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Responses of Bacterial and Fungal Community Structure to Different Rates of 1,3-Dichloropropene Fumigation

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ABSTRACT

1,3-Dichloropropene (1,3-D) is a well-known nematicidal soil fumigant on many crop species. Currently, little is known about its impact on soil microbial communities using culture-free methods. In this study, we investigated changes in soil bacterial and fungal diversity and composition at two depths (30.5 and 61 cm) in response to management practices of applying 1,3-D at four different rates (103, 122, 140, and 187 liters/ha) relative to an untreated control in potato production fields using 16S rRNA and internal transcribed spacer (ITS) amplicon sequencing. A total of 12,783 operational taxonomic units (OTUs) for 16S and 1,706 OTUs for ITS were obtained. Sequencing revealed that *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Ascomycota* were dominant phyla in soils. Comparing alpha diversity of microbial communities at the different chemical rates with untreated plots showed that bacterial communities in plots treated with 1,3-D fumigation at 140 liters/ha were richer, which was supported by

higher richness indices. Other diversity indices and overall soil microbial community structure were not significantly influenced by any rates of 1,3-D fumigation, although higher bacterial and fungal richness and diversity were observed in posttreatment soils and/or at 30.5 cm. Of the identified microbial families, the differential abundance of 45 bacterial and 24 fungal families was affected by sample depth, 1,3-D rate, or the interaction of sample depth and 1,3-D. The bacterial family *Enterobacteriaceae*, which includes species that specialize in decay of complex carbohydrates, increased in abundance post-1,3-D fumigation, and the fungal family *Ophiocordycipitaceae*, which includes nematode and insect pathogens, decreased, suggesting that the nematode and soil insect death caused by fumigation may selectively impact specific fungal and bacterial families.

Keywords: crop, management, metagenomics, microbiome

Over the past few decades, pesticides, including insecticides, fungicides, nematicides, and herbicides, have been increasingly used to improve crop yield and quality. However, widespread adoption and use of pesticides may have unanticipated negative impacts on nontarget organisms and the surrounding ecosystems (Dennis et al. 2018; Pisa et al. 2015; Sugita et al. 2018; Van der

Sluijs et al. 2015; Zaller et al. 2018). For example, previous studies reported that several pesticides and their degradation products can act as soil pollutants (Sivanesan et al. 2004; Zhang et al. 2016) and applications of pesticides may directly or indirectly alter biological processes in the soil as well as affect soil microbial populations (Ahmed and Ahmad 2006; Imfeld and Vuilleumier 2012; Lewis et al. 2009; Wu et al. 2014). Soil microbial communities support a wide range of ecosystem services required for maintaining soil structure and fertility, supporting carbon, nitrogen, and phosphorus cycling, and removing soil contaminants (Aislabie and Lloyd-Jones 1995; Bending et al. 2007; Dong et al. 2017; Larkin 2003; Singh et al. 2015). Thus, changes to the soil microbial community that occur as a result of agricultural management practices can be used as sensitive indicators of soil processes for evaluation of soil function (Hernández-Allica et al. 2006; Schlöter et al. 2018). Therefore, investigating soil microbial community structure and diversity in response to different crop management strategies, including pesticide application, may aid

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farmers in improving sustainability of their management practices (Maul et al. 2014).

The withdrawal of methyl bromide (MeBr), a chemical that has long been used as one of the premier soil fumigants for managing soilborne pests, prompted research to find ecologically acceptable and efficacious alternatives (Liu et al. 2015). Several fumigants, including 1,3-dichloropropene (1,3-D), chloropicrin, and methyl isothiocyanate generators have been registered as MeBr replacements in many countries (Ajwa et al. 2003; Qiao et al. 2011; Shi et al. 2009). As a well-known nematicide with fungicidal and insecticidal activity, 1,3-D is used in multiple crops. The effects of 1,3-D, when applied alone or in combination with other products, on crop yield, 1,3-D degradation characteristics, and 1,3-D distribution and emission have been extensively studied (Dungan et al. 2003; Ji et al. 2013; Luo et al. 2013; Mao et al. 2017; Qiao et al. 2010, 2011; Qin et al. 2013a, b; Wang et al. 2010).

1,3-D can persist in soils from a few days to several weeks (Dungan et al. 2003; Wang et al. 2010) and may exhibit toxicity to nontarget organisms because of its broad biocidal activity. However, there are relatively few studies that have analyzed the effects of 1,3-D fumigation on the nontarget soil microbial community compared with the number of studies reporting effects on its specific target pests. A previous study used nested PCR-denaturing gradient gel electrophoresis to monitor fungal community structure in bulk soil and spinach rhizosphere soil after 1,3-D fumigation. They found that the fungal community profile and diversity changed 2 months postfumigation but recovered within 6 months postfumigation (Hoshino and Matsumoto 2007). Yet another study investigated bacterial communities in 1,3-D-fumigated soil using 454 pyrosequencing techniques; the authors reported that 1,3-D treatment only increased bacterial diversity and had a suppressive effect on the indigenous soil bacterial populations or abundance at the beginning of the treatments in tomato fields (Liu et al. 2015).

