D and chloropicrin. Additionally, the fumigant metam sodium at full and half rates with root removal was evaluated. Treatments were applied once prior to planting and included: BSM at 3.4 t ha\(^{-1}\) with RR, full rate metam sodium (692 L ha\(^{-1}\); Max Fum) with RR, half rate metam sodium with RR (Min Fum), and full rate metam sodium without RR (Max Fum–RR; control). Data collected included: *P. penetrans* population dynamics in soil and raspberry roots, microbial (bacterial and fungal) communities in soil, raspberry vegetative growth, estimated yield, and fruit total soluble solids (TSS) concentration. Population densities of *P. penetrans* in soil and roots were significantly higher in BSM (983–4801 *P. penetrans* g\(^{-1}\) of root) than in Max Fum and Max Fum–RR (32–802 and 40–1509 *P. penetrans* g\(^{-1}\) of root, respectively) during the first two years of the study. By the end of the study there were no differences in *P. penetrans* population densities among the treatments. Root removal did not affect *P. penetrans* densities as there were no significant differences between Max Fum and Max Fum–RR. Min Fum was more effective than BSM at reducing *P. penetrans* population densities, but not as effective as Max Fum or Max Fum–RR. The only difference among treatments regarding the soil microbial community was observed in the first spring after treatment application, when BSM had a soil bacterial community that differed from the other treatments; this difference did not persist into the next sampling date. The most noticeable differences in bacterial and fungal soil communities were due to season, not treatment. There were no significant differences in raspberry vegetative growth the first summer after planting. Yield or TSS did not differ in the second and third summer after planting. The current data show that Min Fum, BSM amended to soil at this experimental rate, and root removal are not effective in reducing *P. penetrans* population densities, but yield and fruit quality are not compromised under the conditions of the experiment.

**1. Introduction**

Floricane red raspberry (*Rubus idaeus* L.) is a valuable crop for the economy of the United States Pacific Northwest (PNW). In 2016, Washington and Oregon produced over 36 million kg of processed red raspberry valued at $58 million (USDA-NASS, 2017). Washington leads the nation in the production of red raspberry for processing. Although total acreage of red raspberry has increased, crop longevity has decreased. Previously, plantings produced for 12 years or longer, but it is now common to remove plantings after only four or five years due to poor production (Hummer and Hall, 2013; Walters et al., 2011). The decline in raspberry crop longevity has been attributed to several...
factors, including pathogenic fungi and oomycetes, pathogenic bacteria, viruses, plant-parasitic nematodes, nutrient deficiencies, and improper soil pH (Merwin et al., 2001).

Pratylenchus penetrans (Cobb) Filippiev and Schuurmans Stekhoven is one of the most important and ubiquitous soilborne pathogens in PNW raspberry production fields and is thought to be a large contributor to the decline in crop health and longevity (McElroy, 1977; Zasada et al., 2015). Pratylenchus penetrans is a migratory endoparasite that moves between soil and plant roots. Using a stylet, this nematode feeds on plant roots resulting in reduced nutrients and water uptake by the plant. Pratylenchus penetrans can survive for extended periods of time in either the soil or roots after a planting has been removed (Kroese et al., 2016).

In the PNW, when a current raspberry planting is deemed unhealthy or too low in productivity, it is common for the grower to remove the planting and replant the same site to raspberry the following spring season. Land suitable for raspberry production is limited and growers have few alternative crops that are of equal value. As part of this replant process, chemical fumigation of the soil commonly occurs for the management of P. penetrans. Nearly all growers in the region practice soil fumigation citing their primary concern as plant-parasitic nematodes (Rudolph et al., 2017). The most commonly used soil fumigants in PNW raspberry production are chloropicrin and 1,3-dichloropropene (1,3-D). Metam sodium is also registered for use in raspberry, but is not widely used because equipment for application is not widely available. Chloropicrin and 1,3-D have been shown to be effective at immediately suppressing P. penetrans, but populations rebound to pre-treatment levels after one to three years (Béalir, 1991; Mazzola et al., 2015; Walters et al., 2017). In addition to limited suitable land for raspberry production and fumigant products, growers face increasing restrictions with respect to the use and application of fumigant products, such as buffer zones, fumigant management plans, posting requirements, and worker protective equipment (US-EPA, 2014).

Chemical fumigation does not address the issue of declining soil quality or soil health. Soil quality is defined as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans" and includes chemical, physical, and biological attributes (Kären et al., 1997; USDA-NRCS, 2017). The commonly used fumigant products are broad-spectrum and non-selectively suppress beneficial microbial populations as well as the intended pathogen populations (Collins et al., 2006; Gamliel et al., 2000). Many soil microorganisms improve soil quality by helping to suppress soilborne pathogen populations, cycle soil nutrients, and breakdown soil organic matter, which contributes to the formation of soil aggregates which help maintain and improve soil structure (Doran et al., 1996). The PNW raspberry grower community needs alternative soilborne disease management practices that can help address the growing economic, regulatory, and biological issues associated with soil fumigation.

