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Microbial Community Response to Fumigation in Potato Soils

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Master of Science

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ABSTRACT

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Soil microorganisms have a variety of beneficial and deleterious effects on plants, impacting such processes as plant growth, soil nutrient cycling, crop yield, disease resistance and tolerance to an array of biotic and abiotic stressors. The disruption of soil microbial community structures, particularly when beneficial soil biota are altered, has been shown to reduce crop yield and leave plants susceptible to disease. Long-term disruption of microbial communities may occur with repeated fumigation, being the application of gaseous pesticides, in agricultural soils. For this reason, we characterized bacterial, fungal, oomycete and nematode populations in paired fumigated and nonfumigated potato fields located in Idaho, Oregon, Washington and Minnesota. Samples were taken at three distinct timepoints: one before a fall fumigation event and two others at important stages in potato production, row closure and vine death. Soil biota populations were assessed by targeting the 16S, 18S and ITS1 gene regions. FunGuild, a database capable of guild and trophic assignment of fungal lineages, was used to sort fungal OTUs in different trophic modes. Fungal analyses indicated an increase in relative abundances of saprotrophic fungal populations and a decrease in pathotrophic fungal populations, both during row closure. Principally, the fungal genera of *Humicola* and *Mortierella* were responsible for the increase of saprotrophs while *Alternaria* decreased the most for pathotrophs. Other fungi occupying multiple trophic modes, such as *Fusarium*, also decreased during row closure. We found that fumigation treatments, in combination with various pesticide and fertilizer applications, alter both alpha- and beta- bacterial soil diversity although certain treatments, i.e. chloropicrin, may alter bacterial populations more than other treatment types such as metam-sodium. Nematode populations were likewise distinct at each location with soils from Boardman, OR, Minidoka, ID and Pine Point, MN with these having higher levels of nematodes associated with better soil health, i.e. Dorylaimidae. Conversely, nematodes associated with plant pathogenesis were found in higher relative abundances at Minidoka, ID and Quincy, WA. In this study, we characterize the populations of bacteria, fungi, oomycetes and nematodes with an emphasis on fungal taxa. We found that relative abundances of fungal trophic modes vary temporally. Additionally, we catalogue several other high abundance taxa with seasonal differential abundances whose functional capacity in potatoes remain uncharacterized.

Keywords: DNA extraction, soil microbiome, soil biota, fumigation, trophic modes

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Microbial Community Response to Fumigation in Potato Soils

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Potato Grant Info

INTRODUCTION

In 2016, 44.1 billion pounds of potatoes, *Solanum tuberosum*, were produced in the United States of America, valued at a total of $3.06 billion dollars (USDA, 2017). But despite the considerable market value for potatoes, the input costs for producing 44.1 billion pounds of potatoes is high, with one of the largest expenses being pesticide application. But even with large-scale application of pesticides, market loss due to diseases can still be high. For instance, a 2001 survey conducted by Guenthner showed that despite potato growers applying an estimated $77.1 million dollars of fungicides, specifically to control the late-blight pathogen *Phytophthora infestans*, $210.7 million dollars' worth of potatoes were still lost to the selfsame pathogen (Guenthner *et al.*, 2001). Notably, Guenthner's estimate solely assessed the economic impact of late-blight, excluding prominent potato diseases like common scab, early blight, dry rot and black dot, among others.

Yearly, growers' monetary input of fumigants, herbicides and other pesticides to potato fields eclipses hundreds of millions of dollars. While no nationwide statistic of fumigant weight applied exists, it is telling that Washington state alone accounted for 6.6 million pounds of 1,3-dichloropropene and 11.6 million pounds of Vapam®, both being fumigants with broad biocidal activity affecting target and non-target soil biota alike (Tu, 1993, Macalady *et al.*, 1998). But despite the widespread use of fumigation and other pesticide application methods in potato production, acreage affected by soilborne pathogens appears to be increasing, with some growers

reporting up to a 50% increase in their acreage affected by soilborne pathogens over the past decade (Personal communication, Noah Rozensweig). This indicates that although fumigation and other forms of pesticide application are effective for interim maintenance of pathogen populations, long-term application may be disadvantageous in controlling soilborne pathogenesis in *S. tuberosum*. In part, this could be because chemical applications alter to the at-large soil microbiome which, being the soil microbiome, has been demonstrated to play a pivotal role in plant defense, nutrient cycling and other plant services (Berendsen *et al.*, 2012, Lakshmanan *et al.*, 2014).

Several proposed hypotheses offer explanations as to how and why long-term heavy fumigation may affect the soil microbiome in a way that it is detrimental to plant health. One such proposal, central to the disease rate narrative of amplified disease risk, is that some fumigated fields that have increased disease incidence rates may be a consequence of unbalanced or diminished soil microbial populations (Garbeva, 2005, Mendes *et al.*, 2011). Justifications for this explanation include a) that the removal of anti-pathogenic microflora from the environment protects pathogens from predation, b) that reductions in beneficial microorganisms concedes previously filled habitat niches to pathogens and c) that repeated fumigant applications select for fumigant-degrading bacterial populations who, in turn, diminish the effectiveness of applied fumigants (VanderZaag, 2010, Penton *et al.*, 2014, Gómez Expósito *et al.*, 2017).

Long-term increased pathogen potential is but one example of how fumigant-disturbed soil microbial populations may affect potato plants. As an example, fumigation has been shown to reduce microbial biomass or change microbial populations to levels affecting carbon and nitrogen cycles (Yamamoto *et al.*, 2008). In some cases, these alterations of microbial populations resulted in lower marketable yield crops and stunted growth patterns (Hiltunen *et al.*,

2017). The affected populations typically include nitrogen-fixing bacteria, many of which have populations that are slow to re-establish, resulting in populations more vulnerable to fumigation (Li *et al.*, 2017). Conversely, many soil biota have shown resistance to broad-spectrum fumigants, such as actinomycetes with Vapam®, which results in a disproportionally high actinomycete population compared to vulnerable soil microorganism populations (Sinha *et al.*, 1979).

Nitrogen fixation and pathogen protection are but two traits microflora may confer on their hosts. Other important services include increased rates of plant growth and crop yield, improved soil nutrient cycling and and tolerance to an array of biotic and abiotic stressors (Ortíz-Castro *et al.*, 2009, Busby *et al.*, 2017). Some of these microbial ecosystem services are general, indicating a level of non-transferability between soils, and are attributable to factors such as microbial beta- and alpha-diversity, clade, or total microbial biomass (Weller *et al.*, 2002). Each of these factors may be changed or reduced by fumigation or other methods of pesticide applications (Collins *et al.*, 2006).

At other times, beneficial plant-microbe interactions may be specific, meaning attributable to an individual organism or select group of microorganisms, and these may be transferable between soils (Weller *et al.*, 2002). For instance, in the case of potato disease resistance, specific isolated strains of nonpathogenic *Streptomyces spp.* have been shown to be effective biocontrols of common scab, both in terms of incidence and severity, albeit dependent on environmental conditions and cultivar type (Weller *et al.*, 2002, Wanner *et al.*, 2014). Of importance, when considering the potential of specific bacterial inoculants to promote plant growth or other functions, the microbe must be considered in a host-specific manner. Simply because *Streptomyces spp.* reduces common scab in one population of potatoes does not mean it

will serve a similar function in other plant species or even other potato cultivars (Wanner *et al.*, 2014). In fact, non-host-specific microbial interactions may incur negative plant responses (Wanner *et al.*, 2014).

These host-specific and non-host-specific plant-microbial relationships may indicate a tight, co-evolutionary relationship between plant and microbe. These could be at an individual, specific plant-microbe level or show a relationship between higher or lower proportions of a clade according to the plant (Johnston-Monje & Raizada, 2011, Kinkel *et al.*, 2011). When considering how a plant might select for distinct microbial populations, it has been shown that plant have unique exudate profiles. The exudates, in microcosm analyses, preferentially enrich specific microbes (Doornbos *et al.*, 2012). In potatoes, studies have found that certain potato cultivars have distinct exudate profiles which may partially explain why, even at a cultivar level, different potato cultivars have unique microbial populations, independent of location (İnceoğlu *et al.*, 2012).

The function of root exudates is not only to attract specific microbes but is also produce to antimicrobial defense exudates (Doornbos *et al.*, 2012). These roots exudates protect against pathogenic fungi, oomycetes, bacteria, viruses and nematodes. Though all these types of pathogens affect *S. tuberosum*, fungi and oomycetes are the principal perpetuators of wide-scale crop loss. The most prominent fungal and oomycete pathogen species that infect potato plants includes *Pythium ultimum*, *Alternaria solani*, *Phytophthora infestans*, *Spongospora subterranea*, *Helminthosporium solani*, *Verticillium dahliae* and *Rhizoctonia solani*. Each of these are soil-borne plant pathogens reside in low abundances in soils until the proper environmental conditions are met. In some instances, pathogens of *S. tuberosum*, such as root lesion nematodes,

stubby root nematodes and fungus *Rhizoctonia solani*, act complementarily to aggravate disease (Björsell *et al.*, 2017).

For these reasons, we aim to characterize the soil microbial communities of *S. tuberosum* in agricultural soils and assess interactions between plant and soil biota. We do so with a special interest in understanding how fumigant-disturbed soils may stimulate divergence or exacerbate existing differences in the composition of soil fungal and bacterial communities as well as other soil biota. Furthermore, we assess communities across of temporal gradient, focusing on three important stages within potato agriculture: prefumigation, the timepoint before a fall fumigation event; row closure, when the canopy of potato plants in adjacent rows overlap; and vine death, when photosynthesis, plant growth and carbohydrate transfer from leaves to tuber declines as the plant safeguards its resources from pathogens. Our primary research aims are to 1) profile soil biota composition of potato fields under traditional and organic management practices under field conditions, 2) profile these communities across different potato growing regions, 3) compare the effects of treatment on soil biota community composition and 4) track temporal changes in soil biota communities. We hope that our findings will enable us to better understand potato soil microbe-plant interactions, an essential aspect for furthering sustainable, healthy potato production under various agricultural management systems.

