The effect of fumigation on nematode communities in California almond orchards

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Summary – Fumigants, such as 1,3-dichloropropene and chloropicrin, have become key to pre-plant pest management in almond production. Whilst the use of these fumigants has become increasingly restricted due to human health concerns, less is known about their below-ground non-target effects in orchards and how nematode communities recover from fumigation over time. In this study, replicated trials compared 1,3-dichloropropene + chloropicrin to non-treated controls in two almond orchards in California, USA. Nematode communities, nematode indices and nematode metabolic footprints were quantified soon after fumigation and for 2 years afterwards. Fumigation reduced the Herbivore Metabolic Footprint in year 1, and populations of Pratylenchus vulnus in year 3. Fumigation also reduced populations of larger omnivores and predators, resulting in lower levels of the Structure Index at one site. Populations of fungal-feeding nematodes were more adversely affected by fumigation than bacterial-feeding nematode populations. At both sites, fumigation still influenced nematode community composition 2 years after treatment application.

Keywords – 1,3-dichloropropene, bioindicators, chloropicrin, Meloidogyne, Mesocriconema xenoplax, nematode populations, non-target fauna, Pratylenchus vulnus, Prunus dulcis, soil food web, USA.

Almond (Prunus dulcis) production relies on pre-plant soil fumigation to control plant-parasitic nematodes and other diseases (Browne et al., 2006). There are three main nematodes of concern in almonds (Micke, 1996): root-knot nematode (Meloidogyne spp.), ring nematode (Mesocriconema xenoplax) and root-lesion nematode (Pratylenchus vulnus). During feeding, these nematodes damage the root system, reducing the uptake of water and nutrients, resulting in stunted trees with reduced yield (Micke, 1996). Since the phase-out of the commonly used fumigant methyl bromide, due to its depleting effects on ozone levels, 1,3-dichloropropene (1,3-D) has been increasingly used as a non-greenhouse gas-emitting alternative (Ibekwe, 2004; Small, 2008). It is commonly combined with 35% chloropicrin under the trade name Telone C-35©. Although 1,3-D has been one of the most widely used fumigants since 1970, recently California has restricted annual applications due to public health concerns (Marks, 2016). Compounding the problem, the effects of fumigation are often influenced by site factors such as soil texture, moisture and organic matter content (Lembright, 1990; Collins et al., 2006). To manage pests better and to understand potential non-target effects, more information is needed on how fumigants interact with soil factors to influence nematode communities.

Nematodes serve as useful indicators of soil ecosystem functioning and soil food web responses to disturbances, such as fumigation (Bongers, 1990; Ettema, 1998; Bongers & Ferris, 1999; Ekschmitt et al., 2001). Since nematodes are primary and intermediate consumers in soil food webs, the abundances of their feeding guilds reflect those of primary decomposers, such as bacteria and fungi (Ferris & Matute, 2003). Due to their permeable cuticle, nematodes are in direct contact with soil pollutants and are considered suitable bio-indicators in agricultural systems (Yeates et al., 1991; Neher, 2001; Biagini & Zullini, 2008). The sensitivity of nematode communities to disturbance can be explained by differences in their life hist-
tory strategies, which can be classified along a coloniser-persister (Cp) scale (Bongers, 1990; Bongers & Ferris, 1999).

Nematode indices integrate the relative abundances of nematode groups, their feeding habits, and positions on the Cp-spectrum. For example, communities with more predators and omnivores with higher Cp values will tend to have higher values of the Maturity Index (MI), which reflects the successional stage of the nematode community (Ettema & Bongers, 1993) and the Structure Index, which estimates food web complexity (Ferris et al., 2001). Nematode communities with greater abundance of opportunistic nematodes with low Cp values will tend to have higher values of the Enrichment Index (EI), which indicates the level of responsiveness of the food web to increased available resources, including the activity of primary detrital consumers (Ferris et al., 2001). The Channel Index is determined by the relative quantities of bacterial grazing and fungal grazing nematodes, in Cp-1 and Cp-2 groups, respectively, which gives insight into whether decomposition is proceeding more through bacterial or fungal channels and how management may differentially affect these groups (Ferris et al., 2001).

Fumigation can cause dramatic shifts in nematode communities. In another cropping system, strawberries, applying 1,3-D and chloropicrin reduced fungal-feeding nematodes and increased opportunistic bacterial-feeding nematodes, although the total number of microbial-feeding nematodes remained unchanged (Sánchez-Moreno et al., 2010). Since soil fungal populations are more vulnerable to fumigation than bacterial communities (Stromberger et al., 2001), these shifts may have resulted from changes in the nematodes food resources. Higher trophic level nematodes, which also have high Cp values, are often negatively influenced by fumigation, but may be able to recover after a year or more (Sánchez-Moreno et al., 2010; Timper et al., 2012). Other studies found that the effects of soil fumigation on soil organisms, including non-target nematodes, varied with soil texture (Collins et al., 2006) and that the application of organic amendments can alleviate the effects of soil fumigation on soil microbial communities (Dungan et al., 2003).

The current study compared nematode communities from two experimental almond orchards where fumigation was applied. We hypothesised that fumigation would reduce nematode community structure and that fungal-feeding nematodes would be more adversely affected by fumigation than bacterial-feeding nematodes. Greater understanding of how fumigation influences orchard nematode communities could lead to better pest management and understanding of non-target effects.