The use of advanced high-throughput sequencing techniques has led to an explosive accumulation of research data and has vastly improved our understanding of the microflora, including species data of unculturable microorganisms in soil (Bai et al. 2014). However, despite the widespread use of 1,3-D as a common practice to reduce the impact of nematodes on many different crops, there are relatively few recent studies that have documented the impact of 1,3-D on the soil microbial community using culture-free methods. The aim of this study was to assess the response of soil microbial communities to the application of four rates of 1,3-D (103, 122, 140, and 187 liters/ha), and the associated soil disturbance, in an Oregon potato field relative to an untreated control, and to determine if 1,3-D impacts on microbial community structure varied between two soil depths.

MATERIALS AND METHODS

Site description, experimental design, and soil sampling. This field experiment was conducted at the Hermiston Agricultural Research and Extension Center (HAREC) south of Hermiston, Oregon during the fall of 2016. The field soil is a fine sandy loam with approximately 65% sand, 25% silt, 10% clay, and less than 1% organic matter. The previous crop at this location was winter wheat. In early September 2016, fall tillage practices prior to experiment establishment included ripping to a depth of 46 cm, disking twice, and roller-harrowing. The field was watered prior to 1,3-D application to obtain proper soil moisture to a depth of 30.5 cm, assessed by the “feel method” as directed by the pesticide label.

Three replicates of four experimental treatments and one untreated control were arranged in a randomized complete block design. Plot sizes were 9.1 m wide by 122.9 m long. Broadcast

applications of 1,3-D were shank injected at a depth of 46 cm by a commercial applicator. The four treatments included different rates of 1,3-D at 103, 122, 140, and 187 liters/ha. A disc and cultipacker, in combination, directly followed the shank, in tow, to seal the soil surface following application. A 9.1 m border treated with 1,3-D at 122 liters/ha was established around the experiment to reduce edge effects within the experiment. Applications of 1,3-D occurred on 13 September 2016. Air temperature at start of application was approximately 4°C and soil temperature at 20 cm was approximately 19°C. Since the purpose of this was to examine how the microbial community was affected by farm management practices, the nontreated control did not have additional tillage associated with 1,3-D application.

Soil was sampled on 12 September 2016 prior to 1,3-D application and on 17 October 2016 following application. The post-treatment sample was taken after planting the cover crop since our goal was to determine if 1,3-D application effects could be detected after common farming operations, such as irrigation and cover crop planting, that typically occur shortly after 1,3-D application. As a fall cover crop, winter wheat was drilled into the entire experimental plot in the week of 5 October 2016. The wheat was watered by center pivot irrigation (ca. 0.64 cm) immediately after planting and an additional 2 cm of precipitation occurred prior to the second soil sampling date. To sample soils, field plots were divided in the center, resulting in two subplots of 9.1 m wide by 61.5 m long. On each sample date and within each subplot, soil samples were obtained at each of 10 locations at two depths using a zig-zag sampling scheme. Soil samples were collected by two people, the first person collected soil to 30.5 cm depth and was followed by a second person who collected soil from the same location to a 61 cm depth. A standard 2.2 cm × 53 cm soil probe (AMS, American Falls, ID) was used to collect all soil samples. The soil from each of the 10 locations within a subplot and for each sample depth were bulked together in a plastic bag and stored at 4°C until further processing. In the laboratory, approximately 2 g of each soil sample was added to 3 ml of RNeasy (ThermoSci) in a 15-ml Falcon tube (Fisher Sci, Pittsburgh, PA) and samples were shipped to Colorado State University for molecular processing.

Samples were stored at –20°C until DNA extractions occurred. To prepare samples for extraction, each was centrifuged at 2,500 × g for 5 min and RNeasy was removed. DNA from soils were then extracted using the RNeasy PowerSoil Total RNA Kit and RNeasy PowerSoil DNA Elution Accessory Kit (MoBio Laboratories, Carlsbad, CA), following manufacturer’s instructions with a few modifications. Modifications included adding 2 g of soil to each bead tube and a reduction in elution buffer from the suggested 100 to 50 µl.

DNA samples were sent to the University of Minnesota Genomics Center for library preparation and Illumina sequencing of the V4-16S ribosomal RNA (rRNA) gene region using primers V4_515F (GTGCCAGCMGCCGCGGTAA) and V4_806R (GGACTACHVGGGTWTCTAAT) and ITS2 of the internal transcribed spacer (ITS) region using primers ITS3 (GCATCGATGAAGAACGCAGC) and ITS4 (TCCTCCGCTTATTGATATGC). Libraries were then sequenced on a MiSeq PE 300.

Sequence data analysis and statistics. Reads from the forward primer or reverse primer of 16S rRNA and ITS in a few samples were of low quality, thus these reads were discarded (Supplementary Table S1). The remaining reads from either the 5′ or 3′ end were used for sequence data analysis. Contigs were created from the overlapping read-pairs from the other samples, and sequences and PCR errors were then reduced using mothur (<https://www.mothur.org/wiki>), in which contigs containing >312 base pairs for 16S rRNA or >355 base pairs for ITS were removed.

Duplicate sequences and sequences with chimera were also removed by mothur. The resulting optimized sequences were taxonomically classified against SILVA (silva.nr_v128.fasta) or UNITE ITS (UNITEv6_sh_dynamic_s.fasta) databases, and identified to kingdom, phylum, class, order, family, and/or genus levels using the Ribosomal Database Project classifier (Wang et al. 2007) at 80% threshold. Subsequently, sequences were clustered by distance-based greedy clustering method with VSEARCH (Westcott and Schloss 2015) as implemented in mothur and were classified into operational taxonomic units (OTUs) with 97% sequence similarity. OTU abundances with sums = 0 were removed after the sparse abundance (count ≤1) was replaced with 0, and the resulting matrices were used in further analysis.