Brassicaceae seed meal (BSM) may be a suitable alternative to address some of the soilborne pathogen and fumigant issues in red raspberry production. BSM is what remains after the oil is extracted from various brassicaceae seeds. Plants in the Brassicaceae family produce glucosinolates which can then breakdown to several products including: oxazolidinethiones, nitriles, thiocyanates, and various isothiocyanates (ITCs) (Kirkegaard and Sarwar, 1998). While isothiocyanates vary in volatility and solubility depending upon chemical structure, in general they have been shown to have bactericidal, fungicidal, and nematicidal properties (Brown and Morra, 1997; Kirkegaard and Sarwar, 1998; Zasada and Ferris, 2002). These naturally-occurring ITCs are similar in structure to methyl-isothiocyanate (MITC), the breakdown product of metam sodium (Cox, 2006; Matthiesen & Kirkegaard, 2006).

The use of Brassicaceae plants as cover crops, green manures, or BSM to suppress soilborne pathogens is called biofumigation (Kirkegaard and Sarwar, 1998); this study focused on BSM. The application of BSM is flexible and does not require additional fertilizer. Additionally, BSM has been shown to indirectly suppress soilborne pathogens by altering soil biology (Cohen and Mazzola, 2006; Mazzola et al., 2015). Several studies have evaluated BSM for P. penetrans suppression under controlled conditions (Zasada et al., 2009; Gitot et al., 2013) or in other crop production systems (Cohen et al., 2005; Mazzola et al., 2015). The ability of BSM to suppress P. penetrans in the raspberry production system has not been demonstrated.

Another potential preplant strategy to managing fields infested with P. penetrans is root inoculum removal (DeVetter et al., 2018; Rudolph and DeVetter, 2015). When growers decide to replant a raspberry field, all the infrastructure is removed, such as trellising wire and posts, the canes are mowed, chopped, and incorporated into the soil. This is followed by soil fumigation which may occur within days or months after a planting is removed. In this system, P. penetrans inoculum has the potential to reside in the field in the form of colonized roots from the previous planting. Additionally, roots and crowns can serve as a barrier to fumigant penetration reducing the overall level of nematode control. Pratylenchus penetrans can reside in soil and old plant material for months and sometimes years (Davis and MacGuidwin, 2000; Kroese et al., 2016). Taking the additional step of physically removing old root material containing P. penetrans from the field could reduce the rates of future infection of raspberry by P. penetrans and improve the effectiveness of other preplant management practices.

Another potential alternative P. penetrans management strategy is metam sodium. Although also a broad spectrum soil fumigant like 1,3-D and chloropicrin, metam sodium is not commonly used in the United States PNW raspberry production system. Previous studies have found that metam sodium does suppress P. penetrans (Fallahi et al., 1998; Miller, 1977). However, there is minimal information regarding the efficacy of metam sodium for managing P. penetrans in raspberry.

The objective of this study was to identify alternatives to traditional preplant soil fumigation with 1,3-D and chloropicrin in the raspberry production system in the PNW. Specific objectives were to: 1) compare a minimum rate of metam sodium to the maximum labeled rate for P. penetrans management, 2) determine if root removal prior to fumigation with metam sodium improved P. penetrans management, 3) evaluate the potential of BSM in combination with root removal for P. penetrans population suppression, and 4) evaluate the impacts of the above treatments on soil chemical and biological parameters of soil quality.

2. Materials and methods

2.1. Experimental design

The experiment was conducted from the fall of 2014 to the fall of 2017 (36 months) in a replanted, commercial ‘Cheimaius’ red raspberry field in Lynden, WA, USA (latitude 48° 59′ 36.6″ N, longitude 122° 23′ 08.2″ W). The experiment was established on 0.06 ha area, comprised of one continuous row of raised bed with 3 m of spacing between the experimental bed and the adjacent beds. Prior to this experiment, the field was planted to ‘Cheimaius’ raspberry and there was a history of high population densities of P. penetrans (> 1500 P. penetrans g⁻¹ of root; Zasada and Walters, 2016). The grower removed the above-ground portion of the raspberry plants and all infrastructure associated with the previous planting (posts, wire, drip tape, etc.) was removed prior to treatment application. Treatment plots (9 m × 2 m) were established. Prior to treatment application, eight soil cores, 2.5 cm diameter and 20 cm deep were randomly collected from each plot, combined and thoroughly mixed to make one composite sample. The composite sample was then split into three subsamples to determine baseline data. One set of subsamples was submitted to Brookside Laboratories, Inc. (New Bremen, OH, USA) for soil chemistry analysis. The soil in this field is a Kickerville silty loam, with a pH of 5.7, 6.9% organic matter (OM), a cation exchange capacity (CEC) of 13.2 meq 100 g⁻¹, 204 kg ha⁻¹ of estimated nitrogen (N) release, 173.8 mg kg⁻¹ of phosphorus (P), and 191.2 mg kg⁻¹ of potassium (K). A second set of subsamples was sent to USDA-ARS Horticultural Crops Research Unit.
(Corvallis, OR, USA) for *P. penetrans* extraction and quantification (see below). The mean population density in raspberry roots in this field was 3839 *P. penetrans* g$^{-1}$ of root (1874–9074 *P. penetrans* g$^{-1}$ of root). The third set of subsamples was stored in a freezer at −10 °C for future DNA extraction and molecular analysis (see below).