METHODS

Sampling design

Soil cores were collected from fumigated and non-fumigated potato fields located in four different states: Washington, Oregon, Idaho and Minnesota. Fumigated fields were managed with either a combination or individual treatment of chloropicrin, Vapam® or Telone II. Two fields were selected in Washington and Oregon; one field was fumigated and the other non-fumigated. Three Minnesota fields were collected, two being fumigated and one being non-fumigated. In Idaho, four fields were selected, representing two different growing seasons with each growing season having a fumigated and non-fumigated field. The total number of fields sampled was eleven.

In each field, soil core sampling consisted of ten sites per field, with each sample site spaced at minimum distances of 100 meters apart. Five subsamples were taken at each sample site. Each subsample was taken with a soil probe (2.5 cm diameter) at a depth of 10 cm. Subsamples were then combined into one sample to convey a more accurate spatial representation of microbial diversity at each site. To measure microbial change temporally, sampling events occurred prior to fumigation, at row closure, and vine death representing 30 samples per growing season. Following sample collection, all soil samples were stored on ice, promptly shipped to Brigham Young University, homogenized, and stored in a -20 C freezer until further processing.

DNA Extraction

Soil biota DNA was extracted using the MoBio PowerSoil® DNA Isolation Kit (Qiagen, Hilden, Germany) following standard kit protocols with slight modifications as recommended by Lindhal (Lindahl *et al.*, 2013). Samples were homogenized with a Vortex-Genie 2 Mixer (Scientific Industries, Bohemia, NY, USA) at a setting of 10 for 15 minutes. Templates were quantified with an ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and then concentrated to 30 ng/µl.

Sequencing

Three separate amplicon libraries were generated to assess different populations: general eukaryotes, bacteria and fungi.

To assess general eukaryote populations, we targeted the V9 hypervariable region of the nuclear 18S rRNA region (Figure 1). Amplicon libraries were created using primers 1391F (5'-GTACACACCGCCGTC-3') and EukBr (5'-TGATCCCTTCTGCAGGTTCACCTAC-3') with primer EukBr containing a unique, 12-bp multiplex identifier (Amaral-Zettler *et al.*, 2009, Fadrosh *et al.*, 2014). Each primer contained either a forward or reverse Illumina adapter, primer pad and primer linker (Table 1). Multiplex identifiers were selected as recommended by Earth Microbiome Project (http://www.earthmicrobiome.org). Thermocycler conditions followed parameters consistent with Earth Microbiome Project's 18S Illumina amplicon protocol with slight modifications to optimize conditions for a different PCR master mix, Invitrogen™ AccuPrime™ Pfx SuperMix (ThermoFisher Scientific, Waltham, MA, USA). Initial denaturation was set at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45 seconds,

annealing at 55.6°C for 60 seconds, and elongation at 72°C for 90 seconds. A final elongation step was set for 72°C for 10 minutes and all samples were held at 4°C.

In bacteria, the V4 hypervariable region of the 16S rRNA gene (Figure 1) was targeted using primers 16Sf (5'-GTGCCAGCMGCCGCGGTAA-3') and 16Sr (5'-GGACTACHVGGGTWTCTAAT-3'). Both primers, 16Sf and 16Sr, contained a series of repeating 8-bp barcodes which, in combination, facilitated a dual-indexed Illumina sequencing approach (Caporaso *et al.*, 2012, Kozich *et al.*, 2013). A forward or reverse Illumina primer, linker region and primer pad were also included on both 16Sf and 16Sr. Invitrogen™ AccuPrime™ Pfx SuperMix was likewise used for the generation of 16S amplicons. Thermocycler conditions were the same as the general eukaryote approach except that no modification was made to the annealing temperature. Initial denaturation was 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 60 seconds, and elongation at 72°C for 90 seconds. A final elongation step was set for 72°C for 10 minutes and all samples were held at 4°C.

For molecular identification of fungal lineages, the nuclear internal transcribed spacer 1 (ITS1) region was amplified in a two-step PCR approach (Figure 1). Initial PCR amplification utilized an oligo containing the forward primer ITS1F_KYO1 (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-NNNXXX-CTHGGTCATTTAGAGGAASTAA-3') with 3-mer, 4-mer, 5-mer or 6-mer Ns and the forward Illumina sequencing primer fused to the 5' end. The reverse oligo consisted of primer ITS2_KYO2 (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-NNNXXX-TTYRCTRCGTTCTTCATC-3'), 3-mer, 4-mer, 5-mer or 6-mer Ns, and the reverse Illumina sequencing primer (Toju *et al.*, 2016). PCR amplification was performed with Accuprime Pfx

SuperMix with the following parameters: initial denaturation at 94°C for 2 min, 35 cycles at 98°C for 10 sec, 50°C for 30 sec, 68°C for 50 sec, with a final extension of 68°C for 5 min and a final holding temperature of 4°C. A final PCR was utilized to ligate Illumina adapters and barcodes. A second PCR process utilized forward fusion Illumina primer consisting of the P5 Illumina adaptor, an 8-mer barcode, and the 5' end of the sequencing adaptor (5'-AATGATACGGCGACCACCGAGATCTACAC-XXXXXXXX-TCGTCGGCAGCGTC-3'). The reverse fusion Illumina primer consisted of the P7 Illumina adaptor, an 8-mer barcode, and the 5' end of the sequence adapter (5'-CAAGCAGAAGACGGCATACGAGAT-XXXXXXXX-GTCTCGTGGGCTCGG). The PCR parameters were as follows: initial denaturation at 94°C for 2 min, 8 cycles at 98°C for 10 sec, 50°C for 30 sec, 68°C for 50 sec, with a final extension of 68°C for 5 min and a final holding temperature of 4°C.

Following amplification of either library, normalization of amplicons occurred using SequalPrep™ Normalization Plate (96) Kit (Invitrogen, Carlsbad, CA, USA). All multiplexed samples will be pooled and have their concentration quantitated on a Qubit™ 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA, USA). Following quantitation, all Idaho V4 16S rDNA samples, save for prefumigation samples from Acequia, ID, and Washington and Acequia, ID eukaryote all samples were shipped to Michigan State University where they underwent further quality control. At Michigan State University, all samples were tested for size distribution, size confirmation and PCR artefacts using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Library quantification using Kapa PCR was utilized prior to sequencing (Kapa Biosystems, Wilmington, MA, USA). Paired-end sequencing (2x250) was carried out on the Illumina MiSeq sequencer at Michigan State University (Illumina Biotechnology, San Diego, CA, USA). Equivalent sequence prep procedures were performed at the Brigham Young

University sequencing center on the Illumina HiSeq 2500 platform (2x250) as (Illumina Biotechnology, San Diego, CA, USA). To increase sequencing efficiency, samples with high levels of primer dimers had primer dimers removed via PippinPrep (Sage Science, Beverly, MA, USA).

Data Analysis

Illumina sequence reads were demultiplexed according to Illumina protocol at the Brigham Young University sequencing center. Following demultiplexing, sequence reads were analyzed within QIIME (v. 1.9.1), an open-source software package capable of target metagenomic microbial community analysis. Prior to introducing reads into QIIME, we removed primers and barcodes with a custom, in-house script. Then, within QIIME, we joined paired-end reads by using fastq-join with default parameters (Aronesty, 2011). Fastq-join output was then demultiplexed and checked for chimeras using the vsearch pipeline (Rognes *et al.*, 2016). Within vsearch, the option called —non_chimeras_retention was changed from its default condition to intersection. By doing so, sequences flagged as chimeric by either of vsearch's *de novo* or reference-based approaches were removed. Following chimera checking, all demultiplexed sequences were clustered into operational taxonomic units (OTUs) but by different methods. 16S,18S and ITS1 reads were clustered by applying a 97% similarity threshold under default parameters with uclust (Edgar, 2010). For 16S rRNA reads, representative OTUs were assigned taxonomy by means of uclust with reference sequences retrieved from the SILVA 128 database (Quast *et al.*, 2013). Unlike the 16S rRNA regions, the ITS1 fungal and 18S eukaryote reads were taxonomy OTUs via RDP assignment with 0.50 confidence. The dynamic fungal UNITE database was used for ITS1 fungal reads and the SILVA 128 database was used for 18S reads

(Kõljalg *et al.*, 2005, Wang *et al.*, 2007, Yilmaz *et al.*, 2014). Because fungal primers ITS1F_KYO1 and ITS2_KYO2 amplify oomycetes, an existing, modified oomycete database was merged with the UNITE database to allow for taxonomic identification of oomycetes (Sapkota & Nicolaisen, 2015). Phylogenetic trees were constructed by FastTree 2.1.3 (Price *et al.*, 2009) for 16S and 18S OTUs. Because the fungal ITS1 region is a poor chronometer, no phylogenetic trees were generated for fungal ITS1 dataset.