Plots with low sequences (12 plots with <10,000 sequences for 16S rRNA and 20 plots with <4,000 sequences for ITS; Supplementary Table S2) were removed, and rarefaction curves, distribution of sequencing depth, and microbial community composition of classified phylum, class, order, and families (>1%) were produced in R (<https://www.rstudio.com>). Alpha diversity indices including Shannon diversity, InvSimpson diversity, observed

richness, and rarefied richness estimates interpolating the minimum sample-specific sequencing depth were calculated with the aid of R packages phyloseq (McMurdie and Holmes 2013) and vegan (Oksanen et al. 2013). Analysis of variance and Tukey's method were used to compare the mean differences of alpha diversity indices among treatments.

OTU and taxon abundances were normalized with the cumulative sum scaling (CSS) method (Paulson et al. 2013). The CSS normalized matrices after calculation of Bray-Curtis dissimilarity were subjected to nonmetric multidimensional scaling (NMDS) and hierarchical clustering analysis, respectively. Three-dimensional NMDS ordination plots were generated and heatmaps of the top 15 families of bacterial or fungal communities comparing treatment effects were made by averaging and clustering the samples collected from the same treatments with unweighted pair group method with arithmetic mean utilizing the Bray-Curtis similarity index. Furthermore, OTU and taxon abundances on the differences between pre- and posttreatment at the family level (subtractions between post- and pretreatment) were ranked and subjected to a linear mixed-effects regression model with depth and chemical rates as covariates,

TABLE 1
The comparison of alpha diversity indices of microbial communities by analysis of variance^a

Targeted gene	Indices	Parameters	F value	P value
16S rRNA	Vegan richness	Sampling time	4.7851	0.0314*
		Depth	6.7913	0.0108*
		Chemical rate	3.4858	0.0109*
		Sampling time × depth	0.8807	0.3506
		Sampling time × rate	0.7702	0.5475
		Depth × rate	0.5155	0.7245
		Sampling time × depth × rate	0.7844	0.5383
	Observed richness	Sampling time	3.7628	0.0556
		Depth	4.4264	0.0382*
		Chemical rate	2.4700	0.0504
		Sampling time × depth	0.9062	0.3437
		Sampling time × rate	1.7405	0.1482
		Depth × rate	0.3663	0.8321
		Sampling time × depth × rate	0.4995	0.7361
	Shannon	Sampling time	13.1944	0.0005*
		Depth	0.8301	0.3647
		Chemical rate	1.9916	0.1027
		Sampling time × depth	3.8883	0.0518
		Sampling time × rate	0.5247	0.7178
		Depth × rate	0.2062	0.9343
		Sampling time × depth × rate	0.6130	0.6544
	InvSimpson	Sampling time	8.5970	0.0043*
		Depth	2.1258	0.1484
		Chemical rate	0.7859	0.5374
		Sampling time × depth	5.8130	0.0180*
		Sampling time × rate	0.9076	0.4632
		Depth × rate	0.3572	0.8384
		Sampling time × depth × rate	0.7334	0.5716

(Continued on next page)

^a α = 0.05; * indicates a significant difference.

using rate 0 (untreated control plots) as reference. Differentially abundant families were also determined by comparisons between depth and among chemical rates with untreated control plots. These analyses were performed with the aid of R packages including lme4, metagenomeSeq, vegan, and DESeq2. Statistical significance was assessed at the 0.05 level for all statistical analyses performed.

RESULTS

Alpha diversity of the microbial community. *Bacterial community.* After clustering and alignment based on a 0.97 threshold across all samples, we found a total of 12,783 OTUs for 16S rRNA with a range of 386 to 1,440 OTUs present in each sample. The lowest number was in the PT2912 sample (DNA extracted at the depth of 30.5 cm after the plot was applied with 1,3-D at the rate of 187 liters/ha), while the highest number was in the PT2724 sample (DNA extracted at the depth of 61 cm after the plot was fumigated with 1,3-D at the rate of 140 liters/ha) (Supplementary Fig. S1). Of the identified OTUs, some were exclusively detected in either 1,3-D-fumigated samples ($n_{103 \text{ liters/ha}} = 907$, 7.88%; $n_{122 \text{ liters/ha}} = 1,073$, 9.33%; $n_{140 \text{ liters/ha}} = 1,307$, 11.36%; $n_{187 \text{ liters/ha}} = 1,021$, 8.87%) or control samples ($n_0 \text{ liters/ha} = 378$, 3.29%), while 59.27% of OTUs were shared by 1,3-D-fumigated

and control samples (Supplementary Fig. S2). The sequence depth for all of the samples ranged from 17,429 to 56,088 for bacterial communities. The sequencing depth of 16S rRNA for most samples did not yield saturation, as shown by the rarefaction curves.