Experimental treatments were arranged in a completely randomized design (CRD) and replicated four times. Each treatment plot consisted of an area of 9 m long and 2 m wide; the total area of each plot was 16.7 m$^2$. A buffer area 3 m long was established between each treatment plot. Treatments were applied only once prior to replanting. Treatments included: brassicaceous seed meal (3.4 ha$^{-1}$ ground *B. juncea* ‘Pescadero Gold’ mustard meal; Farm Fuel Inc., Watsonville, CA, USA) with raspberry root removal (treatment designation: BSM), full rate fumigation of metam sodium (692 L ha$^{-1}$; Vapam®, Newport Beach, CA, USA) with raspberry root removal (treatment designation: Max Fum), minimum rate fumigation (346 L ha$^{-1}$) with raspberry root removal (treatment designation: Min Fum), and full rate fumigation (692 L ha$^{-1}$) with no root removal which served as the control (treatment designation: Max Fum-RR). The rate of BSM applied was the recommended rate given by the company. Because of the high pathogen pressure in this field and the necessity for commercial raspberry production, it was not possible to include a nontreated control in the experimental design. Few raspberry growers in the region go without preplant soil fumigation (Rudolph et al., 2017).

Raspberry root removal occurred on 29 Sept 2014 and was achieved by using a nursery plant lifter (Plant Lifter 72; Lundey Manufacturing, Tolna, ND, USA) to lift leftover plant roots from a depth of 30 cm to the soil surface. The roots brought to the surface were then collected by hand and removed from the field. The seed meal was applied to the soil surface and then incorporated to a depth of 15 cm using a walk-behind rototiller on 29 Sept 2014. Soil fumigation was conducted by the grower on 10 March 2015 with a custom-built applicator that applied the fumigant at a depth of 40 cm using six duck-foot shanks with a nozzle on each shank. The shanks were attached to a rotovator (Super Cobra; Maschio Gaspardo, Campodarsego, Italy) which had an additional 13 nozzles for applying the fumigant. The fumigant was applied and incorporated at a depth of 20 cm. A roller was pulled behind the tractor to pack the soil immediately after fumigant application. The field was replanted to ‘Cheminais’ raspberry on 16 April 2015 on a 61 cm in-row spacing. The raspberry planting was then managed by the commercial grower throughout the experiment, using conventional irrigation and fertilizer strategies common for the region (PNW Extension, 2007).

### 2.2. Raspberry growth and yield assessment

No crop was harvested during the summer of 2015 because the plants were too young; floricanse raspberries do not produce their first crop until the year following a spring planting. Vegetative growth was measured on 14 July 2015 by counting the number of primocanes on five randomly selected plants in each treatment plot; primocane height of the three tallest canes for each of those five plants was also determined. Due to the extended nature of fruit production, a single floricanse raspberry field can be machine harvested 12–20 times within a single growing season (usually lasting the month of July). For this reason, a yield estimation method (Daubeny, 1986) was conducted on the raspberry crop on 10 June 2016 and 14 June 2017. Three raspberry plants per treatment plot were randomly selected and the total floricanse number from each plant were counted. The number of laterals on two floricanes from each of the selected plants were counted. Then, the fruit, including buds, flowers, and green fruit on two fruiting laterals in five different fruiting zones were counted. Early, mid-season, and late ripe raspberry fruit collections occurred on 10 and 21 June, and 5 July 2016 and 6, 15, and 25 July 2017. At each sampling date, 30 raspberries were randomly selected from each treatment plot, weighed, and frozen for future total soluble solids (TSS) analysis. To perform TSS analysis, the fruit from each treatment plot and time point was macerated in a sample mesh bag (Agila™, Inc., Elkhart, IN, USA) and the juice was strained into a test tube. Three drops of undiluted juice were placed on a digital refractometer (Palm Abbe digital refractometer, Model #PA201; MISCO, Solon, OH, USA) for each measurement. Juice from each treatment plot and time point was analyzed three times, the value of each was recorded, and the mean of the three values was calculated.