All taxonomy files, OTU tables and tree files generated in QIIME were input to R (Team, 2014). RStudio was used as a graphical user interface (Team, 2015). In R, we used the PhyloSeq package to combine the input files into a single object and to rarefy all samples (McMurdie & Holmes, 2013). Because PippinPrep expunged Ascomycetes from fungal samples, we removed all fungal ITS1 samples that had PippinPrep performed on them which consisted of samples from Boardman, OR and Quincy, WA. Samples from these locations were not included in fungal analyses. Fungal, eukaryote and bacterial data were treated as separate samples with each of these being rarefied according to their ability to capture diversity. This was estimated by generating alpha rarefaction plots in R. We generated nDMS plots in R, choosing nMDS as a multidimensional scaling method due to its ability to handle sparse datasets and its ability to calculate with non-Euclidean distances.

Fungal functional and guild identity was predicted using FunGuild (Nguyen *et al.*, 2016). Following guild assignment, differential abundances of fungal taxa were tested across different combinations of sites, seasons, treatments and other factors using the R package ANCOM (Weiss *et al.*, 2017). Adonis values were calculated with the R package vegan (Oksanen *et al.*, 2007) with false discovery rates being controlled using FDR correction with a p-value of 0.05.

Soil Analysis

Assessment of physiochemical soil properties at the BYU Environmental Soil Analytical Lab (Brigham Young University, Provo, UT, USA). Characteristics known to affect soil microorganism populations such micro- and macronutrient availability, texture analysis and organic matter was performed. Organic matter was assessed using the Walkley and Black method (Walkley & Black, 1934). Electrical conductivity was measured from a saturated paste of the soil using the RC-16C Conductivity Bridge (Beckman Instruments, Brea, CA, USA) and the pH was estimated using the same paste (Page *et al.*, 1982). Ca++, Mg++ and Na+ were also assessed from the paste by running a dilution of the sample extract with an interference chemical through an AAnalyst 200 (PerkinElmer, Waltham, MA, USA.). Particles sizes, i.e. texture analysis, was performed by measuring percent salt, silt and clay with a hydrometer-based method (Day, 1965). Potassium was also analyzed on the AAanlyst 200 according to methods outlined by Schoenau (Schoenau & Karamanos, 1993). Phosphorus was assessed by the traditional Olsen methods (Olsen, 1954).

RESULTS

Fungi

In total, 653,310 fungal reads, representing 126 samples with each sample being rarefied to 5,185 reads, were submitted into the R workflow (Figure 2). Fungal samples were removed if they were below 5,185 sequence reads or if they underwent PippinPrep at the BYU sequencing center because the PippinPrep protocol selected against Ascomycetes before their being submitted to sequencing. Effectively, this removed all fungal samples from Boardman, OR and Quincy, WA, save for several samples from Washington, but left all Idaho and Minnesota samples intact. For the remaining samples, NMDS analysis of fungal β-diversity revealed distinct fungal profiles at all locations (all p-values < 0.05). This was even the case for paired fields in which, at every timepoint, the populations of the fungi formed unique clusters with NMDS analysis (Figure 3). When comparing alpha diversity metrics, samples from Wadena, MN and Pine Point, MN had higher observed values of OTUs as well as higher Chao1 and ACE estimates.

Importantly, when initially using the unedited UNITE database for taxonomic assignment, 43.38% of reads registered as the fungal class Basidiomycota were unassigned (Table 2). Additionally, order, family, genus and species percentages of unassigned basidiomycete sequences were 63.17%, 63.19%, 63.54% and 64.36%. For ascomycetes, these percentages of unassigned reads were 4.96%, 20.2%, 24.21%, 25.63% and 52.4%. We then blasted high abundance unassigned basidiomycetes reads on NCBI and found that most unassigned basidiomycete reads matched with the oomycetes *Phytophthora* and *Pythium*. We then appended an oomycete database to the existing UNITE database and re-classified the

taxonomy of reads. Upon doing so, we found that unassigned reads for Basidiomycota was reduced to 2.52%, 2.89%, 2.94%, 3.34% and 7.3%. For Ascomycota, these numbers were 5.79%, 6.86%, 12.37%, 13.92% and 26.37% (Table 2). When comparing the percentage of OTUs that changed, from class to species levels, Ascomycota changed -2.87%, -2.35%, -2.17%, -1.91% and -0.84% (Table 3). For Basidiomycota, raw percentage changes in OTU assignment were 1.79%, 3.03%, 2.86%, 3.11% and 1.45%. The comparatively small change in OTU assignment, relative to percent changes in read assignment, indicates that misclassified OTUs were typically in high abundance and were not included within the fungal divisions of Ascomycota or Basidiomycota.

FunGuild assignment

FunGuild was used to assign functional ecological guilds and trophic modes to all fungal and oomycete genera. Of 1951 reported OTUs, 681 of those OTUs were assigned a trophic mode in the FunGuild output. Trophic modes assigned consisted of pathotroph, saprotroph, symbiotroph or a combination of the three. Running PERMANOVA analyses across different sampling timepoints revealed pathotroph populations were lowest during row closure when compared to prefumigation and vine death ($p < 0.05$). At the same time, saprotroph populations were highest during row closure as compared to prefumigation and vine death ($p < 0.05$). ANCOM, which is used to evaluate differential taxon relative abundances of microorganisms, in our case OTUs, across different assigned variables, revealed that the principal saprotroph responsible for the increase in saprotroph genera during row closure were *Humicola* and *Schizothecium* (Table 4). When assessing levels of pathotrophs, populations of *Alternaria* and *Fusarium* where mainly responsible for higher populations of pathotrophs during prefumigation and vine death.

We also ran ANCOMs comparing all fields and we did so considering each paired field as two distinct fields. In total, when considering every combination of locations, ANCOM calculated significant W scores for 89 different fungal OTUs (Figure 7). Common potato pathogenic fungal genera which were called as differentially abundant in ANCOM were *Alternia*, *Cadophora*, *Colletotrichum*, *Fusarium* and *Verticillium*, each being assigned significant W-scores greater than 100. ANCOM results showed that paired field sites had few differentially abundant OTUs that were assigned a trophic mode. Most differences, as would be expected based off our fungal NMDS plots, were between different locations.

Eukaryote community and diversity

In total, 5,147,769 Eukaryote 18S reads were obtained from the QIIME workflow. Each sample was rarefied to a depth 20,000 reads. Samples comprising less than 20,000 reads were removed. Within each sampling location the seasonal variation of β-diversity and treatment effects on β-diversity were measured. The two seasons sampled, Spring 2016 and Fall 2016, were significantly different ($p < 0.001$). When comparing the effect of treatment at each sampling time point there was likewise a significant difference with each p-value below 0.001. Not enough data was received for the North Dakota or Oregon samples to draw reasonable conclusions on β-diversity. The Earth Microbiome Project primers had deep enough taxonomic resolution to identify several prominent oomycete genera, of which common potato pathogens *Pythium* and *Phytophthora infestans* were included. Each of these genera had corresponding OTUs that significantly differed across all sampling locations. Of all states, Washington had the highest rates of *Phytophthora* ($p < 0.05$).

Nematode community and diversity

Nematode communities were assessed using 18S rRNA assigned OTUs. Initially, 18S-generated OTUs were rarefied to 20,000 reads and then subset to only contain nematode-assigned OTUs. No further rarefaction or editing of the reads was undertaken because we considered the differences in the number of nematode reads amongst total V9 rarefied reads to be significant as it indicated that those nematodes occupied either a smaller or greater percentage of the total eukaryotes in a sample. In total, 77,843 nematode reads were recovered with a total of 305 OTUs. Alpha rarefaction curves indicated that we had sufficient depth to capture sufficient alpha diversity (Figure 2).

Although most analysis was done at an order level, there was one species hit that we found particularly interesting: *Heterodera schachtii*. We find this interesting because *H. schachtii* is a pathogen of sugar beet, which was only reported in the crop history for Acequia and Minidoka, ID. Predictably, the OTU representing *H. schachtii* was only present in Acequia and Minidoka, ID. H. *schachtii*, initially identified in QIIME as *Globodera pallida*, was identified with a blast search on NCBI. In total, 20 *G. pallida*-assigned query sequences were blasted in NCBI resulting in a 100% match with *H.* schachtii, as well as *H. glycines* and *trifolii*, but only a 92% match with *G. pallida*. *H. schachtii* was only found in our Acequia, ID samples.

Nematode distribution and relative abundances were of special interest because of their role in nutrient cycling, potato plant pathogenesis and microbivory. Overall, samples were dominated by the orders *Tylenchida* or *Rhabiditis* (Figure 9). However, when compared to other locations, *Tylenchida* and *Rhabditida* in Pine Point samples were offset by populations of *Dorylaimida*, *Areaolaimida*, *Monhysterida* and *Mononchida*. Higher populations of *Araeolamida* and *Monhysterida* may be reflective of the conventional practices used by the Minnesota farmers

which may be connected to the fact that *Araeolamida* and *Monhysterida* are free-living and do not overwinter. Of all samples, Minidoka, ID consistently had the highest rates of *Tylenchida*, which is commonly associated with pathogenesis (Figure 9).

Population trends were not consistent for nematodes at different field locations. For samples at our Acequia, ID location, prefumigation samples had a significantly higher population of *Tylenchida* ($p < 0.05$) than during row closure and vine death. Also, at Acequia, ID, populations of *Tylenchida* increase from row closure to vine death. The same trend is seen for the Vapam®-fumigated at Wadena, MN. This same trend, however, does not appear to be statistically significant ($p > 0.05$) at Boardman, OR, Minidoka, ID or Pine Point, MN. When considering populations of *Dorylaimida*, their populations were lowest Quincy, WA and Wadena, MN.