Materials and methods

Experimental design

To characterise how soil communities responded to fumigation in almonds, experiments were set up in two orchards on sandy loam soils known to be infested with root-knot (Meloidogyne spp.), root-lesion (P. vulnus) and ring (M. xynoplax) nematodes. The first experiment was located in Merced County, CA, USA, near the town of Ballico and the second experiment in Stanislaus County, CA, USA, near the city of Modesto. Hereafter, the two sites are simply referred to as Merced and Stanislaus after the counties where they were located. While the previous crop at Merced was almond on ‘Nemaguard’ rootstock, the Stanislaus site followed two generations of peach orchards that spanned 40 years or more. At both sites, the previous orchard was removed mechanically in the autumn of 2014 and the soil ripped 0.9-1.2 m deep to remove remaining roots and break up hardpan. All plots remained fallow from the autumn of 2014 to the spring of 2015. In the spring of 2015, almonds were replanted at both sites with ‘Nemaguard’ almond rootstocks grafted to the scion ‘Nonpareil’. The spacing of almonds was 4.9 × 6.7 m at Merced and 4.9 × 6.6 m at Stanislaus. Almonds were irrigated at Merced by a double inline drip tube and at Stanislaus by microsprinklers. Almonds were planted either in non-fumigated soil, or soil that had been fumigated in the autumn of 2014 with 1,3 dichloropropene (1,3-D):chloropicrin 63:35, which was applied at a rate of 600 kg ha⁻¹ using a 3.6 m wide rig centred on the tree row according to standard practice. The randomised complete block design comprised five blocks of 12 trees. Within each block, two plots were created, each of six trees, with one plot treated with fumigant, while the remaining plot was maintained as a non-fumigated control (for a total of ten experimental plots for each site).

Soil sampling, Nematode extraction and identification

Sampling focused on the raised berm where almond trees were planted. This area was treated with a combination of pre-plant and post-plant herbicides annually and remained weed free for the summer and autumn during the experiment. Nematode samples were collected in

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mid-October of each year and followed a protocol similar to Shurtleff & Averre (2000), with the first sampling taking place after fumigation but before tree planting in 2014. For each of the ten plots, four soil cores (25.4 mm diam., 45.7 cm depth) were taken from within the dripline of each of four trees and pooled for analysis, for a total of ten samples at each site (five fumigated and five controls). Nematodes were extracted from 200 ml of moist field soil using a sieving and decanting technique followed by sugar centrifugation (Barker, 1984) with an extraction efficiency of ca 64%. The total number of nematodes in each sample was counted, and the first 200 encountered on a slide were identified. Most nematodes were identified to the genus level according to Bongers (1988) and feeding classifications and functional guilds assigned according to Yeates et al. (1993) and Bongers & Bongers (1998). The abundances were scaled to nematodes (l soil)−1 and used to calculate nematode indices of ecosystem function such as the Channel, Enrichment and Structure Indices according to Ferris et al. (2001). Nematode indices that measure the response of the nematode community to environmental stress, such as the Maturity Index and the Plant Parasitic Index, were also calculated (Bongers, 1990).

Nematode metabolic footprints include weightings of each group’s size-dependent metabolic activity, estimating the contribution of nematodes to various ecosystem functions (Ferris, 2010). Building on the nematode indices, which take into account Cq group and abundance, nematode metabolic footprints use estimates of body size from published morphometric parameters and assumptions about carbon utilised in biomass production and respiration (Ferris, 2010; Ferris et al., 2012). Calculated footprints included the nematode Bacterial and Fungal Metabolic Footprints, as well as the Structure and Enrichment Footprints. Calculations of indices and metabolic footprints were completed using the R, shiny package, NINJA: ‘Nematode INdicator Joint Analysis’ (Sieriebrienikov et al., 2014). Nematodes in the family Tylenchidae have been classified mostly as epidermal root hair feeders (Yeates et al., 1993) but some genera also feed on fungi (Okada et al., 2002, 2005; Okada & Kadota, 2003). Therefore, in calculating nematode indices, the abundance of Tylenchidae was split, with half categorised as plant parasites (Cp-2) and half as fungal feeders (Cp-2), reflecting the uncertainty of feeding preferences in this group.

Soil analysis

Soils were analysed for soil properties which could potentially affect nematode distribution such as sand, silt and clay fractions (particle size), labile soil organic matter and soil nitrogen (N) and carbon (C) content (Hodson et al., 2014; Margenot & Hodson, 2016). For mineral soil properties, which were not expected to change from year to year, samples were analysed from year 3 of sampling only (2016). Soil was dried at 60°C, and sieved to 2 mm. Soil particle sizes were determined by laser diffraction on a Beckman-Coulter LS-230 Particle Size Analyzer (Eshel et al., 2004). Finely ground soil was analysed for total N (%) and C (%) on a Europe Hydra 20/20 isotope ratio mass spectrometer at the University of California Davis Stable Isotope Facility. Labile soil carbon (POXC) was measured in both years 2 and 3 of sampling (2015 and 2016). A subsample of ground soil from all plots and sites was analysed for POXC following Culman et al. (2012). Briefly, triplicate samples of 2.5 g soil were oxidised with 0.02 mol l−1 KMnO4 with 2 min shaking followed by 10 min incubation and non-reduced Mn7+ quantified by colorimetry.

Statistical analysis

All analyses of nematode communities and ecological indices were performed in R v.3.1.1 (R Core Team, 2014). For biological data, we used the package lme4 (Bates et al., 2015) to perform a linear mixed effects analysis of the relationship between each sampled block, treatment, time and nematode indices. As fixed effects, we entered Treatment. As random effects, we had intercepts for year, sample location, blocks and orchard row. Assumptions of normality and homoscedasticity were assessed by visual inspection of residual plots and nematode indices were occasionally log transformed in models to meet these assumptions (graphs report non-transformed values). P values were obtained using the package lmerTest (Kuznetsova et al., 2017) and posthoc Tukey comparisons were done with the package multcomp (Hothorn et al., 2008) applying the false discovery rate adjustment. Similar models were run with year as a fixed effect, to test for increases in nematode abundances over time. Differences in indices and the relative abundance of nematode groups within individual years were compared by Kruskal-Wallis tests (denoted by the coefficient Chi), since with the lower sample sizes in individual years, data violated assumptions of normality. Kruskal-Wallis tests were also used to test for differences in soil variables between the sites and between treatments within an individual site. Since nematode data were non-normally distributed, often including zeros or outliers, medians are reported in the tables and
figures, along with either the minimum and maximum values or the interquartile range.