### 2.3. Soil chemical, *Pratylenchus penetrans*, and microbial measurements

Eight soil cores, 2.5 cm diameter and 20 cm deep, were collected from the raised bed in all treatment plots in the spring and fall of each year. The eight soil cores were combined to make one composite sample for each treatment plot. Each composite sample was mixed thoroughly and split into three subsamples. One subsample was sent for chemistry analysis (Brookside Laboratories, Inc.), another subsample was stored in a freezer at −10 °C for future DNA extraction and molecular analysis, and the third subsample was used for *P. penetrans* extraction and quantification. Mixed stages of *P. penetrans* were extracted for 5 days from a 50 g subsample of soil using the Baermann funnel extraction method (Zasada et al., 2015). Collected *P. penetrans* were counted and identified using a stereoscope at ×40 magnification. Population densities are expressed as number of *P. penetrans* 100 g$^{-1}$ of soil. To determine *P. penetrans* population densities in roots, roots from the raspberry crop were collected from three randomly chosen plants in each treatment plot, using a square-blade shovel in the spring and fall of each year. Roots < 2 mm in diameter were preferentially selected for *P. penetrans* extraction, rinsed free of soil, and placed under intermittent mist for 5 days (Zasada et al., 2015). Extracted roots were oven dried for 1 week at 70 °C and then weighed. Nematodes were identified and counted as described above and are expressed as number of *P. penetrans* g$^{-1}$ of dry root.

To prepare for soil DNA extraction, frozen soil samples were moved to a refrigerator overnight to thaw. Once thawed, DNA was extracted from 5 g of soil from each plot using the PowerMax Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) per the manufacturer’s instructions. For bacterial analysis, DNA from each sample was normalized to a concentration of 5 ng µL$^{-1}$. Bacterial 16S rDNA was amplified using the forward primer S-D-Bact-0341-b-s-17 and reverse primer S-D-Bact-0785-a-A-21 (Klindworth et al., 2013) modified to contain partial Illumina MiSeq adapter sequences. The 16S Metagenomic Sequencing Library Preparation protocol created by Illumina® (San Diego, CA, USA) was followed for amplicon PCR prior to submission to the sequencing center for subsequent sample preparation and sequencing. For fungal analysis, DNA was normalized to a concentration of 200 ng µL$^{-1}$ using Amicron Ultra centrifugal filters (0.5 mL; EMD Millipore, Billerica, MA, USA). Fungal DNA was amplified using the forward primer ITS1-F (Gardes and Bruns, 1993) and a reverse primer ITS2 (White et al., 1990) modified to contain partial Illumina MiSeq adapter sequences. A similar preparation protocol was used for fungal amplification as was used for bacterial amplification. All amplified samples were sent to Oregon State University Center for Gene Research and Biocomputing (Corvallis, OR, USA) for sample preparation and paired-end 300 bp sequencing on an Illumina® MiSeq sequencer.

### 2.4. Data analysis

Treatment by year interaction analyses were performed followed by individual year analyses. *Pratylenchus penetrans*, soil chemical, and raspberry yield and TSS data were subjected to statistical analysis using Statistical Analysis System (SAS) statistical software (Version 9.3; SAS Institute Inc., Cary, NC, USA). Alpha was set at 0.05 for all data. All data were initially subjected to an analysis of variance test (ANOVA) with Tukey as the post-hoc test. Much of the nematode data had unequal variance. When this occurred, means were transformed using log
(x + 10) and reanalyzed. When this transformation did not correct unequal variance, data were analyzed using a Kruskal-Wallis non-parametric test. All data are presented with original means, even when transformations or non-parametric methods were performed.

Illumina MiSeq sequencing data received from the sequencing facility was processed through mothur software version 1.38.1 (Schloss et al., 2009) to filter low quality sequences, join paired-end reads, classify sequences, and generate phylotype tables. Phylotype abundances were analyzed using STAMP V2 (Parks et al., 2014). R statistical package (R Core Team, 2017) with the “vegan” package (Oksanen et al., 2017) was used to generate non-metric multidimensional scaling (NMDS) ordinations and to test sample groupings by calculating permutation multivariate analysis of variance (PERMANOVA) for sampling date and treatment.