Bacteria

In total, 15,600,301 bacterial 16S reads were obtained from the QIIME workflow. Each sample was rarefied to a depth 10,248 reads. Samples comprised of less than 10,248 reads were removed. Overall, across all sites, 6316 OTUs showed significant differences across all sampling locations although many of these OTUs were unassigned. When considering overall diversity, each sampling location had unique eukaryote profiles ($p < 0.001$).

Bacterial β-diversity estimates were affected by many factors. The strongest factor was that of location. Across all sites, each population was unique ($p < 0.05$) with, predictably, Western and Midwest samples having closer clusters (Figure 4). Both Idaho sites, Minidoka and Acequia, were comprised of similar bacterial populations but were distinct from Quincy, WA

and Boardman, OR field sites ($p < 0.05$). The two Minnesota sites, Pine Point and Wadena, were also statistically different from each other ($p < 0.05$).

Although location was the dominant factor in determining soil microbial composition, barcode bias, as introduced by the second Schloss barcode, was a strong factor as well. The barcode bias may have been caused sequence artefacts to be introduced into the sequences. Because of barcode biases, we amended several default parameters within the QIIME pipeline to reduce bias. Such alterations of the QIIME pipeline included a) setting stricter parameters for determining whether a sequence read was chimeric and b) removing sequence reads if they were flagged as chimeric in during either *de novo* or reference-based chimera checking. Furthermore, low abundance reads, from singletons to tripletons, were removed within R. We justify the removal of low abundance reads from our dataset because low abundance microorganisms typically play a less prominent role in the soil ecosystems as well as the likelihood of low abundance reads being spurious.

In addition to the second barcode and location, treatment effect also influenced soil bacterial composition, albeit situationally. It was only at one site, Pine Point, MN, that all paired fields differed in the bacterial populations each season. During prefumigation at Pine Point, populations of *Spartobacteria* were proportionally high and these populations decreased during row closure and vine death in both the chloropicrin-treated and nonfumigated fields (Figure 6). During row closure, the chloropicrin-treated field had high populations of *Thermolephilia* and *Acitnobacteria* while classes categorized as “other” were the lowest of any other site.

Seasonal variation in the relative abundances of bacteria also limited to select sites. Neither of the Idaho sites, Minidoka or Acequia, experienced seasonal variation in the bacterial composition. Only Wadena, MN and Pine Point, MN sites were considered to be significant.

Bacterial populations

At a phylum level, community relative abundances across all samples were predominately *Proteobacteria* (28.78%), *Acidobacteria* (20.49%), *Actinobacteria* (11.93%), *Bacteroidetes* (10.40%), *Chloroflexi* (7.20%), *Verrucomicrobia* (5.30%), *Gemmatimonadetes* (4.28%), *Planctomycetes* (3.77%), *Firmicutes* (3.52%) and *Nitrospirae* (1.03%). The most abundant classes of bacteria were *Alphaproteobacteria* (13.90%), *Blastocatellia* (7.21%), *Sphingobacteriia* (7.18%), *Actinobacteria* (6.96%), *Betaproteobacteria* (6.96%), *Gammaproteobacteria* (6.03%), *Deltaproteobacteria* (3.78%), *Thermoleophilia* (3.46%), *Spartobacteria* (3.33%) and *Bacilli* (3.19%).

DISCUSSION

Taxonomic assignment of fungi with the UNITE database

The fungal UNITE database is the current standard in metagenomics for assessing fungal lineages based on the internal transcriber spacer region. The database, however, only contains fungal identifiers and does not include closely related lineages such as oomycetes. Oomycetes are commonly classified as lower fungi but occupy a distinct taxonomic lineage from fungi despite being oomycetes, often having a comparable functional role in ecosystems as fungi. Despite their different taxonomic ranks, fungal-specific primers targeting the ITS1 region have been shown to amplify both oomycetes and true fungi. The results of this, as is the case with our data, is that both oomycete and fungal ITS1 sequences are input into the sequence process and their sequences are, in return, intermingled with fungal-amplified reads.

We found that this combination of fungal and oomycete reads, when only using the UNITE database for taxonomic assignment, heavily affects taxonomic assignment in the QIIME pipeline by mis-assigning taxa, leaving OTUs unassigned and by yielding poor taxonomic assignment, particularly within the fungal phylum Basidiomycota. Within the phylum Basidiomycota, before adding oomycetes to the UNITE database, 43.48% of all reads at a class level were unassigned with that number rising to 63.17% at an order level (Table 2). However, with the addition of oomycetes to the UNITE database, the percentage of unassigned basidiomycetes at the class and order level decreased to 2.52% and 2.89%, roughly a difference of 40.96% and 60.28% for each. For Ascomycetes, there was an increase in unassigned reads at a class level. However, order, family and genus-level classification assignments of Ascomycetes experienced a roughly two-fold increase (Table 2). It should be noted that despite the increase in assigned reads, there was not a similar trend for the percentage of OTUs that were given an assignment. For instance, Ascomycota had more unassigned OTUs after the incorporation of the oomycetes to the UNITE database, whereas basidiomycetes had a slight increase in the percentage of their OTUs assigned (Table 3). This indicates that the majority of OTUs that were either previously unassigned or received deeper taxonomic identification had higher prevalence within the ecosystem.

This finding is important because most studies that use the UNITE database do not append other ITS lineages of closely related to fungi to the UNITE database. Because oomycetes are common throughout soil samples, particularly in agricultural fields, we searched studies focusing on ITS analysis of fungi in agricultural soils which used the UNITE database. Of the five studies we investigated which use the same combination of primers and were also conducting with soils, no amendment to account for any non-fungal lineages was made with the

UNITE database. Of the 20 studies we considered that solely used the UNITE database, none of these accounted for non-fungal lineages in their data analysis unless non-fungal, off-target lineages were removed with NCBI BLAST searches, which was not reported in any study. Speculatively, for these studies, it is possible that they lost taxonomic assignment depth for ascomycetes and basidiomycetes, mis-assigned taxonomy to a number of OTUs, and failed to classify organisms such as oomycetes which play an important functional role similar to fungi in their target ecosystems. We suggest that target metagenomics analyses focused on using the ITS region, particularly with soil analyses, must incorporate closely related lineages to fungi so that they increase depth of taxonomic assignment, have more accurate taxonomic assignment, and that they identify other functionally important organisms which may be sequenced even if so-called "fungal specific" primers are used.

Finally, it should be noted that, for taxonomic assignment, the Wang classifier method, which is also known as the rdp method and which is the primary assignment method used in the mothur pipeline, was used and that similar trends may not be seen for other common QIIME taxonomic assignment methods such as blast, uclust, rtax or other methods.

FunGuild assignment of taxa

We measured changes in FunGuild-assigned genera over a temporal gradient during prefumigation, row closure and vine death (Table 4). Across all seasons we found that saprotrophs had their highest relative abundances during row closure ($p < 0.05$) which coincides with lower relative abundances of pathotrophs at same timepoint ($p < 0.05$). Saprotrophic genera with increased relative abundances during row closure included *Humicola* and *Mortierella* (Figure 8). An increase in genus *Humicola* is intriguing because *Humicola* has been found to

confer disease resistance against *Alternaria* and *Phytophthora* (Ko *et al.*, 2011). *Humicola* and *Schizothecium* have also been found to be common in other potato molecular analyses and had higher populations in potato soils considered to be healthy (Lu *et al.*, 2013). Though relatively little is known about the function of genus *Mortiella* within the rhizosphere, the fungus is associated with disease-suppressiveness and growth patterns (Bonito *et al.*, 2016). *Mortierella*, a fungus with a broad saprotrophic range, is known to have anti-pathogenic properties towards bacterial potato scab pathogens, being various *Streptomyces spp.*, especially under low pH conditions (Tagawa *et al.*, 2010). However, it has also been found that *Mortierella* has indirect negative effects on plant growth by inhibiting plant growth (Hong-wei *et al.*, 2012, Qin *et al.*, 2017). In some studies, *Mortierella* has been found to be the predominant genus in potato soils (Qin *et al.*, 2017). *Mortierella* is consistently associated with disease-suppressive soils towards different fungi, bacteria and nematodes (Giné *et al.*, 2016, Gómez Expósito *et al.*, 2017). In soil of vanilla orchids, hyper-suppressive soils to pathogen to Fusarium wilt disease was associated with high relative abundances of *Mortiella spp.*, with some soils having a relative abundance of 37% (Xiong *et al.*, 2017).

Lower relative abundances of pathotrophic fungi during row closure comes as no surprise. This is because the earliest reports of disease in potato fields typically comes shortly after row closure. This is because the potato foliage creates a canopy, protected from the sun, suitable for pathogenic expansion, especially when coupled with increased temperature. Pathotrophic fungi with lower relative abundances were *Fusarium* and *Alternaria*. Populations of *Fusarium* have been reported to be lower in ridge planting management in potato agricultural soils (Qin *et al.*, 2017). Although *Fusarium* is responsible for dry rot in *S. tuberosum* it should be noted that most *Fusarium* lineages are not pathogenic. As recognized by FunGuild, *Fusarium* is

listed as both a pathotroph and saprotroph. It should be noted that there were several high abundance fungal genera that were not characterized by the FunGuild database. These include the genera *Fusicolla*, *Guehomyces* and *Nectria*.