All alpha diversity indices, except the observed richness, differed significantly between pre- and posttreatments (Table 1; Supplementary Fig. S3). The indices were higher in the posttreatment than in the pretreatment samples ($\text{Diff}_{\text{Pre-Post}} = -59.98$, $P_{\text{VeganRichness}} = 0.0314$; $\text{Diff}_{\text{Pre-Post}} = -0.30$, $P_{\text{Shannon}} = 0.0005$; $\text{Diff}_{\text{Pre-Post}} = -1.46$, $P_{\text{InvSimpson}} = 0.0043$). Bacterial communities were more complex at 30.5 cm depth compared with 61 cm depth, which was supported by higher vegan richness and observed richness at 30.5 cm ($\text{Diff}_{61-30.5} = -94.32$, $P_{\text{VeganRichness}} = 0.0382$; $\text{Diff}_{61-30.5} = -71.66$, $P_{\text{Observed}} = 0.0108$). The InvSimpson diversity index was higher at 30.5 cm in the posttreatment compared with the pretreatment ($\text{Diff}_{\text{Pre30.5-Post30.5}} = -2.94$, $P_{\text{Pre30.5-Post30.5}} = 0.0017$).

Comparing rarified richness of the bacterial communities at the different chemical rates showed that bacterial OTUs were more diverse and abundant in plots treated with 1,3-D fumigation at a rate of 140 liters/ha than in the untreated control (0 liters/ha) or in plots treated with 1,3-D fumigation at 122 liters/ha ($\text{Diff}_{140-0} = 143.18$, $P_{140-0} = 0.0112$; $\text{Diff}_{140-122} = 132.89$, $P_{140-122} = 0.0226$). The observed richness of the bacterial community in plots treated with

TABLE 1 (Continued from previous page)

Targeted gene	Indices	Parameters	F value	P value
Internal transcribed spacer	Vegan richness	Sampling time	17.7685	<0.0001*
		Depth	1.8584	0.1766
		Chemical rate	0.6611	0.6208
		Sampling time $\times$ depth	0.8544	0.3581
		Sampling time $\times$ rate	0.6568	0.6238
		Depth $\times$ rate	1.0595	0.3821
		Sampling time $\times$ depth $\times$ rate	0.3106	0.8701
	Observed richness	Sampling time	11.7935	0.0009*
		Depth	0.6957	0.4067
		Chemical rate	0.8251	0.5130
		Sampling time $\times$ depth	0.2054	0.6517
		Sampling time $\times$ rate	0.4387	0.7803
		Depth $\times$ rate	1.2643	0.2910
		Sampling time $\times$ depth $\times$ rate	0.2652	0.8995
	Shannon	Sampling time	33.4277	<0.0001*
		Depth	8.3777	0.0049*
		Chemical rate	0.7229	0.5788
		Sampling time $\times$ depth	1.1747	0.2817
		Sampling time $\times$ rate	0.6346	0.6393
		Depth $\times$ rate	0.8590	0.4923
		Sampling time $\times$ depth $\times$ rate	1.2551	0.2946
	InvSimpson	Sampling time	24.4643	<0.0001*
		Depth	7.7894	0.0066*
		Chemical rate	1.2047	0.3153
		Sampling time $\times$ depth	1.5618	0.2150
		Sampling time $\times$ rate	0.8981	0.4692
		Depth $\times$ rate	0.6347	0.6392
		Sampling time $\times$ depth $\times$ rate	0.7586	0.5553

1,3-D at a rate of 140 liters/ha was greater than in control plots ($\text{Diff}_{140-0} = 201.67$, $P_{140-0} = 0.0403$). Shannon and InvSimpson diversity indices between 1,3-D-treated and control plots ranged from 0.04 to 0.34 ($P_s > 0.0803$) and 0.16 to 1.22 ($P_s > 0.5947$), respectively, indicating no significant differences of bacterial community profiles were observed (Supplementary Table S3).

Fungal community. A total of 1,706 OTUs for ITS with a range of 21 to 398 OTUs was present in each sample. The lowest number was observed in PR0212 (DNA extracted from samples collected at a depth of 30.5 cm in an untreated control plot), while the highest number was in PT0912 (DNA extracted at a depth of 30.5 cm after treatment with 1,3-D at the rate of 140 liters/ha). Among the identified fungal OTUs, a portion was exclusively detected in either 1,3-D-fumigated samples ($n_{103 \text{ liters/ha}} = 28$, 1.82%; $n_{122 \text{ liters/ha}} = 73$,

4.74%; $n_{140 \text{ liters/ha}} = 409$, 26.58%; $n_{187 \text{ liters/ha}} = 148$, 9.62%) or control samples ($n_0 \text{ liters/ha} = 284$, 18.45%), while 38.79% of OTUs were shared by 1,3-D-fumigated and control samples. Sequence depth for all the samples ranged from 4,046 to 41,240 for fungal communities. Compared with the bacterial rarefaction curves, sequencing depth of ITS region was adequate as highlighted by the saturated curves.

All four alpha diversity indices differed significantly between pre- and posttreatment (Table 1). The mean indices were higher in communities of posttreatment samples than in pretreatment samples ($\text{Diff}_{\text{Pre-Post}} = -19.81$, $P_{\text{VeganRichness}} < 0.0001$; $\text{Diff}_{\text{Pre-Post}} = -34.54$, $P_{\text{Observed}} = 0.0009$; $\text{Diff}_{\text{Pre-Post}} = -0.43$, $P_{\text{Shannon}} < 0.0001$; $\text{Diff}_{\text{Pre-Post}} = -1.91$, $P_{\text{InvSimpson}} < 0.0001$; Supplementary Fig. S7). In addition, the mean of Shannon and InvSimpson index were higher at 30.5 cm

Fig. 1. Bacterial community composition at family rank (>1%) presented by plots with different rates of 1,3-dichloropropene applied at two depths in the pre- and posttreatment.

than at 61 cm ($\text{Diff}_{61-30.5} = -0.20$, $P_{\text{Shannon}} < 0.0049$; $\text{Diff}_{61-30.5} = -1.08$, $P_{\text{InvSimpson}} < 0.0066$). 1,3-D fumigation with different rates did not affect alpha diversity indices (Table 1). For example, differences of vegan and observed richness indices between treated and untreated soils ranged from -4.93 to 5.80 ($P_s > 0.9325$) and -8.31 to 17.08 ($P_s > 0.8112$); ranges of differences on Shannon and InvSimpson indices between treated and untreated plots were -0.15 to -0.009 ($P_s > 0.6602$) and -1.10 to -0.30 ($P_s > 0.3900$), respectively.