For year 3 measurements, non-metric multidimensional scaling (NMDS) was conducted to determine the degree to which site factors and treatments still influenced nematode community composition. A Chao distance measure for the abundance of nematode groups was run using the metaMDS function in the vegan package (Oksanen et al., 2018). Abundance data was Wisconsin double standardised and square root transformed. Correlations between the NMDS ordinations of nematode communities and treatment were tested with loop permutations (999) in the envfit function. The final stress value of the reported analysis, which measures the level of disagreement between the 2-D configuration and predicted values from the regression, was 0.06 for Site Merced and 0.09 for Stanislaus. To examine the relationship between nematode abundance and environmental data, two-tailed Spearman rank correlations were performed.

**Results**

**Soil properties**

Although both classified as sandy loam soil, the two sites differed slightly in specific soil characteristics (Table 1). The Stanislaus site had less sand compared to Merced (Chi = 10.6, \( P < 0.01 \)), particularly coarse sand (Chi = 4.2, \( P = 0.04 \)) and was higher in % C (Chi = 14.3, \( P < 0.01 \)), % N (Chi = 14.3, \( P < 0.01 \)) and C:N (Chi = 5.5, \( P = 0.02 \)). For the two time points in which the labile soil C fraction, POXC, was measured, levels increased between year 2 and year 3 at Merced (Chi = 9.1, \( P < 0.01 \)), while levels remained similar at Stanislaus. In both years 2 (Chi = 13.7, \( P < 0.01 \)) and 3 (Chi = 9.6, \( P < 0.01 \)), POXC was 75 and 48% higher at Stanislaus compared to Merced, respectively (Table 1).

**Nematode communities**

A total of 22 nematode taxa were identified in the two almond orchards (Table 2). The most abundant groups across locations and treatments were bacterial feeders such as *Rhoditidis* (with relative abundances as high as 38%), *Acrobeloides* (up to 61%), as well as the fungal-feeding *Aphelenchoides* (up to 68%). Nematodes in the family Tylenchidae, which can be either fungal feeders or root hair feeders, were also very common, accounting for up to 20% of all nematodes at Merced in year 2. While the overall community composition of the two sites was similar, Stanislaus had higher median abundance of pest nematodes such as *Paratylenchus* (Chi = 12.1, \( P < 0.01 \)) and Tylenchonchus (Chi = 13.9, \( P < 0.01 \)) in year 3 (Supplementary Table S1).

At Merced, fumigation decreased values of indices that measure ecological structure and maturity, although these effects were variable between years (Table 3). In year 1, fumigated plots had ten times lower levels of the Structure Index than controls (Chi = 4.5, \( P = 0.03 \)). Although levels of the Structure Index dropped to zero in year 2 for both treatments, by year 3 fumigated plots again had Structure Indices five times lower than controls (Chi = 3.9, \( P = 0.04 \)), largely driven by reductions in the relative abundance of large predatory Mononchidae (Supplementary Table S1, Chi = 4.5, \( P = 0.03 \)). While only one of the five fumigated samples had Mononchidae, they were present in abundances of greater than 50 (l soil)\(^{-1} \) in all but one of the control samples.

| Soil properties | Merced County | Stanislaus County |
|-----------------|---------------|-------------------|
| Å       | 0.34 ± 0.03   | 0.92 ± 0.66*    |
| Å       | 0.04 ± 0.003 | 0.10 ± 0.006*    |
| Å       | 8.14 ± 0.29   | 8.85 ± 0.14*    |
| Å       | 0.99 ± 0.08   | 0.77 ± 0.07    |
| Å       | 28.74 ± 1.31  | 37.80 ± 1.56*    |
| Å       | 70.27 ± 1.35  | 61.42 ± 1.59*    |
| Å       | 13.02 ± 0.87  | 13.58 ± 0.47    |
| Å       | 12.02 ± 0.79  | 11.02 ± 0.29    |
| Å       | 20.02 ± 0.75  | 18.35 ± 0.68    |
| Å       | 18.32 ± 1.72  | 13.48 ± 1.48*    |
| Å       | 147.51 ± 24.86 | 597.67 ± 30.50* |
| Å       | 280.91 ± 18.73 | 536.86 ± 37.95* |

Values are means ± standard error. For particle size classifications, clay (0.041-2.0 \( \mu \)m), silt (2-47.94 \( \mu \)m), sand (47.94-2000 \( \mu \)m), very fine sand (62.5-125 \( \mu \)m), fine sand (125-250 \( \mu \)m), medium sand (250-500 \( \mu \)m), and coarse sand (500-1000 \( \mu \)m). Asterisks denote statistical differences at the \( P < 0.05 \) level by Kruskal-Wallis tests.
Table 2. Nematode taxa identified from soil samples (0-46 cm depth) taken from almond orchards in Merced and Stanislaus Counties, CA, USA, during 2014-2016.

| Nematode Taxa         | Bacterivores | Fungivores | Herbivores | Predators/Omnivores |
|-----------------------|--------------|------------|------------|---------------------|
| Panagrolaimus         |              | Aphelenchoides | Meloidogyne | Qudsianematidae     |
| Rhabditis             |              | Aphelenchus  | Trichodorus | Mesodorylaimus      |
| Cephalobus            |              |             | Tylenchidae | Microdorylaimus     |
| Eucephalobus          |              |             | Paratylenchus | Discolaimus        |
| Acrobeles             |              |             | Tylenchorhynchus | Mononchidae      |
| Acrobeloides          |              |             | Pratylenchus |               |
| Prismatolaimus        |              |             | Xiphinema   | Mesocriconema      |

Table 3. Median nematode indices for soil samples (0-46 cm depth) taken from almond orchards in Merced County, CA, USA and Stanislaus County, CA, USA.