Bacteria

Bacterial populations were found to be influenced by season. Although our variable season was influential, we predict that a stronger influence on the bacterial populations, acting as a confounding variable, was the influence of the vegetative stage of the plant. The reason we hypothesize this is support by a recent analysis performed by Pfeiffer et al, 2017. In Pfeiffer's study, he evaluated the rhizosphere microflora of *Solanum tuberosum* in its center of origin, the Central Andean Highlands, under varying environmental conditions of which include, different soil characteristics, climatic conditions, elevation and agricultural practices (Pfeiffer *et al.*, 2017). Under these circumstances, Pfeiffer investigated the taxonomic composition and core microbiome which revealed vegetation stage as the most important indicator of the microbial composition. In his study, three vegetation stages were sampled which were emergence, flowering and senescence. In our study, none of our timepoints coincided with Pfeiffer's sampling schedule as our timepoints had more to do with important timepoints in agricultural management and not with plant development. Furthermore, although sampled the soil before fumigation, a time when there will be no potato planted, only two of our sampling points were when the potato was planted. Thus, our two timepoints are not enough to draw any reasonable conclusion from this.

An explanation as to why some treatment effects may be different would be the drastic differences in fumigation approaches, pesticides applied, the amount and rate of pesticide

application and more. Before proceeding further, it should be noted that only at one site, Acequia, Idaho, can the data be accurately assumed. This is because the sampling efforts of the local farmers and researchers did not follow directions as instructed. Only the Acequia, Idaho site did this. For this site, we found that there was a p-value < 0.05. During the first timepoint, prefumigation 2015, there was no difference between the paired fumigated and non-fumigated potato fields. This is important because it allows for differences in populations to be attributed to the treatment itself. In an important note, the same field site contained different fungal populations to begin with. This means, for the fungi, that no direct comparison for field treatments can be made.

Bacterial populations

We were not surprised to find a high relative abundance of the genus *Devosia* within our samples as *Devosia* has been identified in potatoes in other metagenomic analyses (Barnett *et al.*, 2015). This, likely, bodes well for our potato samples and yields because *Devosia* is associated with nitrogen-fixation.

In Minnesota soil samples, *Spartobacteria* were found in higher abundances than in non-Minnesota soils. *Spartobacteria* are typically abundant in soils however relatively little is known of their function. *Spartobacteria*, however, have been found to persist off of plant polysaccharides and recently have been found to colonize the roots and rhizosphere of rice (Hernández *et al.*, 2015).

Treatment effects on bacteria

It is interesting to note that bacterial populations responded differently to the treatments which does indicate varying levels of effectiveness. For the Acequia, ID bacterial populations it was determined that fumigation had no meaningful effects on their population ($p > 0.05$). The overriding variable in this situation was season which may, as will be discussed, be more correlated with the fact that one sampling timepoint had no plant in the soil and then the other two timepoints represent different life stages of the plant.

Overall, our results show either diminutive or no changes in bacterial populations at each site according the treatments of, individual or in combination with each other, Vapam®, Telone II, and chloropicrin. Each of these fumigants, previously, have been previously demonstrated to have severe and immediate changes to soil microbial populations. An explanation for the lack of change in bacterial populations may be found in a paper by Kato (Kato *et al.*, 2015). In this paper, in a controlled environment, Kato surveys changes in bacterial population in response to fumigation during time intervals for a period of 24 weeks. During this 24-week period Kato found that despite rapid initial change following treatment, bacterial populations appeared almost normal after the 24-week period. One explanation for our results is that our sampling window was long enough that bacterial populations had sufficient time to stabilize post-treatment. Another could be a failure of our experimentation; however, this explanation seems unlikely because of statistical trends we see in our eukaryote and fungal data (i.e. clustering by location, season, treatment and other factors). This seems to indicate that our experimental methods were procedurally consistent and sound.

Fungal populations

Fungal richness and phylogenetic diversity for fungi is higher in lower *temperatures* and higher moisture content (Pellissier *et al.*, 2014). One of the more revealing results from our analyses was that no fungal population at any field site, whether paired or cross-state, save in Acequia, Idaho, was the same. When bacterial populations at Minidoka, ID were the same, the fungal populations in the same fields were different. There are many potential explanations as to why this phenomenon would occur. The simplest explanation might be that fungi are less globally distributed than bacteria. Thus, even random, small-scale regional differences could account for initial fungal population differences. Another explanation might be that plant recruitment or repulsion of soil microbiota via root exudation may be stronger for bacteria than fungi. Thus, plant selection for individual or populations of fungi may be weak when compared to plant recruitment of specific bacterial lineages and populations.

FunGuild assignment of Acermonium

In order to estimate the functional diversity of our samples, we used a program called FunGuild which uses taxonomic data to parse OTUs into predetermined ecological guilds (Nguyen *et al.*, 2016). From FunGuild, we were able to separate out OTUs into three different trophic modes (pathogen, saprotroph and symbiotroph), assign a guild and a confidence ranking to the possibility of that rank, information on growth morphology and, when possible, a trait. Our data shows that row closure coincides with the highest rates of saprotrophs. This is noteworthy because saprotrophs, along with arbuscular mycorrhizal fungi, have been shown to play a role in the nitrogen cycle for plants (Hodge & Fitter, 2010, Jin *et al.*, 2012).

The presence of one fungal genera, *Acremonium spp.*, is especially interesting because of its role in mycoparasitism. Recently, *Acremonium strictum* was found to have a close association with *Helminthosporium solani*, which is the causal agent of silver scurf in potatoes (Rivera-Varas *et al.*, 2007). It has been found that *A. strictum* inhibits the growth of *H. solani*. In either case, the high abundance of *Acremonium* in the soils may indicate that it is the presence of a mycoparasite which keeps in check the populations of other fungal pathogens.

Nematodes

The economic impact of yield reduction from nematodes is a global issue. In the United Kingdom, potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, are responsible for an estimated $70 million lost per annum {Nicol, 2011 #345} and a decrease of 9% in production (DEFRA 2010). In the United States, a 1999 study revealed that 5-10 percent of land where potatoes were grown in Washington was lost due to phytopathogenic nematodes despite almost 75 percent of all land being treated with a nematicides (Koenning *et al.*, 1999). Total global crop losses due to phytonematodes have been estimated to be as high as $80 billion which is likely a higher figure today (Handoo, 1998).

Detection of phytonematode infection remains challenging because symptoms, such as cysts and lesions, may appear belowground in the root or tuber without any above-ground symptoms save reduced growth. Some innocuous symptoms such as nutrient and water uptake, which leave no apparent symptoms, may be entirely overlooked (Nicol *et al.*, 2011). In some cases, nematodes will spur secondary pathogenesis which is when nematodal infection facilitates fungal or bacterial infestation (Powell, 1971). Because symptoms are difficult to track, accurate crop estimations or yield loss from nematodes is hard to determine. The most prominent potato

nematode pathogens are potato cyst nematodes (PCNs), being of the order Tylenchida with two main species-*Globodera rostochiensis* and *G. pallida*. Potato cyst nematodes have been known to decrease yields as much as 50 percent (Nicol *et al.*, 2011). The best way to control PCNs is by growing PCN-resistant potato varieties (Nicol *et al.*, 2011).

Other nematode species that are pathogenic and a concern to growers include: *Pratylenchus brachyurus*, *P. penetrans*, *Ditylenchus destructor*, *Meloidogyne incognita*, *Belonolaimus longicaudatus*, *Paratrichodorus* and *Trichodorus* species. Typically, most nematode potato pathogens are in the order of Tylenchida. Overall, *Tylenchida* had the second highest relative abundance of all nematodes just behind *Rhabditida*. In our Minidoka, ID samples, percentages of *Tylenchida* comprised the highest average relative abundance of nematodes with a relative abundance of near 40% across all seasons (Table 5). Correlated with this, at Minidoka, ID sites, there were significantly lower populations of *Rhabditida* with average to high levels of *Dorylaimida*. No disease information was reported from growers in Minidoka, ID during this time.

Populations of *Rhabditida* were especially high at the Quincy, WA, Wadena, MN and Acequia, ID locations while there were lower relative abundances at Minidoka, ID. Populations of *Rhabditida* were significantly lower during prefumigation at Pine Point, MN when compared to row closure and vine death ($p < 0.05$). As some *Rhabditida* species are microbivores, especially bacterial-feeding, the *Rhabditida* in our soil samples may directly act on the soil microbiome (Parfitt *et al.*, 2005). In fact, high proportions of bacterial-feeding nematodes, when compared to the total nematode population may affect soil biological activity and net nitrogen mineralization (Parfitt *et al.*, 2005).

Implications for crop management.

Some factors possibly influencing bacterial relative abundances in our study include differences in fumigant applications, the fumigant itself, time of application, rate, soil temperature, and soil moisture (Table 6). Each of these variables will affect how microbial populations respond to treatment and how they vary in effectiveness (Hamm *et al.*, 2003). For example, fumigation with metam sodium (MS) has been shown to have more severe effects on fungal populations than the common fumigant 1,3-dichloropropene (Hamm *et al.*, 2003). At the same time, though MS is effective against fungi, if nematodes are an issue, treatment with MS alone will likely be insufficient for controlling disease and increasing yields (Ingham *et al.*, 2000, Ingham *et al.*, 2000). As each growing region of potato faces its own unique environmental and disease challenges, it is likely that the farmers and collaborators selected treatments specific to their growing region or the history of their fields. Unique challenges to different growing regions make the idea of a universal best fumigation treatment difficulty at best and makes drawing conclusions of treatments, even if they are the exact same treatments, across growing regions difficult to assess.

Though populations of bacteria were relatively consistent throughout all sampling locations, the samples at Pine Point and Wadena, MN displayed higher levels of *Spartobacteria* and *Thermoleophilia*. The role that these classes play in the soil ecosystem is relatively unknown; however, they are commonly found in the rhizosphere and they associate with plant root chemoattractants. As both *Spartobacteria* and *Thermoleophilia* have been found to be associated with potato roots, along with other agricultural crops, their plant interactions should be explored in greater depth (Bergmann *et al.*, 2011, vAN OvERbEEk, 2013). Currently, we are unsure why these populations were higher in the Minnesota samples, and as to why they were

highest at prefumigation before having their relative populations diminish, but more should be explored to understand their role and, if beneficial, to maintain their population levels.