3. Results

3.1. Raspberry growth and yield assessment

Four months after planting, there were no significant differences in either primocane number or height among treatments. The following summer, approximately 15 months after planting, estimated fruit yield in Max Fum was 33% higher than the average estimated BSM yield, but differences were not significant (Fig. 1). There were no significant differences in fruit TSS (data not shown). In the final summer, 27 months after planting, there were no significant differences in yield (Fig. 1) or TSS among treatments (data not shown). When yield was considered across years, the average yield across treatments was similar. The TSS across treatments in 2017 was significantly higher compared to TSS across treatments in 2016 (data not shown).

3.2. Soil chemical measurements

There were very few significant differences in soil chemistry attributes among treatments at all seven sampling points during the experiment. In the fall of 2016 (18 months after planting), CEC was more than 17% higher in Max Fum-RR (18.3 meq 100 g⁻¹) than in Min Fum and Max Fum (15.2 and 15.0 meq 100 g⁻¹, respectively; P = 0.02). The CEC in the spring of 2017 was also significantly different among treatments (P = 0.03). The CEC in Max Fum was nearly 30% higher compared to that of Max Fum-RR (14.6 and 10.5 meq 100 g⁻¹, respectively). Max Fum was not significantly different than BSM or Min Fum (data not shown). There were no significant differences in pH, OM, estimated soil N release (N), P, or K among treatments at any sampling date (data not shown).

3.3. Pratylenchus penetrans population densities in raspberry roots and soil

Soon after planting and fumigation, and six months after BSM application, there were no P. penetrans found in the soil of any of the treatments (Table 1). Six months after planting, the average P. penetrans density in raspberry roots in BSM was nearly 25 fold higher than that of Max Fum-RR and 30 fold higher than Max Fum (P = 0.01; Table 1). A similar trend was observed with P. penetrans population densities in soil, but differences were not significant (P = 0.09; Table 1).

In the spring of 2016, 12 months after planting and fumigation, and 18 months after BSM application, P. penetrans average population densities in raspberry roots remained the highest in BSM (P < 0.001; Table 1). This density was more than 6 fold higher than in both Max Fum-RR and Max Fum. Pratylenchus penetrans average population density in soil was also significantly higher in BSM compared to all other treatments (P = 0.0002; Table 1). In the fall of 2016, 18 months after planting and fumigation and 24 months after BSM application, the BSM treatment had a 33% decrease in average P. penetrans per gram of root compared to six months earlier. However, roots collected from BSM treatment plots still had a greater average population density than those collected from Max Fum and Max Fum-RR (P = 0.0017; Table 1). This pattern was similar in the soil population densities (P = 0.0022; Table 1).

In the spring of 2017, 24 months after planting, raspberry roots collected from BSM had a P. penetrans population density six times greater than the mean population density from raspberry roots grown in Max Fum (P = 0.02; Table 1). The population densities of both treatments were not significantly different than those of Min Fum or Max Fum-RR. Soil populations of P. penetrans were not significantly different among treatments (P = 0.73; Table 1). By the fall of 2017, 36 months after BSM application and 30 months after fumigation, there were no differences in P. penetrans population densities among the treatments in either roots or soil (P = 0.35 and 0.52, respectively). Throughout the study, Max Fum performed nearly identical to Max Fum-RR in its ability to suppress P. penetrans.

3.4. Soil microbial community structure

During the 36 months of the study, differences in bacterial composition were greatest among sampling dates (P = 0.001), rather than among treatments (P = 0.4; Fig. 2). The pretreatment soil bacterial community was noticeably distinct compared to all other sampling dates, but sampling date differences were significant even when excluding pretreatment samples (P = 0.001). Despite the strong seasonal variation in bacterial community composition, there were significant differences among treatments. Results were narrowed to genera that were detected by 25 sequences or more. Pseudomonas spp., Gemmatimonas spp., and unclassified Rhizomicrobium spp. were all more abundant in Max Fum-RR compared to BSM (P ≤ 0.042; Fig. 4). Nitrospira spp., unclassified Gammaproteobacteria, and unclassified Rhodospirillales were more abundant in BSM than Max Fum-RR (P ≤ 0.046; Fig. 4).

Sampling date was also significant with respect to differences in the soil fungal composition (P = 0.001; Fig. 3); differences among treatments were not observed (P = 0.575). When fungal taxa were considered within the two most extreme treatments, BSM and Max Fum-RR, unclassified Nectriaceae, Mycocellicola, unclassified Amphiphilaceae, and unclassified Chytridiomycota were all more abundant in Max Fum-RR compared to BSM (P ≤ 0.028; Fig. 5). Ascomycota, Cryptococcus, and Pyrenomycetae were more abundant in BSM than
RR = soil fumigant metam sodium applied at a rate of 692 L ha⁻¹

4. Discussion

In this multi-year field trial, BSM applied at the recommended rate of 3.4 t ha⁻¹ did not suppress P. penetrans population densities in raspberry soil or roots. The other non-chemical management alternative, root removal, also did not reduce population densities of P. penetrans in soil or roots. Applying metam sodium at the lowest rate recommended on the label (Min Fum) did not suppress population densities of P. penetrans equal to the labeled maximum application rate of metam sodium. The soil treatments had short term effects on soil chemistry and microbial communities. The overwhelming differences among microbial community structure were observed across sampling dates, not among treatments within sampling dates.