| Nematode index                  | 2014 C | 2014 F | 2015 C | 2015 F | 2016 C | 2016 F |
|---------------------------------|--------|--------|--------|--------|--------|--------|
| Merced County, CA, USA          |        |        |        |        |        |        |
| Maturity Index                  | 1.9    | 1.7    | 2.0    | 1.9    | 2.0    | 1.9    |
| Min-Max                         | 1.7-2.0| 1.6-2.0| 1.5-2.1| 1.7-2.0| 2.0-2.3| 1.7-2.2|
| Maturity Index 2-5              | 2.1    | 1.7*   | 2.0    | 2.0    | 2.3    | 2.0*   |
| Min-Max                         | 2.0-2.1| 1.6-2.0| 2.0-2.2| 2.0-2.0| 2.2-2.4| 2.0-2.3|
| Plant Parasitic Index           | 2.2    | 2.2    | 2.7    | 2.1    | 2.2    | 2.0*   |
| Min-Max                         | 2.0-2.6| 2.0-2.5| 2.4-3.5| 2.0-2.7| 2.1-2.3| 2.0-2.2|
| Channel Index                   | 56.5   | 30.4   | 27.3   | 24.5   | 31.0   | 24.0   |
| Min-Max                         | 29.6-74.7| 20-91.2| 7-100  | 2.1-100| 19.5-56.4| 9.5-81.4|
| Enrichment Index                | 55.0   | 74.8   | 38.5   | 30.6   | 58.2   | 50.0   |
| Min-Max                         | 50.4-72.8| 51.6-79.3| 22.1-82.8| 27.6-62.4| 36.7-65.7| 44.1-71.8|
| Structure Index                 | 11.7   | 0.0*   | 0.0    | 0.0    | 43.4   | 8.4*   |
| Min-Max                         | 0-13.6 | 0-2.3  | 0-31.1 | 0-2.4  | 38.4-57.4| 2.8-44.3|
| Enrichment Met. Ft.             | 178.1  | 284.3  | 122.6  | 78.6   | 357.6  | 640.5  |
| Min-Max                         | 79-449 | 152-929| 9-1837 | 61-2168|        |        |
| Structure Met. Ft.              | 60.2   | 0.0    | 0.0    | 0.0    | 176.1  | 15.6   |
| Min-Max                         | 0-139 | 0-47.8 | 0-95   | 0-6.2  | 51.9-405| 3.7-265.4|
| Herbivore Met. Ft.              | 6.6    | 0.9*   | 363.3  | 3.9    | 29.8   | 21.9   |
| Min-Max                         | 1.6-8.4| 0-4.2  | 181-1202| 0-2296 | 5.1-62.1| 6.9-111.3|
| Fungal Met. Ft.                 | 36.4   | 27.4   | 9.2    | 15.4   | 49.0   | 21.6   |
| Min-Max                         | 14.5-51.8| 18.2-93.7| 2.7-71.9| 12.7-25.5| 19.4-68.2| 19.4-276|
| Bacterial Met. Ft.              | 149.1  | 268.7  | 174.2  | 137.4  | 487.4  | 1135.0 |
| Min-Max                         | 78.6-433| 60.8-906| 41.8-1855| 65.8-3355| 363-823| 94.3-2146|
| Predator Met. Ft.               | 0.0    | 0.0    | 0.0    | 0.0    | 0.0    | 0.0    |
| Min-Max                         | 0-8.6 | 0-0    | 0-44.9 | 0-0    | 0-321.8| 0-161.6|
| Omnivore Met. Ft.               | 51.6   | 0.0    | 0.0    | 0.0    | 0.0    | 0.0    |
| Min-Max                         | 0-138.8| 0-47.8 | 0-21.4 | 0-0    | 0-0    | 0-0    |
| Total nematodes (l soil)−1      | 803.9  | 643.4  | 1105.0 | 1790.0 | 2260.0 | 2440.0 |
| Min-Max                         | 413-1017| 390-1296| 585-3140| 1405-6030| 2180-3540| 1020-8480|
| % Herbivores                    | 8.0    | 1.6**  | 20.0   | 1.4    | 13.5   | 8.9    |
| Min-Max                         | 5.5-13.3| 0-5.4  | 13.3-33.8| 0-39.5 | 3.2-44.2| 3.8-29.2|
| % Fungal-feeding                | 57.8   | 60.0   | 13.6   | 8.7    | 19.9   | 24.5   |
| Min-Max                         | 46.3-72.4| 41.1-93.3| 6.7-28.6| 2.5-27 | 14.1-35.9| 11.5-38 |
| % Bacterial-feeding             | 29.7   | 40.0   | 68.2   | 90.7   | 58.3   | 55.3   |
| Min-Max                         | 13.2-48.1| 5.1-53.6| 46.9-80 | 39.7-97.5| 29.3-72 | 36-84.6 |
Table 3. (Continued.)