We also show that fungal populations vary considerably from site to site, more so than bacteria. We were surprised that samples from Minidoka and Acequia, ID were so different when comparing the distances between the sites (~5 miles). Furthermore, no paired fields for fungi were clustered wholly together despite being adjacent to each other. This uniqueness should indicate to growers that even close to or adjacent fields may need to be treated individually for the fungal populations. Furthermore, we show that relative abundances of fungi associated with pathogenic trophic modes are lowest during, or at least preceding, row closure. Typically, after row closure is when fungal pathogenesis is first seen in the potato growing year. If there were high amounts of a certain pathogen, for example *Alternaria solani* which causes early blight in potatoes, then that pathogen should have a high relative abundance at this timepoint. In our circumstance, no farmers reported incidences of disease that would indicate an outbreak of a fungal pathogen. Neither does our data indicate this as such either.

REVIEW OF LITERATURE

Global population estimates predict a human population of 9 billion by the year 2050, necessitating increased food production via improved yield efficiency or more land for agriculture (Godfray *et al.*, 2010, Tilman *et al.*, 2011). Lamentably, various stressors such as climate change, rising disease rates and soil exhaustion challenge growers to maintain, let alone improve yields, while available land becomes scarcer, or previously arable land succumb to soil

exhaustion. In many regards, total available cultivatable land can be thought of as a non-renewable, limiting resource to food production. This makes sustainable agriculture acutely important in the long-term for feeding the burgeoning human population.

One important component of sustainable agriculture is the soil microbiome which plays a role in plant health, disease tolerance, nutrient cycling and abiotic stressor tolerance. Unfortunately, our understanding of plant-soil-microbial interactions, both in terms of function and identity, remains inadequate. Plant-soil-microbial interactions are important in sustainable agriculture because they influence plant growth, nutrient cycling, phenology, disease resistance and plant tolerance to an array of abiotic stressors (Ortíz-Castro *et al.*, 2009, Wagner *et al.*, 2014, Busby *et al.*, 2017). As climate change, anthropogenic disruption and other stressors continue to exacerbate problems concomitant to sustainable agriculture, beneficial soil microbes may be able to dampen or render null the stressors through a variety of means.

These soil microbes, notably bacterial or fungal, remain largely undefined in terms of identity and function. This is, largely, due to the inordinate quantity of soil microbial taxa as well as growth mediums inadequate for the culturing diverse microbial taxa (Blackwell, 2011). However, with the advent of next generation sequencing, the identity and functional capacity of soil microorganisms and plant-soil-microbial profiles have begun to be rapidly characterized, principally in cash crops.

Microbial diversity and total microbial biomass are associated with disease-suppressive soils

Rhizospheric microorganisms are vital at every chapter of a plant's life. They may be involved in such processes as promotion of seed germination, sustained plant nutrition and root

nodule formation (Berner *et al.*, 1999, Kondorosi *et al.*, 2013). Indeed, these microbes, both endophytically and ectophytically, are intimately involved in an elaborate soil-plant ecosystem. Consequently, it should come as no surprise that microorganisms may also play a key role in plant defense mechanisms against pathogens. This microorganism-plant defense system may be a result of a co-evolutionary process (Cook *et al.*, 1995) indicating that microbial-plant interactions play a part in natural selection. This might mean that plant domestication, the removal of the plant from pre-agricultural soils and cultivation practices may expose the plant to a variety of plant pests, particularly soil pathogens (Wissuwa *et al.*, 2009, Pérez-Jaramillo *et al.*, 2016).

Our knowledge of the mechanisms by which rhizospheric microorganisms are involved in plant defenses is incomplete. After all, the rhizosphere is a complex network of macro and micronutrients, microecosystems, soil biota and more. Even when exclusively considering soil biota, the bulk of organisms remain uncharacterized by both identity and function (Wissuwa *et al.*, 2009). However, one variable which has been demonstrated to be a valid indicator for disease-suppression is soil microbial diversity.

Evidence of microbial diversity's influence on disease-suppression is validated by diversity gradient analyses. In one such study, wheat roots were inoculated with diluted, undiluted, and gnotobiotic suspensions of wheat rhizosphere inocula (Matos *et al.*, 2005). Wheat plants were incubated for a fixed time and inoculated with uniform cell suspensions of *Pseudomonas aeruginosa*, an opportunistic pathogen. One-week post-inoculation, wheat roots were sampled for colony forming units of *P. Aeruginosa*. A strong inverse relation between *P. aeruginosa* and the dilution gradient was observed, indicating that microbial diversity may indicate disease-suppression in soils (Matos *et al.*, 2005). Protocol for creating an operable diversity gradient in this manner, as well as further supporting evidence of results, have been

confirmed by sequencing diluted microbial communities (Franklin & Mills, 2006) (van Elsas *et al.*, 2012, Vivant *et al.*, 2013, Yan *et al.*, 2015). It should be noted that these studies generally only observe colonization and infection by a single pathogen rather than a suite of pathogens, as would occur in natural ecosystems.

The importance of these diversity gradient analyses is to isolate, as far as they can, diversity as the major factor in disease suppression. As these gradations are artificially made it could be that field experiments, which cannot limit as many confounding factors to microbial diversity, may yield different results. Though natural ecosystem studies of microbial diversity and disease-suppressiveness are largely limited to correlation, an overwhelming body of literature substantiates findings from artificial diversity gradient analyses.

Though microbial diversity has been shown in both artificial and natural ecosystem settings, there are many other factors that must be considered when assessing disease-suppressiveness. In a review by Janvier, Janvier listed approximately 23 abiotic, 6 quantitative microbial, 7 diversity and structure of microbial community, and 6 microbial activity parameters, that have been shown to have either positive or negative correlations with disease-suppressiveness (Janvier *et al.*, 2007). Since 2007, it is likely that more parameters have been established. This is especially so for soil microbiota which play a vital role in disease-suppression (Mendes *et al.*, 2011, Berendsen *et al.*, 2012, Chaparro *et al.*, 2012, Mendes *et al.*, 2013). In many ways, rhizosphere microbiota act as a blockade against invading plant pathogens through various mechanisms.

While microbial diversity receives the most attention as a source of soil disease-suppressiveness, total microbial biomass is also an indicator of general soil suppressiveness. One hypothesis as to why microbial biomass is indicative of disease-suppressiveness is that resource

competition eliminates the resources necessary for pathogen expansion. A common practice which increases rhizospheric microbial biomass is the application of organic amendments (Janvier *et al.*, 2007). Though correlation between high soil microbial diversity with total disease-suppressiveness has been shown, mechanisms by which this happens are still being hypothesized (Brussaard *et al.*, 2007).

One hypothesized mechanism by which high microbial diversity drives disease-suppressiveness was characterized by Mendes when showed that plants recruit beneficial soil microbes to counter soilborne pathogens (Mendes *et al.*, 2011). These soil microorganisms in this study include γ-Proteobacteria and Pseudomonadaceae which work by producing chlorinated lipopeptide encoded by NRPS genes. Although, individual genera, such as the two above, have been shown to have an individually suppressive nature, there are other genera that are not active except with the proper consortia of microorganisms (Garbeva *et al.*, 2011). This was evidenced in 2011 when Garbeva found that *Pseudomonas fluorescens* Pf0-1 was significantly more antagonistic in the presence of Gram-negative bacteria as compared to Gram-positive bacteria. When Gram-positive bacteria were present, with or without Gram-negative bacteria, *Pseudomonas fluorescens* Pf0-1 produced a weaker yet more universally inhibiting antibiotic. This antibiotic was not transcribed when just Gram-negative bacteria were present.

Mechanisms of microbiotal antagonistic effects on pathogens

One reason that the soil biota of potatoes is important to understand is because plant-associated bacteria and fungi have antagonistic potential to pathogens such as bacteria, fungus, and nematodes (Krechel *et al.*, 2002). Methods of antagonism fit neatly in two general categories: general suppression, which involves a suite of microorganisms, and specific

suppression, which involves a definite group or individual microorganism suppressing an explicit set of pathogens (Janvier *et al.*, 2007). Examples of specific suppression abound while an understanding of general suppression is not.

There are many different mechanisms reported by which soil microbes may confer resistance to pathogens. One mechanism, reported by Pieterse, involves root-associated mutualists such as *Pseudomonas*, *Bacillus*, and *Trichoderma* that sensitize the plant immune system without direct activation of the immune system. Thus the plant exists in a heightened sense of awareness against pathogens but does not produce costly defenses (Pieterse *et al.*, 2014).

It is important to note that just because a biocontrol agent has been shown to provide resistance to a plant does not mean that it will under all conditions. In a recent study conducted by Jonathan Cray, he found that varying environmental conditions allow a biocontrol (Bacillus sp. JC12GB43) to either inhibit a pathogen (i.e. fusarium or phytophthora) or to promote pathogenic growth (Cray *et al.*, 2016). Cray then concludes that studies looking at biocontrols such as Bacillus sp. JC12GB43 should be at least a year long to ensure analyses under diverse environmental conditions.