Microbial community composition. Bacterial community. Out of the quality-filtered reads, 58.9, 52.1, 31.8, 22.5, and 8.1% could be taxonomically classified to phylum, class, order, family, and genus level for 16S rRNA, respectively. When considering the bacterial community composition at phylum, class, order, and family level (relative abundance $> 0.1\%$; Supplementary Fig. S4), the *Proteobacteria* was the most abundant phylum across all samples. The other dominant phyla were *Firmicutes* and *Actinobacteria*. Further comparison of the microbial communities was conducted at the class level. Eight classes were screened (relative abundance $> 0.1\%$) in all samples and the most abundant classes were *Gammaproteobacteria*, *Alphaproteobacteria*, *Firmicutes*_unclassified, *Actinobacteria*, and *Bacilli*. Of the most abundant bacterial taxa, *Gammaproteobacteria*_unclassified was the dominant family, followed by *Enterobacteriaceae*, and *Rhizobiales*_unclassified (Fig. 1).

Bacterial community profiles at the family level were also visualized using a hierarchically clustered heatmap (Supplementary Fig. S5). The samples harvested from the same depth were more closely clustered. Samples collected at 30.5 cm exhibited a distant relationship with samples collected at 61 cm. Conversely, the relationship between pre- and post-samples were mixed, with some pretreatment samples clustering with posttreatment samples. NMDS plots based on Bray-Curtis dissimilarities of bacterial community composition resulted in two clear clusters of pre- and posttreatment samples, at the two depths, and four clear clusters among two depths of pre- and posttreatment samples (Supplementary

Fig. S6). In contrast, neither the overall bacterial composition in samples with different rates of 1,3-D fumigation nor the comparison of these samples from pre- and posttreatment showed separated clusters (Fig. 2).

Fungal community. Out of the quality-filtered reads, 68.9, 64.2, 62.5, 58.3, 47.4, and 19.6% could be taxonomically classified to phylum, class, order, family, genus, and species rank for ITS, respectively. When considering fungal community composition at phylum, class, order, and family level (relative abundance $> 0.1\%$), members of the Ascomycota phylum were the most ubiquitous across all samples (Supplementary Fig. S8). Within this phylum, Eurotiomycetes was the dominant class, followed by Sordariomycetes and Saccharomycetes. Fungal orders identified included Hypocreales, Eurotiales, Coniochaetales, Sordariomycetes_sedis, Sordariales and Pleosporales which are commonly found in soil samples. Of the most common fungal taxa, Nectriaceae was the dominant family, followed by Trichocomaceae, Plectosphaerellaceae, Coniochaetaceae, and Hypocreales_sedis (Fig. 3).

Similarly, fungal community profiles at the family level from 30.5 cm were dissimilar to samples collected at 61 cm, whereas pre- and post-samples were mostly clustered together (Supplementary Fig. S9). In contrast, three-dimensional NMDS plots of fungal community composition resulted in two clear clusters of pre- and posttreatment samples. Clear separations were also observed from samples collected at two depths and within the same depth between pre- and posttreatment (Supplementary Fig. S10). Similar to bacterial community, there were no separations of overall fungal community structure in samples with different rates of 1,3-D applied, nor the samples comparing the different rates between pre- and posttreatment (Fig. 4).

Significant differences in microbial communities at family level.

Bacterial community. To identify the bacterial families with significant abundance differences between treatments, we performed linear mixed-effect regression models on ranked abundance differences (subtractions between post- and pretreatment). Out of 216 bacterial families, a total of 13 phyla with 45 families showed

Fig. 2. Two-dimensional nonmetric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities of bacterial community composition showing community composition **A**, among different rates of 1,3-dichloropropene (1,3-D) applied to plots and **B**, among different rates of 1,3-D applied in pre- and posttreatment.

changes either between two depths, with different rates of 1,3-D applied, or their interactions (Table 2). Of these, three families had higher abundances and four families had lower abundances in the samples collected at 30.5 cm than in samples collected at 61 cm. Interestingly, the abundance of the common family *Enterobacteriaceae* was increased in samples at 30.5 cm.