| Nematode index                     | 2014 | 2015 | 2016 |
|-----------------------------------|------|------|------|
|                                   | C    | F    | C    | F    | C    | F    |
| Stanislaus County, CA, USA        |      |      |      |      |      |      |
| Maturity Index                    | 1.9  | 1.9  | 2.0  | 1.8  | 2.1  | 2.1  |
| Min-Max                           | 1.9-2.0 | 1.4-2.0 | 1.9-2.1 | 1.7-2.0 | 2.0-2.2 | 2.0-2.5 |
| Maturity Index 2-5                | 2.0  | 2.0  | 2.1  | 2.0  | 2.3  | 2.5  |
| Min-Max                           | 2.0-2.0 | 2.0-2.0 | 2.0-2.3 | 2.0-2.0 | 2.2-2.4 | 2.2-2.6 |
| Maturity Index 2-5                | 2.7  | 2.0  | 2.1  | 2.2  | 2.4  | 2.5  |
| Min-Max                           | 2.0-3.0 | 2.0-2.0 | 2.0-2.8 | 2.0-2.7 | 2.0-2.9 | 2.1-2.6 |
| Channel Index                     | 72.1 | 47.0 | 43.2 | 13.7 | 57.7 | 30.2 |
| Min-Max                           | 68.5-100 | 4.3-100 | 15.6-100 | 1.9-18.1 | 5.9-52.8 | 6.9-85.4 |
| Enrichment Index                  | 50.0 | 52.8 | 38.7 | 50.2 | 56.9 | 57.5 |
| Min-Max                           | 43.7-53.1 | 47.6-87.3 | 15.6-59.9 | 19.1-59.3 | 54.6-71.9 | 48.1-79.0 |
| Structure Index                   | 0.0  | 0.0  | 10.7 | 1.6  | 48.6 | 57.7 |
| Min-Max                           | 0-0  | 0-0  | 1.6-45.8 | 0-8.8 | 33.4-58.9 | 35.8-62.6 |
| Enrichment Met. Ft.               | 418.7 | 228.5 | 81.9 | 420.7 | 1723.5 | 640.8 |
| Min-Max                           | 183-1643 | 61-2372 | 27.8-1788 | 59.6-1155 | 1000-2417 | 578-2866 |
| Structure Met. Ft.                | 0.0  | 0.0  | 59.7 | 1.4  | 334.0 | 441.2 |
| Min-Max                           | 0-0  | 0-0  | 2.2-570.3 | 0-120.9 | 185-563 | 153-1098 |
| Herbivore Met. Ft.                | 9.1  | 0.6* | 14.9 | 7.1  | 346.0 | 142.9 |
| Min-Max                           | 4.1-82 | 0.4-9 | 6.2-28.4 | 2.5-14.1 | 66.6-387.9 | 104-836 |
| Fungal Met. Ft.                   | 145.2 | 19.2** | 28.2 | 14.2 | 68.6 | 100.1 |
| Min-Max                           | 127-482 | 13-104 | 8.6-67.2 | 2.2-31.6 | 23.7-357.4 | 28.9-432.8 |
| Bacterial Met. Ft.                | 347.9 | 226.2 | 169.1 | 514.2 | 2092.0 | 891.7 |
| Min-Max                           | 48-1335 | 13-2395 | 60.4-2064 | 353-1322 | 1071-2946 | 451-081 |
| Predator Met. Ft.                 | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| Min-Max                           | 0-0  | 0-0  | 0-200 | 0-14.6 | 0-33 | 0-46.2 |
| Omnivore Met. Ft.                 | 0.0  | 0.0  | 58.2 | 0.0  | 121.4 | 359.5 |
| Min-Max                           | 0-0  | 0-0  | 0-317.8 | 0-87.6 | 0-314.4 | 29-1064 |
| Total nematodes (l soil) −1       | 2955.0 | 995.0 | 1945.0 | 2680.0 | 10524.0 | 6340.0 |
| Min-Max                           | 2225-9765 | 380-1905 | 610-4990 | 1450-3940 | 8420-15534 | 5260-13008 |
| % Herbivores                      | 3.6  | 1.3  | 12.3 | 4.6  | 38.2 | 24.4 |
| Min-Max                           | 1.0-6.8 | 0.1-11 | 7.5-15.6 | 1.2-12.5 | 20.1-48.4 | 13.7-56.2 |
| % Fungal-feeding                  | 64.7 | 45.8 | 17.0 | 6.5  | 7.4  | 21.6 |
| Min-Max                           | 55.2-79.6 | 11.1-90.7 | 13.4-21.7 | 2.0-10.8 | 3.6-39.2 | 4.9-43.8 |
| % Bacterial-feeding               | 30.4 | 50.6 | 67.4 | 89.0 | 47.5 | 32.1 |
| Min-Max                           | 18.3-41.2 | 9.3-89.0 | 61.8-75.5 | 76.7-95.9 | 35.6-53.9 | 15.58.8 |

Statistical differences from Kruskal-Wallis rank sum tests are denoted by * for $P < 0.05$ and ** for $P < 0.01$. C = control, F = fumigated. Nematode metabolic footprints (Met. Ft.) are expressed as $\mu$g C (l soil)$^{-1}$.

(Chi = 4.5, $P = 0.03$) and 3 (Chi = 4.8, $P = 0.03$), but not in year 2 (Table 3).

At Merced, fumigation treatments decreased plant-parasitic nematodes in the first year but did not alter ratios of bacterial and fungal feeders (Table 3). In year 1, fumigation reduced the relative abundance of plant-parasitic and root-herbivore nematodes (Chi = 6.8, $P < 0.01$), and also reduced the Herbivore Metabolic Footprint (Chi = 4.8, $P = 0.03$), due to lower numbers of Tylenchidae (Supplementary Table S1, Chi = 5.7, $P = 0.02$). By year 3, fumigated trees had lower numbers of Paratylenchus (Chi = 3.5, $P = 0.05$) and also Pratylenchus (Chi = 5.1, $P = 0.02$), which contributed to slightly lower Plant Parasitic Indices compared to controls (Table 3, Chi = 5.1, $P = 0.02$). In mixed models, the relative abundance of bacterial feeders (Fig. 2A, $F = 7.6$, $P = 0.01$) as well as the Bacterial Metabolic Footprint (Table 3, $F = 6.7$, $P = 0.02$) increased over time although there were no significant effects due to treatment.
Fumigation effect on nematodes in almond orchards

**Fig. 1.** The nematode Maturity Index calculated from soil samples (0-46 cm depth) taken from almond orchards in A: Merced County; B: Stanislaus County, CA, USA. Bars are medians, boxes the upper and lower quartiles (25 and 75%), lines are the minimum and maximum values and outliers are dots.

**Fig. 2.** The relative abundance (percent of total) of the nematode community comprised by bacterial feeders. Soil samples (0-46 cm depth) were taken from almond orchards in A: Merced County; B: Stanislaus County, CA, USA. Statistical differences from Kruskal Wallis rank sum tests at the $P<0.05$ level are denoted by asterisk (*). Bars are medians, boxes the upper and lower quartiles (25 and 75%), lines are the minimum and maximum values and outliers are dots.

By contrast, fungal feeders decreased in relative abundance over time (Fig. 3A, $F = 9.2, P < 0.01$).