Soil fumigation with Telone C-35 (65% 1,3-D and 35% chloropicrin) of 323 L ha⁻¹ without root removal. Applying metam sodium at the lowest rate equivalent to Telone C-35 because of the effective suppression of P. penetrans for over two years. Our results follow what has previously been observed by both Bélair (1991) and Walters et al. (2017); fumigation suppresses P. penetrans populations initially, but populations rebound after one to three years. There are many reasons to adopt the use of metam sodium over products containing chloropicrin and 1,3-D. Metam sodium application is less expensive than Telone C-35 (Walters et al., 2011). With the proper equipment, growers can apply metam sodium themselves, without the need for custom fumigation. The buffer zone associated with metam sodium applied at the full rate of 692 L ha⁻¹ is equivalent to Telone C-35 deep-shank broadcast applied at the full rate of 323 L ha⁻¹ under the same field conditions (both applications un-tared; US-EPA, 2017a, 2017b).

Soil fumigation using metam sodium without root removal performed as well as the equivalent treatment with root removal, Max Fum-RR (P ≤ 0.036; Fig. 5).

Table 1

<table>
<thead>
<tr>
<th>Treatment¹</th>
<th>Spring 2015</th>
<th>Fall 2015</th>
<th>Spring 2016</th>
<th>Fall 2016</th>
<th>Spring 2017</th>
<th>Fall 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSM</td>
<td>0</td>
<td>51 ± 27¹</td>
<td>300 ± 68²</td>
<td>633 ± 161</td>
<td>693 ± 213</td>
<td>607 ± 138</td>
</tr>
<tr>
<td>Min Fum</td>
<td>0</td>
<td>6 ± 3</td>
<td>76 ± 26b</td>
<td>228 ± 66</td>
<td>509 ± 381</td>
<td>963 ± 303</td>
</tr>
<tr>
<td>Max Fum</td>
<td>0</td>
<td>10 ± 6</td>
<td>7 ± 3b</td>
<td>43 ± 15bc</td>
<td>383 ± 108</td>
<td>989 ± 224</td>
</tr>
<tr>
<td>Max Fum-RR</td>
<td>0</td>
<td>0</td>
<td>21 ± 10b</td>
<td>45 ± 31c</td>
<td>359 ± 98</td>
<td>815 ± 394</td>
</tr>
</tbody>
</table>

P. penetrans of raspberry root

<table>
<thead>
<tr>
<th>Treatment²</th>
<th>Spring 2015</th>
<th>Fall 2015</th>
<th>Spring 2016</th>
<th>Fall 2016</th>
<th>Spring 2017</th>
<th>Fall 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSM</td>
<td>983 ± 408a</td>
<td>4778 ± 746a</td>
<td>3165 ± 355a</td>
<td>4801 ± 427a</td>
<td>2786 ± 470</td>
<td></td>
</tr>
<tr>
<td>Min Fum</td>
<td>310 ± 273ab</td>
<td>1509 ± 224a</td>
<td>1529 ± 802ab</td>
<td>2139 ± 1199ab</td>
<td>4083 ± 841</td>
<td></td>
</tr>
<tr>
<td>Max Fum</td>
<td>32 ± 17b</td>
<td>629 ± 175b</td>
<td>143 ± 62bc</td>
<td>802 ± 457a</td>
<td>5176 ± 4138</td>
<td></td>
</tr>
<tr>
<td>Max Fum-RR</td>
<td>40 ± 33b</td>
<td>557 ± 243b</td>
<td>135 ± 110c</td>
<td>1509 ± 518ab</td>
<td>3396 ± 703</td>
<td></td>
</tr>
</tbody>
</table>

1 Treatment abbreviations: BSM = brassicaceous seed meal soil amendment applied at a rate of 3.4 t ha⁻¹ with root removal, Min Fum = soil fumigant metam sodium applied at a rate of 346 L ha⁻¹ with root removal, Max Fum = soil fumigant metam sodium applied at a rate of 692 L ha⁻¹ with root removal, Max Fum-RR = soil fumigant metam sodium applied at a rate of 692 L ha⁻¹ without root removal.

2 Values are the mean of 4 replications ± standard error.

3 Values followed by the same letter in the same column are not significantly different from one another at P ≤ 0.05.

4 No root samples were collected at this sampling date because raspberry had only recently been planted.

Max Fum-RR (P ≤ 0.036; Fig. 5).