There were 2, 2, 17, and 14 families that had decreased abundances, while 3, 4, 6, and 3 families had increased abundances in response to 1,3-D fumigation at rates of 103, 122, 140, and 187 liters/ha, respectively. Of the families with decreased abundances, 11 families (*Blastocatellaceae*_(Subgroup4), *Subgroup_6_fa*, *Subgroup_6_unclassified*, *Acidimicrobiales_unclassified*, *Corynebacteriales_unclassified*, *Saprospiraceae*, *KD3-93*, *Rhodospirillales_unclassified*, *Betaproteobacteria_unclassified*, *Bacterioboraceae*, and *Coxiellaceae*)

had reduced abundance in plots with 1,3-D at 140 liters/ha. The abundances of 11 families (*Chthonomonadales_fa*, *Bacteroidales_unclassified*, *Cyanobacteria_unclassified*, *Clostridiaceae_1*, *Selenomonadales_unclassified*, *0319-6A21*, *Planctomycetaceae*, *Comamonadaceae*, *Gammaproteobacteria_unclassified*, *Proteobacteria_unclassified*, and *Chthoniobacteriales_unclassified*) were only negatively affected by 1,3-D at 187 liters/ha. There were three families, including *C0119_fa*, *Tepidisphaeraceae*, and *UA11_fa*, that had lower abundances in plots where 1,3-D was applied at rates of 140 and 187 liters/ha. The abundances of *Firmicutes_unclassified* and *Rhodospirillaceae* decreased in plots treated with 1,3-D at rates of 103 and 140 liters/ha, as well as a decreased abundance of *Rhizobiales_Incertae_Sedis* in plots treated with 1,3-D at rates of 122 and 140 liters/ha. However, one family,

Fig. 3. Fungal community composition at family rank (>1%) presented by each plot with sampling depth and different rates of 1,3-dichloropropene applied in the pre- and posttreatment.

Methylophilaceae, was lowered in response to 103 liters/ha, but its abundance increased in plots with fumigant at a rate of 122 liters/ha. In contrast, the abundances of five families (*Flavobacteriales_unclassified*, *Obscuribacterales_fa*, *Bacillaceae*, *Polyangiaceae*, and *Xanthomonadales_unclassified*) increased in plots with 1,3-D at rates of 103, 122, or 140 liters/ha, while families including *Cytophagia_unclassified*, PHOS-HE5, *Enterobacteriaceae*, and an Unknown_Family in phylum *Verrucomicrobia* were positively affected by more than one rate. *Enterobacteriaceae* was the only family with consistent increasing abundances with higher rates of 1,3-D fumigation.

Certain families including *Bacteroidales_unclassified*, *Cytophagia_unclassified*, *Simkaniaceae*, *Chlamydiales_unclassified*, *Selenomonadales_unclassified*, and *Alphaproteobacteria_unclassified* displayed either more or less abundance in plots at 30.5 cm with applications of 1,3-D using different rates when compared with soil samples collected at 61 cm in untreated control plots.

Fungal community. We examined 139 fungal families where 24 families that belong to six phyla showed changes either between sample depths, with different rates of 1,3-D applied, or their interactions (Table 3). Most of these families that increased or decreased in abundance belong to Ascomycota. There were two families with abundances that differed between the two sample depths. *Ophiocordycipitaceae* had higher abundances in soil samples collected at 30.5 cm and the abundance of *Cordycipitaceae* was lower when compared with fungal community abundances from soil samples collected from 61 cm.

There were two, three, seven, and four families that decreased in abundance, while one, two, one, and one family(s) increased in abundance in response to 1,3-D fumigation at rates of 103, 122, 140, and 187 liters/ha, respectively (Table 3). Of the families with increasing abundances, *Microascales_unclassified*, *Lophiostomataceae*, and *Hyaloscyphaceae* increased after fumigating with 1,3-D at rates of 103, 140, and 187 liters/ha, respectively, while *Chaetomiaceae* and *Montagnulaceae* both increased after application of 122 liters/ha. Among these families identified with decreasing

abundances, *Ophiocordycipitaceae* was the only family that displayed consistent decreasing trend in response to 1,3-D fumigation at 122, 140, and 187 liters/ha.

The abundances of six families, including *Arachnomycetaceae*, *Lophiostomataceae*, *Microascales_unclassified*, *Montagnulaceae*, *Ophiostomataceae*, and *Claroideoglomeraceae*, increased in plots at 30.5 cm with applications of 1,3-D at different rates when compared with soil samples collected at 61 cm in untreated control plots. In contrast, seven families (*Botryosphaeriaceae*, *Coniochaetales_unclassified*, *Cordycipitaceae*, *Gymnoascaceae*, *Onygenaceae*, *Ophiocordycipitaceae*, and *Malasseziales_unclassified*) showed decreasing trends of abundances in plots at 30.5 cm with applications of 1,3-D when compared with soil samples in untreated plots at 61 cm depth. *Ophiocordycipitaceae* was negatively affected at the upper level of soils through fumigation, regardless of the amount of 1,3-D applied.

DISCUSSION

Soil microflora is vital for healthy soil and is a source of genetic and metabolic diversity (Wang et al. 2015), but application of fumigants has become an essential practice to protect many crops from soilborne pathogens, nematodes, and weeds. Soil microorganisms experience disturbance (i.e., transient or intransient effects) upon application of fumigants, soil amendments, and other agricultural practices (Singh et al. 2015), but soil can be a robust ecosystem and fumigants may not have a long-term impact on the overall microbial community. We designed our experiment to determine if we could detect the impact of 1,3-D on the soil community approximately 1-month postfumigation, when 1,3-D treatment was included as part of typical farming operations.

We observed that posttreatment soils had higher bacterial and fungal richness and diversity than pretreatment soils, even in the pre- and post-control plots (Table 1). This suggests that moderate disturbance such as incorporation of cover crop into the entire experimental plot, and the irrigation and precipitation that occurred

Fig. 4. Two-dimensional nonmetric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities of fungal community composition showing community composition **A**, among different rates of 1,3-dichloropropene (1,3-D) applied to plots and **B**, among different rates of 1,3-D applied in pre- and posttreatment.