In the Stanislaus orchard, the response of the nematode community to fumigation was less than Merced, and varied between years. One consistent trend was that for both treatments the Maturity Index increased over time during the 3 years measured (Fig. 1B, Chi $= 13.9, P < 0.01$). However, there were no statistically significant differences in the Maturity Index between treatments within individual years.

Fumigation decreased the Herbivore Metabolic Footprint and groups of plant-parasitic nematodes at Stanislaus in certain years. In year 1, the Herbivore Metabolic Footprint was 15 times higher in control plots compared to fumigated plots (Table 3, Chi $= 4.8, P = 0.03$). There were no statistically significant differences in plant parasites in year 2. Abundances of *Paratylenchus*, *Tylenchorhynchus* and *Pratylenchus* had increased by year 3, and these genera also had higher relative abundance in controls than fumigated treatments (Supplementary Table S1; Chi $= 3.8, P = 0.05$).

The Bacterial Metabolic Footprint increased over time at the Stanislaus site and bacterial-feeders responded positively to fumigation at certain time points. Similar to
Merced, the Bacterial Metabolic Footprint increased over time for both treatments ($Z = 10.0, P < 0.01$) in mixed effects models. Bacterial feeders made up a higher proportion of the nematode community under fumigated trees than controls in year 2 (Table 3, Chi $= 5.3, P = 0.02$) and included groups such as *Panagrolaimus*, *Rhabditis*, *Cephalobus*, *Eucephalobus*, *Acrobeles* and *Acrobeloides*. The most abundant genus was *Acrobeloides*, with a median relative abundance of 61.0% in fumigated plots compared to 44.4% in controls (Supplementary Table S1, Chi $= 5.3, P = 0.02$).

In contrast to bacterial-feeding nematodes, fungal-feeding nematodes decreased over time in the Stanislaus orchard and responded negatively to fumigation (Fig. 3, $Z = 15.5, P < 0.01$). In year 1, soon after the orchard had been established and fumigated, fungal-feeding nematodes were still quite numerous, making up 64.7% of all nematodes in control treatments and 45.8% in fumigated treatments. Fungal Metabolic Footprints, with a median 19.2 $\mu$gCs sample$^{-1}$ in fumigated treatments, were 6.5 times lower than controls, which had a median Fungal Metabolic Footprint of 145.2 $\mu$gCs sample$^{-1}$ (Table 3, Chi $= 6.8, P < 0.01$). In year 2, the relative abundance of fungal feeders was still higher in control plots than fumigated plots (Chi $= 5.3, P = 0.02$). By year 3 there were no statistical differences in fumigation between the treatments.

**Fig. 3.** The relative abundance (percent of total) of the nematode community comprised by fungal feeders. Soil samples (0-46 cm depth) were taken from almond orchards in A: Merced County; B: Stanislaus County, CA, USA. Statistical differences from Kruskal Wallis rank sum tests at the $P < 0.05$ level are denoted by an asterisk (*). Bars are medians, boxes the upper and lower quartiles (25 and 75%), lines are the minimum and maximum values and outliers are dots.

**RELATIONSHIP BETWEEN NEMATODE COMMUNITIES, SOIL PROPERTIES AND FUMIGATION**

By the third year of the experiment at Merced, nematode communities were still influenced by fumigation according to NMDS analysis ($P = 0.01$, $R^2 = 0.51$), mainly through differences in herbivorous and predatory nematode groups (Fig. 4). For example, control treatments were associated with predatory nematodes in the family Mononchidae and the relative abundances of the plant-parasitic nematodes *Paratylenchus* and *Pratylenchus*. Correlations showed that the abundances of certain bacterial feeders varied with soil physical and chemical factors. For example, the abundance of *Acrobeles* increased with the percent medium sand (Rho $= 0.68$, $P = 0.03$). *Rhabditis* increased with soil C (Rho $= 0.62$, $P = 0.05$) and soil N (Rho $= 0.66$, $P = 0.04$), while *Prismatolaimus* decreased with these factors (Rho $= -0.67$, $P = 0.03$, Rho $= -0.70$, $P = 0.03$, respectively).

At Stanislaus in year 3, nematode communities were also influenced by fumigation according to NMDS analysis (Fig. 5, $R^2 = 0.35$, $P = 0.01$). In the ordination, plant-parasitic nematodes, such as *Pratylenchus* and *Tylenchorhynchus*, were associated more with control treatments, as were certain bacterial feeders such as *Cephalobus* and *Prismatolaimus*. In correlations, sev-
Fig. 4. Nonmetric multidimensional scaling analyses of soil nematode communities from soil samples (0-46 cm depth) taken from an almond orchard in Merced County, CA, USA, and their relationship to fumigation treatment in 2016.

Fig. 5. Nonmetric multidimensional scaling analysis of soil nematode communities from soil samples (0-46 cm depth) taken from an almond orchard in Stanislaus County, CA, USA, and their relationship to fumigation treatment in 2016.

eral nematode groups showed relationships with environmental site variables. Larger-bodied *Mesodorylaimus* increased with the percent sand (Rho = 0.77, P < 0.01). When sand was broken down into smaller size fractions, *Acrobeloides* (Rho = 0.68, P = 0.03) increased with the percentage fine sand and *Rhabditis* (Rho = 0.71, P = 0.02) with the percentage very fine sand (62.5-125 μm). *Rhabditis* also increased with the C:N ratio of the soil (Rho = 0.72, P = 0.02). Nematodes in the family Tylenchidae, which have unclear feeding preferences, were associated with increased C (Rho = 0.76, P = 0.01), N (Rho = 0.65, P = 0.04) and the carbon pool POXC (Rho = 0.83, P < 0.01).

**Discussion**

The goals of this study were to quantify changes in nematode community structure associated with fumigation. Nematodes serve as useful biological indicators of changes in the soil environment (Ritz et al., 2009) since they represent all trophic levels, co-vary with the abun-
dance of other soil biota such as mites (Sánchez-Moreno et al., 2009) and respond rapidly to changes in management (Kapp et al., 2013). This study suggests that treatment with 1,3-D and chloropicrin influences nematodes communities up to 2 years after fumigation, decreasing the abundance of fungal-feeding and predatory nematodes, but occasionally increasing bacterial-feeding nematodes compared to non-treated controls.