Monocultivation is a common practice among potato growers. Though inherent dangers of monocultivation are well documented (i.e. Irish potato famine), an increasing body of literature validates the hypothesis that prolonged monoculture can convert disease-conducive soils to disease-suppressive soils (Cook *et al.*, 1995). Early observations of the association between prolonged monocultivation and disease-suppressiveness in potatoes appeared in Washington and Michigan where common scab, caused by pathogenic *Streptomyces scabies*, was near absent (Shipton, Liu 1995). The role of biological organisms was determined to be the

driving force of this suppression since monoculture soils conferred take-all resistance in polyculture soils whereas polyculture soils conferred no resistance to monoculture soils.

The identification of specific microbes associated with soil disease-suppression has already been discussed. A further discussion of this should be how these microbes cause suppression. Of these, non-pathogenic *Streptomyces spp.* was the most important indicator of suppression.

Studies demonstrating association of soil biota with disease suppressive soils

Soil is a mixture of minerals, organic matter, gases, liquids, and biota. Any one of these soil components can affect general soil disease suppressiveness. We, however, are most interested in the ability of soil biota to affect disease suppressiveness. Several experiments have been conducted that show the influence of soil biota on soil disease suppressiveness. One way this has been demonstrated is by removing, both fully and partially, biota from soils by way of pasteurization (Haas & Défago, 2005, Mendes *et al.*, 2011). Pasteurization at incremental degrees demonstrates incremental disease conducive effects when inoculated post-pasteurization. Another way this has been shown is by testing soils with similar physical-chemical traits from the interior of a disease-suppressive field and soil from the margins of the field (Mendes *et al.*, 2011). Although soil properties are roughly the same there is a large difference in suppressiveness. When no fungal pathogen was present these fields showed no difference in suppressive or conducive traits. Another way that scientists have tested for microbial influence in disease suppression is by gamma irradiation. When soil was gamma-irradiated previously disease-suppressive soils lost these traits (Mendes *et al.*, 2011).

Soil microbial studies focusing on Solanum tuberosum

Overbeek studied the effects of plant growth on bacterial communities (Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato). There are five stages of potato growth: sprout development, vegetative growth, tuber initiation, tuber bulking and maturation. They looked at "total bacteria, actinobacterial and *Pseudomonas* communities in bulk and rhizospehere soils and endospheres." Communities were assessed by group-specific primers. Plant growth stage strongly affected plant-associated communities. Plant growth stage overwhelmed any effect of plant genotype on the bacterial communities associated with potato. They found the genotype and growth stage affected the bacterial composition of potato-associated soils. This was assessed by both cultivation-dependent and cultivation-independent means. Plant growth stage was the biggest factor. It occurred in all cultivars tested. Plant health associated bacteria such as *Pseudomonas* and *Actinobacteria* were also affected-similarly to that of the total bacteria metric.

Fumigation alters soil biota α- and β-diversity

When fumigants are used on soils, they cause changes in the general soil microbial community. These changes in microbial community composition have impacts on the functional diversity and overall soil health. The change in diversity could be due to unequal proliferation of soil microbes or even the complete elimination of microbes upon soil treatment.

In a study by Mowlick in 2010, microbial communities in spinach fields were compared before and after being treated with chloropicrin, disinfected through traditional biological soil management practices, or not being treated whatsoever (Mowlick *et al.*, 2013). Largely, soils recovered their original populations but without members of some major phyla such as

Acidobacteria, Bacteroidetes, and *Planctomycetes* (Mowlick *et al.*, 2013). This suggests that these microbes may be highly-susceptible members of the soil community. Similarly, reports show certain organisms, such as gram-positive bacteria, are more resistant to fumigation (Ibekwe *et al.*, 2001).

A weakness of Mowlick's study is that it only analyzes changes in the microbial community after a single fumigation event. Other studies, however, have looked at the effect of repeated applications. It has been found that repeated applications of chemical fumigants leads to an accelerated loss in microbial diversity after each application (Gamliel and Dotan, 2009).

It should be noted that fumigation will not eliminate all pathogens in the soil. In fact, certain soil microbes are more likely to survive. For instance, it has been reported that certain gram-positive bacteria preferentially survive in Chloropicrin-treated soils (Mowlick *et al.*, 2013). Some of these survivors included thermophilic or acidophilic bacteria groups which demonstrated an ability to adapt to how the fumigant altered the soil profile. This was found previously by Ibekwe who demonstrated that certain *Bacilli* species had higher survival rates than other *Bacilli* post-fumigation (Ibekwe *et al.*, 2001). Similar as to how particular microbes have higher survival or recovery rates in fumigated soils, it has also been shown that some microbes have greater fumigation susceptibility. For instance, Tanaka found in 2003 that Chloropicrin-treated soils had drastically decreased levels of nitrate-oxidizing and ammonium-oxidizing bacteria (Tanaka *et al.*, 2003).

Variation in potato cultivar and disease susceptibility

Our study does not take cultivar into account but is aware of this variable. In a 2011 article by Genet, which tracked disease susceptibility in 133 cultivars and 18 breeding lines it was found that resistance to powdery scab had large variation among different potato cultivars (Genet *et al.*, 2007). Arbitrarily, cultivars had a range of lesions present, varying from very few to high abundance. Future studies should keep this a point of interest as cultivar type affects disease-suppressiveness. High variation was likewise reported Maldonado which compared just two cultivars (Hernandez Maldonado *et al.*, 2013).

There are many potential reasons as to why cultivar type is important in disease-suppressiveness. Perhaps the biggest thing to consider is that the study of disease-suppression in soil cannot be thought of in an isolated system. There are many interactions between the plant and the soil biota in the rhizosphere. It has long been documented that hormones in plants may stimulate mechanisms in soil microbiota. Perhaps, different cultivars have or lack the production of certain hormones that are able to stimulate either disease-specific or disease-general suppressiveness qualities in soil biota. The plants themselves may also lack mechanisms in themselves at just a plant level to defend against disease.

Thus, effects of microbial diversity and total biomass on crops should be studied at both a genus, species, and cultivar level. This is especially so if a GIS-based model is to be employed for potato growers because different cultivars will have different disease thresholds. Garbeva tells us that plant type and soil type are the main two drivers for determining the microbial community structure (Garbeva *et al.*, 2004). Thus, different cultivars having different disease susceptibility makes sense.

But what plant constituents indicate disease susceptibility is unsure. In a recent study by Abo-Elyousr he found positive correlations between cultivar type with high calcium, potassium, and (Abo-Elyousr). Although all known papers are in agreement with high calcium, most papers disagree with the relationship between high potassium and disease susceptibility (Davis J.R., Krištůfek, 1976). It is not only which microbes are present but it may also be the abundance of a specific microbe which is present that is affected by cultivar type (Weinert *et al.*, 2011).

Other aspects of core soil microbiota and their influence: α- and β-diversity

α- Diversity is shown to increase the decomposition rate of organic substrates for soil saprophytic fungi (Setälä & McLean, 2004). However, the higher level of species abundance does not necessarily correlate with maximal decomposition rates. In artificial systems, Setälä incorporated six diversity treatments (1, 3, 6, 12, 24 or 43 taxa) had different levels of decomposition rates. Setälä found that the maximal decomposition rate was found with 6 taxa, above even that of 12, 24 or 43 taxa (Setälä & McLean, 2004). Thus, as we look at our experiment, sheer abundance of taxa cannot be used as a measure of soil productivity. At the same time, low alpha diversity metrics will likely indicate low soil productivity.

Core microbiomes are unique, often at a genotype or cultivar level, and, lesser-so, to clades

Evident from target and functional metagenomic analyses is, because of a shared evolutionary history between plant and soil microbiome, that core soil microbiomes are unique to individual plant taxa, even down to the cultivar level. Although many plant-soil-microbial interactions and soil microbiome characteristics are shared across diverse plant taxa, because of

similar soil profiles, environment and plant-species relatedness, unique microbes or ratios of soil microbial taxa may forecast unique functions of which include disease resistance, especially to plant-specific pathogens, drought tolerance and nutrient cycling.

In 2011, Weinert, using PhyloChips as a method to characterize potato microbial communities, found that (Weinert *et al.*, 2011). In Weinert's study, there were three different cultivars which were grown in a randomized field study at two distant sites in Southern Germany. Of 2432 detected OTUs, 9% of them were found to be unique to a specific potato cultivar. Though this does not represent a substantive number, these OTUs may play a vital role in the unique function of the potato plant (Weinert *et al.*, 2011). As specific cultivars may be more or less resistant to a specific pathogen it could be that a unique relationship with a co-evolved endophyte or relation through exudation may be the source of the resistance.

Common potato pathogens

Pathogen induced potato diseases are affiliated with five major groups: bacterial, fungal, oomycete-derived, viral, and nematode parasitic. Of these five groups, this study focuses on bacterial, fungal, oomycete and nematode parasitic pathogens because their DNA is readily extracted. Because of similarities with fungi, oomycetes will be included in the previously mentioned category. The level of pathogenicity is determined by three main constituents: inoculum density, pathogenic capacity and soil factors which alter the previous two components (Janvier *et al.*, 2007).

Major bacterial potato pathogen genera include Ralstonia, Erwinia, Pseudomonas, Clavibacter, Candidatis, and Streptomyces. Within each genus there are pathogenic and

nonpathogenic species or strains. For instance, within genus *Streptomyces, Streptomyces scabies* RB3II is known to cause common scab; a disease characterized by brown, corky lesions with none to limited above-ground symptoms (Lerat, 2009). However, unlike *S. scabies* RB3II, other *Streptomyces spp.* strains are capable of conferring resistance against pathogens, even *S. scabies* RB3II itself (Liu *et al.*, 1996). When nonpathogenic *Streptomyces spp.* are used as biocontrols in potato fields they may, dependent on environmental conditions and cultivar type, reduce the incidence and severity of common scab (Wanner *et al.*, 2014).