TABLE 2
Bacterial phylum and families with significantly different abundances identified through linear mixed-effect regression models at a depth of 61 cm and no chemical (0 liters/ha) applied as reference levels^a

Phylum	Family	Depth (cm)	Rate applied (liters/ha)				Depth × rate			
		30.5	103	122	140	187	30.5 × 103	30.5 × 122	30.5 × 140	30.5 × 187
<i>Acidobacteria</i>	<i>Blastocatellaceae</i> _(Subgroup4)					–				
	Subgroup_6_fa					–				
	Subgroup_6_unclassified					–				
<i>Actinobacteria</i>	<i>Acidimicrobiales</i> _unclassified					–				
	<i>Corynebacteriales</i> _unclassified					–				
<i>Armatimonadetes</i>	<i>Chthonomonadales</i> _fa					–				
<i>Bacterioidetes</i>	<i>Bacteroidales</i> _unclassified					–				–
	<i>Cytophagia</i> _unclassified	–		+		+	+			+
	<i>Flavobacteriales</i> _unclassified				+					
	PHOS-HE51		+		+	+				
	<i>Saprospiraceae</i>				–					
	KD3-93				–					
<i>Chlamydiae</i>	<i>Simkaniaceae</i>									–
	<i>Chlamydiales</i> _unclassified	+							+	–
<i>Chloroflexi</i>	<i>Chloroflexi</i> _unclassified						+			
	C0119_fa				–	–				
	P2-11E_fa						–			
<i>Cyanobacteria</i>	<i>Cyanobacteria</i> _unclassified					–				
	<i>Obscuribacteriales</i> _fa			+						
<i>Elusimicrobia</i>	MVP-88_fa	–								
<i>Firmicutes</i>	<i>Bacillaceae</i>				+					
	<i>Lachnospiraceae</i>						+			
	<i>Clostridiaceae</i> _1					–				
	<i>Firmicutes</i> _unclassified		–		–					
	<i>Selenomonadales</i> _unclassified					–				–
<i>Nitrospirae</i>	0319-6A21					–				
<i>Planctomycetes</i>	<i>Tepidisphaeraceae</i>				–	–				
	<i>Planctomycetaceae</i>					–				
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i> _unclassified								–	
	<i>Rhodospirillales</i> _unclassified				–					
	<i>Rhodospirillaceae</i>		–		–					
	<i>Rhizobiales</i> _Incertae_Sedis			–	–					
	<i>Comamonadaceae</i>					–				
	<i>Betaproteobacteria</i> _unclassified				–					
	<i>Methylophilaceae</i>	–		–	+					
	<i>Bacterioboracaceae</i>				–					
	<i>Polyangiaceae</i>		+							
	<i>Gammaproteobacteria</i> _unclassified					–				
	<i>Enterobacteriaceae</i>	+		+	+	+				
	<i>Xanthomonadales</i> _unclassified	–	+							
	<i>Coxiellaceae</i>				–					
	<i>Proteobacteria</i> _unclassified					–				
<i>Verrucomicrobia</i>	Unknown_Family	+		+	+					
	<i>Chthoniobacterales</i> _unclassified					–				
	UA11_fa				–	–				

^a *Ps* < 0.05, α = 0.05; +, an increasing abundance; and –, a decreasing abundance.

prior to the post-soil sampling, has a greater impact on bacterial and fungal communities in this soil ecosystem than did fumigation (Galand et al. 2016; Lancaster et al. 2010). Previous research reported that application of 1,3-D only had transient suppressive impacts on indigenous bacteria (Ibekwe et al. 2001; Liu et al. 2015); however, in the present study, increasing rates of 1,3-D to 122 and 140 liters/ha resulted in an enhanced bacterial richness in soils that was not observed in treatments that received 1,3-D at rates of 103 and 187 liters/ha when compared with control plots at the same sampling time. These results suggest that 1,3-D and other environmental factors might collectively drive the shift of bacterial community structure (Santoyo et al. 2017). In contrast, diversity and richness indices of fungal communities were not affected by 1,3-D fumigation which is in agreement with the results obtained by Hoshino and Matsumoto (2007), who studied the impact of 1,3-D on the fungal community in a Dystric-Silic Andisol, light clay spinach field.

Richness indices of bacterial community and diversity indices of fungal community were different in soil samples collected at two depths, with higher indices occurring at 30.5 cm. NMDS was conducted to examine the effect of sampling time, depth, and 1,3-D fumigation on overall microbial community structure at the family level. Clear separations between pre- and posttreatment, at the two depths, and their interactions indicate that the soil microbial

community varied interactively as a function of sampling time and depth. In contrast, the 1,3-D fumigation had no impact on overall microbial community profiles compared with untreated control plots (Figs. 2 and 4) although a few families showed altered abundances in response to 1,3-D fumigation. These findings are consistent with the relationships observed using a hierarchically clustered heatmap, except that microbial community profiles in plots from pre- and post-samples were mixed which might be attribute to similar soil physical properties among pre- and post-samples. It is also possible that temperature and humidity gradients as well as plant biomass influence soil microbial community structure at different depths (Mandakovic et al. 2018; Rui et al. 2015; Št'ovíček et al. 2017).