Regardless of treatment, metrics of nematode community structure increased between years 1 and 3 after fumigation, probably as nematodes recovered from the physical disturbance of orchard establishment and the planted trees began to grow. Preparing for orchard planting is a disruptive process that involves deep tilling and shaping the raised berms where almonds are planted (Flint, 2002). In other cropping systems, similar tillage practices caused reductions in predatory nematodes and increases in bacterial feeders, decreasing levels of the Maturity Index (Lenz & Eisenbeis, 2000; Sánchez-Moreno et al., 2006). However, it has also been found that tillage has a minimal influence on nematode communities (Griffiths et al., 2009). In the current study, some indices of ecological complexity slowly increased after orchard establishment in both fumigated and control plots, such as the Maturity Index.

Fumigation disproportionately affected certain nematode taxa, particularly higher trophic level omnivores and predators; however, the two sites differed in the degree to which this was so. The effects of fumigation were immediately apparent at Merced, with lower levels of the Structure Index, an indicator of food web complexity, in fumigated plots, but other metrics, such as the Maturity Index, were unaffected. By contrast, no large changes were seen at Stanislaus in the Structure Index or the Maturity Index. Wall et al. (2002) noted that the Maturity Index provided contradictory results to other diversity indices and failed to separate sites that showed differences in multivariate analysis. In other studies after fumigation, values of the Maturity Index gradually increased, with differential rates of recovery of nematode species (Ettema & Bongers, 1993). At both sites in the current study, the relative abundance of *Prismatolaimus* was low in years 1 and 2, but increased more in control plots than fumigated plots by year 3. These nematodes, typically classified as generalist bacterial feeders (Yeates et al., 1993), are more sensitive to disturbance, as indicated by their c-p value of 3 (Bongers, 1990), and have been classified by some as omnivores (Ferris et al., 1996). Since these nematodes are relatively common in mature orchards, and were less numerous in fumigated plots at Stanislaus, their abundance could serve as a useful indicator of fumigant effects on nematode communities.

The rate of recovery of predatory and omnivore nematodes may depend on site-dependent field characteristics (Sánchez-Moreno et al., 2010; Timper et al., 2012). In this study, strong effects on the Structure Index were seen at Merced but not Stanislaus. Stanislaus had a lower sand content, at 60%, compared to 70% at Merced. Collins et al. (2006) found that bacteria and fungi were more likely to survive fumigation with metam sodium and 1,3-D in fine-compared to coarse-textured soil, perhaps due to reduced diffusion of the fumigant through soil pore spaces. Compared to Merced, Stanislaus also had nearly three times more soil C and twice the POXC, a labile carbon pool correlated with particulate organic carbon, microbial biomass carbon and soil organic carbon (Culman et al., 2012). As soil organic matter content increases, more volatiles adhere to the surface of the organic matter, reducing available fumigant (Lembright, 1990). The microbial activity promoted by organic amendments can also degrade fumigants (Gan et al., 1998), reducing their effects on microbial communities (Dungan et al., 2003) and therefore their effectiveness. It could be that the history of prior orchard management at Stanislaus contributed to higher soil C, since peach trees are pruned heavily each year and the wood is shredded in place on the orchard.

Nematode communities at both sites were still influenced by fumigation in year 3 according to NMDS ordinations. This was partly due to the effects of plant parasites, such as *Pratylenchus*, which were associated with controls at both sites. While predators such as Mononchidae were negatively associated with fumigation at Merced, omnivorous nematodes such as *Mesodorylaimus* and Qudsianematidae were not dramatically affected by fumigation at Stanislaus, perhaps reflecting their tolerances to disturbance or feeding preferences. Nematodes such as bacterial-feeding *Rhabditis* were correlated with higher soil C and N, possibly relating to increased food resources for these nematodes (Ferris & Bongers, 2006). Similar to other studies in the same region, larger-bodied *Mesodorylaimus* increased with the percentage sand, probably because the larger pore sizes facilitated their movement (Fujimoto et al., 2010; Hodson et al., 2014).

The relative abundance of bacterial-feeding to fungal-feeding nematodes offers valuable insight into primary decomposition pathways in soil environments (Ferris & Bongers, 2006). In general, fungal-feeding nematodes declined after year 1 regardless of fumigation treatment,
probably because tilling during orchard establishment adversely affected fungal populations (Bailey et al., 2002). At Stanislaus, fumigation immediately decreased fungal-feeding nematodes. The Fungal Metabolic Footprint was 7.6 times higher in the controls compared to the fumigated plots and the relative abundance of fungal-feeding nematodes was still more than twice as high the following year. However, it is difficult to explain why this trend was not observed at Merced. Both sites had similar relative abundances of Aphelenchoides, but while Aphelenchus was observed at Stanislaus in year 1, it was not found at Merced until year 2. By contrast, bacterial-feeding nematodes increased over time for both treatments and sometimes responded positively to fumigation. Tillage also can increase bacterial populations and therefore favours increases in r-selected bacterial-feeding nematodes (Ettema & Bongers, 1993). Fumigation may similarly increase bioavailable nutrients by facilitating release of NH$_4^+$-N and phosphate from the soil, altering microbial communities (Rovira, 1976). The increased relative abundance of bacterial-feeding Acrobeiloides, lending support to the hypothesis that this nematode could serve as an indicator of fumigation effects on bacterially-mediated decomposition (Sánchez-Moreno et al., 2010).

Fumigation effectively reduced plant-parasitic nematodes, with both sites showing immediate decreases in herbivorous nematode populations soon after fumigation as measured by the Herbivore Metabolic Footprint. The Plant Parasitic Index, which has been shown to be a useful measure of fumigation efficacy (Lenz & Eisenbeis, 2000), was only higher in controls compared to fumigated plots in Merced in year 3 and no differences were seen at Stanislaus. However, at both sites the relative abundance of the pest species, Pratylenchus, remained low with fumigation.