Fig. 2. Soil bacterial community structure over time from soil collected from red raspberry in Lynden, WA. Each dot represents one soil sample from a treatment replication and is based on genus-level phylotype abundance. Permutational multivariate analysis of variance was used to determine significance among sampling dates. P = 0.001.

Fig. 3. Soil fungal community structure over time from soil collected from red raspberry in Lynden, WA. Each dot represents one soil sample from a treatment replication and is based on genus-level phylotype abundance. Permutational multivariate analysis of variance was used to determine significance among sampling dates. P = 0.001.
Fum. Root removal would be an additional step that a grower would need to take in order to prepare the field for replanting. A separate study has also observed no significant differences in P. penetrans parasitism and raspberry plant growth after root removal (DeVetter et al., 2018). A potential explanation for the lack of efficacy in root removal could be that finer roots are left behind in the field because it is difficult to see them and physically remove them; they are simply too small. Pratylenchus penetrans preferentially resides in these finer roots that are less than 2 mm in diameter (Davis and MacGuidwin, 2000). It is also possible that there were nematodes missed by fumigation deeper in the soil profile (Kroese et al., 2016) and that these nematodes served as a source of inoculum to newly planted raspberry.

BSM did not suppress P. penetrans population densities relative to soil fumigation. It is possible that an increased application rate would have resulted in increased suppression. Previous studies have evaluated the effects of BSM on P. penetrans with varying rates of suppression. Mazzola et al. (2015) observed suppression of P. penetrans populations after incorporating BSM into apple (Malus domestica) field soil at 6.7 t ha$^{-1}$. However, the P. penetrans population densities in the Mazzola et al. (2015) study were much lower than those observed in our study, ranging from 164 to 843 nematodes per root. Pretreatment population densities were 11–30 times higher in this raspberry field than densities reported in the untreated controls in previous apple field studies. There is potentially a density-dependent component to P. penetrans suppression by BSM, with greater efficacy against low P. penetrans population densities compared to high population densities. A similar effect was observed with the use of oxamyl in raspberry, where P. penetrans suppression was more pronounced in fields with low densities compared to high densities (Zasada and Walters, 2016).

Although these studies illuminate the possible success of BSM soil amendments, a grower would need to weigh that potential success against the high cost. The BSM used in this study costs approximately $5900 ha$^{-1}$ based on the recommended rate. This is much more expensive than a custom nontargeted application of Telone C-35 ($2700 ha$^{-1}$) as well as a metam sodium application ($1200 ha$^{-1}$; Walters et al., 2011). However, the recommended rate of BSM was not effective at suppressing P. penetrans in this study. Doubling the rate used in this study would approximate what was applied in the field study conducted by Mazzola et al. (2015) where effective P. penetrans suppression was achieved. Doubling the application rate would double the cost, bringing the cost of BSM to $11,800 ha$^{-1}$; this expense would be difficult for most raspberry growers to assume.

Raspberry yield and total soluble solids were similar among treatments throughout the study. This differs from previous studies in other perennial fruit crops where yield differences were observed in plants grown in BSM-treated soil compared to fumigated soil. Some of those differences may be attributed to the different Brassica species that were included in the BSM amendments. For example, Brassica napus seed was the sole component of the BSM used in an apple replant study (Mazzola and Mullinix, 2005). Across time, treatments in this study with a BSM component resulted in higher yields than an untreated control. In another apple study (Mazzola and Brown, 2010), the efficacy of a B. napus, B. juncea, or S. alba seed meals were compared to Telone C-17 and mefenoxam in a replanted orchard. All BSM treatments resulted in significantly lower apple yield compared to Telone C-17 or mefenoxam. The authors determined that individual cultivar formulations of seed meal would not be an effective treatment against apple replant disease and created a 1:1 mix of B. napus ‘Athena’ and B. juncea ‘Pacific Gold’. In a separate orchard, the authors compared the BSM mix to Telone C-17 (Mazzola and Brown, 2010). Trunk diameter was not significantly different between the two treatments; both had significantly larger trunk diameters than the untreated control. A BSM 1:1 mix of B. juncea and S. alba at 6.7 t ha$^{-1}$ was compared to Telone C-17 in a replanted apple field (Mazzola et al., 2015). The BSM treatment resulted in significantly higher cumulative fruit yield compared to Telone C-17 or the untreated control.