Similar to some previous studies that analyzed microbial community composition in various environments or in response to different treatments (Dong et al. 2017; Guo et al. 2015; Fang et al. 2015; Ma et al. 2016; Liu et al. 2015; Spain et al. 2009), three phyla, including *Proteobacteria*, *Firmicutes*, and *Actinobacteria*, were predominant across all soil samples. It is not surprising that *Proteobacteria*, a genetically and metabolically diverse, environmentally widespread, and ecologically important phylum was most abundant in our soil samples (Liu et al. 2015). Within this phylum, *Gammaproteobacteria* are responsible for utilizing propionate, butyrate, and acetate, and *Alphaproteobacteria*, which could

TABLE 3
Fungal phylum and families with significantly different abundances identified through linear mixed-effect regression models at a depth of 61 cm and no chemical (0 liters/ha) applied as reference levels^a

Phylum	Family	Depth (cm)	Rate applied (liters/ha)					Depth × rate		
		30.5	103	122	140	187	30.5 × 103	30.5 × 122	30.5 × 140	30.5 × 187
Ascomycota	Arachnomycetaceae						+			
	Botryosphaeriaceae		–				–			
	Chaetomiaceae			+						
	Coniochaetales_unclassified				–		–			
	Cordycipitaceae	–		–			–			–
	Gymnoascaceae						–			
	Hyaloscyphaceae					+				
	Hypocreales_unclassified					–				
	Lophiostomataceae				+				+	
	Microascales_unclassified		+				+			
	Montagnulaceae			+				+		
	Nectriaceae				–					
	Onygenaceae									–
	Ophiocordycipitaceae	+		–	–	–	–	–	–	–
	Ophiostomataceae								+	
	Pyrenomataceae		–							
	Saccharomycetales_fam_Incertae_sedis			–						
Basidiomycota	Bulleribasidiaceae				–					
	Malasseziales_unclassified				–				–	
	Schizophyllaceae					–				
Chytridiomycota	Unclassified_Chytridiomycetes				–					
Glomeromycota	Claroideoglomeraceae								+	
Mortierellomycota	Mortierellaceae				–					
Rozellomycota	Unclassified_GS10					–				

^a $P_s < 0.05$, $\alpha = 0.05$; +, an increasing abundance; and –, a decreasing abundance.

decompose glucose were dominant (Garcia-Peña et al. 2011; Huang et al. 2018). *Firmicutes* was the dominant phylum ranked only second to *Proteobacteria*. Many *Firmicutes* produce endospores to survive extreme conditions (e.g., *Bacilli*, *Clostridia*), and are often encountered in soil (Fimlaid and Shen 2015; Janssen 2006). *Actinobacteria*, which play important roles related to organic material turnover and carbon cycling were also ubiquitous in our plots (Nielsen et al. 2014).

Among fungi, the dominant phylum was assigned to Ascomycota in all of the soil samples. This is in agreement with previous studies, which showed that Ascomycota accounted for more of the total sequences in soil samples as demonstrated by Illumina HiSeq sequencer (Dong et al. 2017; Schmidt et al. 2013). Three dominant classes within Ascomycota, Sordariomycetes, Eurotiomycetes, and Saccharomycetes, play critical roles in decomposition and nutrient cycling, and the high abundances of this phylum in the soil samples may be associated with a higher disease suppression ability (Shen et al. 2015).

Notably, more families from bacterial and fungal communities had reduced abundance than families with enriched abundance after 1,3-D fumigation, perhaps due to the broad biocidal activity of 1,3-D. However, most families did not show consistent patterns of increasing or decreasing abundances correlated with 1,3-D fumigation (Tables 2 and 3). Among the microbial families that were significantly increased or suppressed in response to 1,3-D fumigation, most were not dominant families (Tables 2 and 3; Figs. 1 and 3). Of the dominant bacteria families, *Enterobacteriaceae* showed a greater abundance in plots with increased 1,3-D rates. Similarly, Liu et al. (2015) found that *Enterobacteriaceae* were greatly enriched in 1,3-D-fumigated plots compared with their relative abundance in control plots. This family includes many species that grow quickly and that are associated with invertebrates and these species tend to encode enzymes capable of degrading chitin. Death of nematodes and insects caused by 1,3-D may have provided bacteria from this family a nutrient source that supported increased growth of some *Enterobacteriaceae* species. Other dominant bacterial families, *Firmicutes_unclassified* and *Gammaproteobacteria_unclassified* displayed decreasing responses to 1,3-D fumigation. Interestingly, a parasitic fungal family, Ophiocordycipitaceae which belongs to Ascomycota, was negatively affected by 1,3-D fumigant with increasing rates. Some species within the family are nematode and insect parasitic fungi (Simmons et al. 2015; Zhang et al. 2017). It is likely that the decreasing abundance of Ophiocordycipitaceae were positively related to the reduction of nematode and insect populations by 1,3-D fumigants.

In this study, we demonstrated that 1,3-D fumigation did not significantly affect overall microbial community profiles nor microbial richness and diversity, although bacterial richness increased following application of 1,3-D at 140 liters/ha. Species from the bacterial family *Enterobacteriaceae*, which may be able to quickly metabolize dead invertebrates, and species from the family Ophiocordycipitaceae, which may be invertebrate pathogens, were altered in abundance in response to different rates of fumigation treatment. Based on the results, we propose that 1,3-D fumigation has only a minor overall effect on microbial community structure in sandy loam soils. However, future studies that determine if decreases in potential fungal pathogens of invertebrates in the Ophiocordycipitaceae allow nematode populations to recover quickly postfumigation may be warranted.

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