Conclusions

Although multiple measurements within each year or larger sampling sizes may have revealed additional fluxes in nematode communities, this study shows that the effects of pre-plant fumigation in an orchard can persist for several years. It should be noted, also, that this study cannot separate the effects of fumigation from the effects of growing tree roots, since replicated plots were not included with bare soil and no trees. Applying fumigants markedly altered the composition of the nematode food web, reducing fungal feeders and predators, but increasing bacterial feeders at some time points. At both sites, the effects of fumigation, including reduced food web complexity due to loss of predators and sensitive taxa, were still apparent after 2 years.

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**Supplementary Table S1.** Median relative abundance of nematode taxa (L soil$^{-1}$) identified from fumigated (F) plots and controls (C) from sites in Merced County, CA, USA and Stanislaus County, CA, USA, their associated feeding groups and life history coloniser persist (Cp) values.

| Nematode taxa | Feeding group | Cp | 2014 | 2015 | 2016 |
|---------------|---------------|----|------|------|------|
|               |               |    | C    | F    | C    | F    | C    | F    |
| Merced County, CA, USA |               |    |      |      |      |      |      |      |
| Panagrolaimus  | b             | 1  | 0.0  | 0.0  | 0.0  | 3.8  | 4.5  | 2.8  |
| Rhabditis      | b             | 1  | 4.9  | 20.2 | 4.4  | 0.0  | 6.8  | 8.4  |
| Cephalobus     | b             | 2  | 1.3  | 0.5  | 0.0  | 0.0  | 28.2 | 19.3 |
| Acrobeles      | b             | 2  | 0.0  | 0.0  | 2.2  | 1.6  | 4.9  | 2.0  |
| Acrobelesoides | b             | 2  | 5.8  | 2.7  | 37.5 | 55.0 | 0.0  | 0.0  |
| Pristionchus   | b             | 3  | 0.0  | 0.0  | 0.0  | 0.0  | 12.1 | 3.0  |
| Aphelenchoides | f             | 2  | 28.4 | 37.5 | 0.0  | 0.0  | 9.6  | 4.0  |
| Aphelenchus    | f             | 2  | 0.0  | 0.0  | 2.2  | 2.5  | 5.4  | 2.0  |
| Tylennchida    | h             | 2  | 6.2  | 1.8* | 13.6 | 2.7  | 11.2 | 17.8 |
| Paraylenchus   | h             | 2  | 0.0  | 0.0  | 0.0  | 0.0  | 3.8  | 0.0* |
| Pratylenchus   | h             | 3  | 0.0  | 0.0  | 0.0  | 0.0  | 1.3  | 0.0* |
| Meloidogyne    | h             | 3  | 0.0  | 0.0  | 5.2  | 0.0  | 0.0  | 0.0  |
| Trichodorus    | h             | 4  | 0.0  | 0.0  | 2.2  | 0.0  | 0.0  | 0.0  |
| Mesodorylaimus | o             | 4  | 0.7  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| Mononchidae    | p             | 5  | 0.0  | 0.0  | 0.0  | 0.0  | 5.5  | 0.0* |
| Stanislaus County, CA, USA |               |    |      |      |      |      |      |      |
| Panagrolaimus  | b             | 1  | 1.4  | 0.0  | 1.1  | 5.0  | 5.1  | 5.6  |
| Rhabditis      | b             | 1  | 4.5  | 9.6  | 1.7  | 11.2 | 4.1  | 3.2  |
| Cephalobus     | b             | 2  | 7.0  | 3.6  | 4.9  | 3.0  | 16.4 | 13.3 |
| Eucephalobus   | b             | 2  | 0.0  | 0.0  | 1.5  | 1.4  | 0.0  | 0.0  |
| Acrobeles      | b             | 2  | 0.0  | 0.0  | 5.8  | 2.1  | 4.9  | 7.4  |
| Acrobelesoides | b             | 2  | 14.7 | 23.3 | 44.4 | 61.0*| 1.2  | 2.5  |
| Pristionchus   | b             | 3  | 0.0  | 0.0  | 1.0  | 0.5  | 10.0 | 2.0* |
| Aphelenchoides | f             | 2  | 58.7 | 34.9 | 4.3  | 1.4  | 12.1 | 17.4 |
| Aphelenchus    | f             | 2  | 2.9  | 2.3  | 8.8  | 2.9  | 2.3  | 1.2  |
| Tylennchida    | h             | 2  | 4.2  | 2.6  | 6.4  | 1.6  | 4.6  | 6.5  |
| Paraylenchus   | h             | 2  | 0.0  | 0.0  | 2.0  | 1.5  | 14.4 | 18.4 |
| Tylennchorhynchus | h          | 3  | 0.0  | 0.0  | 0.0  | 0.0  | 4.2  | 4.4  |
| Pratylenchus   | h             | 3  | 0.0  | 0.0  | 0.0  | 0.0  | 11.7 | 0.5* |
| Mesoriconea    | h             | 3  | 1.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  |
| Xiphinema      | h             | 5  | 0.0  | 0.0  | 0.0  | 0.0  | 0.6  | 1.9  |
| Qudsianematidae| o             | 4  | 0.0  | 0.0  | 0.0  | 0.0  | 0.6  | 4.2  |
| Mesodorylaimus | o             | 4  | 0.0  | 0.0  | 0.4  | 0.0  | 1.5  | 6.7  |
| Microdorylaimus| o             | 4  | 0.0  | 0.0  | 0.9  | 0.0  | 0.0  | 0.0  |
| Discolaimus    | p             | 5  | 0.0  | 0.0  | 0.0  | 0.0  | 0.2  | 0.1  |

Asterisks denote statistical differences of $P < 0.05$ by Kruskal–Wallace rank sum test. b = bacterial feeders; f = fungal feeders; h = herbivores; o = omnivores; p = predators.