Although differences among treatments were apparent with respect to P. penetrans population densities, few differences were observed regarding the soil bacterial and fungal communities; this was unexpected based upon the results of previous studies. In the first spring after all treatments had been applied, the bacterial community in BSM was distinct from all other treatments. This distinction lasted longer than previously reported 28 days (Wang et al., 2011), but not nearly as long as previous reports of 2 years (Mazzola et al., 2015). However, in our study, bulk soil was collected while in the other studies rhizosphere soil was collected. At the first sampling date, the bacterial communities from BSM-treated soil clustered together while those from metam sodium-treated soil clustered together. Methyl-isothiocyanate (MITC) is the primary breakdown product of metam sodium (Smelt and Leistra, 1974). Approximately 50% of the degradation of MITC is from
biological, rather than chemical processes (Dungan et al., 2002). Specific microorganisms are adapted to have this function and must be present in order to degrade MITC (Verhagen et al., 1996). These soil microbes may be different from those present in soil treated with BSM. However, as MITC degrades, the soil microbial communities present under the different treatments may become more similar over time.

Across sampling dates, the most distinct differences among bacterial communities existed between Max Fum-RR and BSM. Pseudomonas is a genus of gram-negative bacteria that commonly resides in soil or water and was significantly more abundant in Max Fum-RR compared to BSM soil. Some Pseudomonas species are beneficial, such as 2,4-diace-tylylphloroglucinol (2,4-DAPG)-producing fluorescent pseudomonads which can provide biological control of certain soilborne diseases (Keel et al., 1992; Maurohofer et al., 1995). It is difficult to know which Pseudomonas species are represented in this case, but very few bacteria are soilborne pathogens because they typically prefer an opening or wound in order to infect the plant (Raaijmakers et al., 2009). Nitrospira is a genus of nitrite-oxidizing bacteria and is believed to have an important role in nitrification in agricultural soils (Bock and Wagner, 2006). These bacteria help convert N to more plant-available forms that can be readily taken up by plant roots. This genus was more abundant in BSM soil compared to that of Max Fum-RR. This is most likely due to the presence of more N for these bacteria to consume; the BSM contains approximately 6% N (Gale et al., 2006; Snyder et al., 2009). Fungal taxa differences included unclassified Nectriaceae, which was more abundant in Max Fum-RR compared to BSM. Nectriaceae includes several plant pathogens to raspberry, such as Fusarium spp. (Valulkaïte et al., 2008).

Differences have been observed between BSM-treated and fumigant-treated rhizosphere soil microbial communities in previous studies. Mazzola et al. (2015) observed distinct differences in the rhizosphere microbial community two years after pre-treatment treatments were applied. Fumigated soil microbial communities very closely resembled that of the untreated control, whereas BSM soil microbial communities were significantly distinct. Overall microbial diversity was lower in BSM-treated soil compared to fumigated or untreated soil, but certain beneficial microorganisms were more abundant in BSM soil. An interesting commonality between our study and the previous study (Mazzola et al., 2015), was the proportions of sequences representing Trichoderma spp. were significantly higher in BSM-treated soil compared to all other treatments. Trichoderma spp. have been shown to be resistant to the ITCs produced by BSM (Mazzola and Manici, 2012). Trichoderma spp. are imperfect fungi that are commonly found in soil. This genus has been shown to suppress soilborne pathogen populations, such as Phytophthora cactorum and Rosellina necatrix (Porras et al., 2007; Ruano Rosa and López Herrera, 2009). The presence of Trichoderma may partially account for the lack of yield differences among treatments.

The overwhelming difference in the microbial community was across sampling dates or seasons, rather than among treatments. Season can be a stronger influence than management methods, management system (e.g. organic vs. conventional), and spatial variation within a field (Angers et al., 1993; Bosio et al., 1998; Schutter et al., 2001). Land management, particularly cultivation, can also have lasting effects on the soil microbial community (Buckley and Schmidt, 2001). The entire field where our study was conducted was cultivated many times during renovation before treatments were applied; beds were also shaped and soil was moved vertically after treatment application. Regardless of the different treatment applications, cultivation may have had the strongest influence on the soil microbial community structure observed here. This was apparent from the NMDS for both bacterial and fungal communities across sampling dates. Nearly all samples after treatment application clustered away from the pre-treatment samples which suggests that the microbial community did not return to its pre-treatment structure even after more than two years.

While the nonchemical strategies evaluated in this study were not shown to be effective in suppressing P. penetrans in raspberry roots and soil, it was demonstrated that the maximum labeled rate of metam sodium does protect raspberry from P. penetrans. Regardless of the differences in P. penetrans population densities among treatments, yield and TSS were not different. Future research should include evaluating different BSM rates against varying field population densities of P. penetrans as the level of parasitism pressure may play an important role in efficacy of BSM. Future work should also evaluate applying both BSM and metam sodium while shaping the raised beds in order to avoid future soil disturbances that could inhibit product efficacy. This may also improve the ability to find differences in the soil microbial communities among the treatments because the influence of tillage and soil disruption would be lessened.

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