Over 500 *Streptomyces* species have been discovered with several demonstrating pathogenicity to potatoes: *Streptomyces scabies*, *Streptomyces acidiscabies*, and *Streptomyces turgidiscabies* (Barrera *et al.*, 2013). Each species has similar morphology which renders culture-based identification methods impractical except when screened for bioactivity-a time consuming and laborsome process. However, sequencing segments of the 16S rRNA sub-unit, in combination with other genetic regions may allow for discrimination of certain *Streptomyces spp.*, whether pathogenic or nonpathogenic. It should be noted that sequencing a solitary DNA region will not wield enough resolving power to assure the identity of various *Streptomyces*. At a minimum, several different DNA regions should be used. Thus, the presence of *Streptomyces spp.* in a sample, if determined by culture-based methods or via single gene sequencing, does not validate its pathogenicity. The same principle is legitimate for other potato bacterial pathogens.

Like bacteria, ribosomal subunit and internal transcribed spacer region genetic analyses for fungal and oomycete potato pathogens provides insufficient resolving power to assume pathogen presence or absence. Thus, using the 18S rRNA sub-unit for fungi, even in combination with ITS1 or ITS2, is not an appropriate method for identifying fungal pathogens.

This method, however, is slightly more appropriate for making inferences to populations because of using a longer sequence region and less genetic diversity

Major genera of fungal potato pathogens include *Colletotrichum, Alternaria, Cercospora, Sclerotium, Fusarium, Botrytis, Phytophthora, Pythium, Spongospora, Rhizoctonia, Helminthosporium, Verticillium,* and *Sclerotinia*. *in silico* sequence alignment of these fungal genera, whether the ITS1 or V9 hypervariable region, using ClustalW in Geneious confirmed the ability of the V9 hypervariable region to distinguish between said genera (Thompson *et al.*, 2002, Kearse *et al.*, 2012). Species-level discrimination of fungi, *in silico*, was not inherent for the fungal V9 hypervariable region but was discriminatory for ITS1. The same trends were seen with the alignment of oomycetes, being *Phytophthora* and *Pythium*.

A good example of a fungal pathogen is *Verticillium dahliae*. *Verticillium dahliae*, a causal agent of Verticillium wilt-also known as potato early dying syndrome-in potatoes, is a fungal plant pathogen of phylum Ascomycota. A filamentous fungus, *V. dahlia* is a soil-borne contaminant whose symptoms may first be observed in the lower leaves of potatoes. The process of infection for *V.* dahliae begins with colonization of the potato stem base, thence extending to the apices. In greenhouse studies, *V. dahliae* was detected in stem bases 2 weeks post-inoculation and in the apices 4 weeks post-inoculation (Bae *et al.*, 2007). Although similar functionally but distant phylogenetically, oomycetes are common potato pathogens. The most commonly treated oomycete potato pathogen is *Phytophthora infestans* which causes late blight (Guenthner *et al.*, 1999).

Major genera of nematodes that are pathogenic to potato are *Globodera, Pratylenchus, Ditylenchus, Meloidogyne, Belonolaimus, Paratrichodorus,* and *Trichodorus*. Each genus is classified as a potato cyst nematode, root-knot nematode, root-lesion nematode, potato rot

nematode, stubby-root nematode, as well as other ectoparasitic types (Bird). *Pratylenchus* penetrans, a root lesion nematode, is especially interesting because it's co-occurrence with *V. dahliae* to incur potato early dying syndrome. *P. penetrans* does this by creating a primary lesion in the potato which allows for a secondary invasion by *V. dahliae* (Davis, 2014).

Effects of chloropicrin on soil biota

The soil fumigant chloropicrin, CCl_3NO_2, was first registered as a broad-spectrum soil fumigant in the United States in 1975 (Webster *et al.*, 2010). Since that time, chloropicrin's use as a fungicide and nematacide has steadily increased as other pesticides, such as methyl bromide, have been phased out due to concerns with toxicity, high volatility, and ozone depletion. Chloropicrin, like methyl bromide, also has high volatility owing to high vapor pressure-32 mm Hg-leading to concerns of rapid degradation upon application in soils (Wilhelm *et al.*, 1997).

Several factors appear to be the main drivers of chloropicrin degradation in soils: soil temperature, soil moisture, and soil biota (Gan *et al.*, 2000). Interestingly, of each of these factors, soil biota is the greatest contributor to degradation, accounting for an estimated 68 to 92% of degradation (Gan *et al.*, 2000). As reported by Castro, *Pseudomonas* sp. is the main microbe degrader which may wholly dechlorinate chloropicrin in as little as 1 hour (Castro *et al.*, 1983). Chloropicrin, of course, changes the microbial biomass and it also reduces species richness (Rokunuzzaman *et al.*, 2016).

Because *Pseudomonas* sp. are central to chloropicrin degradation we predict that recently chloropicrin-fumigated fields will have greater populations of *Pseudomonas* species. High populations of *Pseudomonas* in fumigated fields versus non-fumigated fields will confirm the

aforementioned phenomenon as well as validate our protocol. If *Pseudomonas* populations are not significantly higher in fumigated versus non-fumigated fields then either too much time existed between fumigation and sampling or fumigation was overall ineffective in reducing soil biota populations (Tsror Lahkim *et al.*, 2000, Hamm *et al.*, 2003, Mowlick *et al.*, 2013).

Effects of Vapam® on soil biota

Another commonly used fumigant, Vapam® HL, also known as metam-sodium, $C_2H_4NNaS_2$, is effective in suppressing nematodes, bacteria, fungus, and other biota. In most soils, metam-sodium generates methylisothiocyanate (MITC) upon application (Di Primo *et al.*, 2003). When testing metam-sodium activity in soils, levels of MITC are used as the actual measurement.

Unfortunately, the more that metam-sodium is applied to a field the more it experiences accelerated degradation, reducing the duration for which MITC maintains its toxicity (Di Primo *et al.*, 2003). The leading hypothesis for accelerated degradation is that repeated metam-sodium application enriches populations of microbes capable of degrading MITC (Di Primo *et al.*, 2003). Other potential mechanisms for accelerated degradation include increased enzymatic activity from degrading microbes and horizontal gene transfer of genes that function in biodegradation (Di Primo *et al.*, 2003).

As has been shown with both Vapam® HL and chloropicrin, there is a strong correlation between the number of applications and rate of fumigant degradation. This association predicts that increasing applications of fumigants will increase the rate of fumigant degradation; and by consequence, lead to inadequate pathogen control. Thus, if farmers augment the number of

fumigant applications they may be inadvertently accelerating degradation processes by virtue of increased biodegrading microorganism populations such as *Pseudomonas* sp. There is no established timeline for which suppression of accelerated degradation consequences may be reversed.

Effects of Telone II on soil biota

Telone II, a combination of 1,3-dichloropropene ($C_3H_4Cl_2$) and 1,3,3-trichloropropene ($C_3H_3Cl_3$) is an effective nematicide. Telone II prevents lesion nematodes, root knot nematodes, and potato cyst nematodes. Since nematodes produce a primary lesion that subsequently permits a secondary lesion and invasion of various fungi or bacteria, Telone II treatment may indirectly reduce co-infecting microorganisms such as *Rhizoctonia solani* and *Verticillium dahliae* (Botseas & Rowe, 1994). Telone II has been shown to affect the nitrogen cycle. Lower nitrate levels appear in Telone II treated soils as compared to non-treated soils (Tu, 1993). Some experiments have have decreased nitrate levels up to eight weeks post-fumigation (Marks *et al.*, 1972). Similar to metam-sodium, Telone II rapidly degrades in soils in the presence of *Pseudomonas spp.* (Poelarends *et al.*, 1998). Degradation of Telone II is also caused by hydrolysis of 1,3-dichloropropene to 3-chloroallyl alcohol, an inactive ingredient (Ou, 1998).

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FIGURES

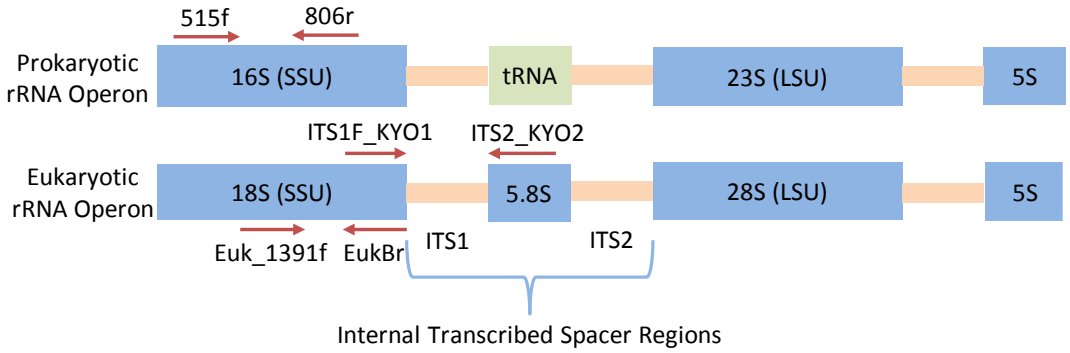

Figure 1. Binding sites for primers on the Prokaryotic and Eukaryotic rRNA operons
Schematic of primer binding sites for the internal transcribed spacer region, 16S rRNA and 18S rRNA operons. Primers 515f and 806r correlate with bacterial-associated organisms. ITS1F_KYO1 and ITS2_KYO2 target both fungi and oomycetes. Euk_1391f and EukBr Earth Microbiome Project primers target eukaryotes generally.

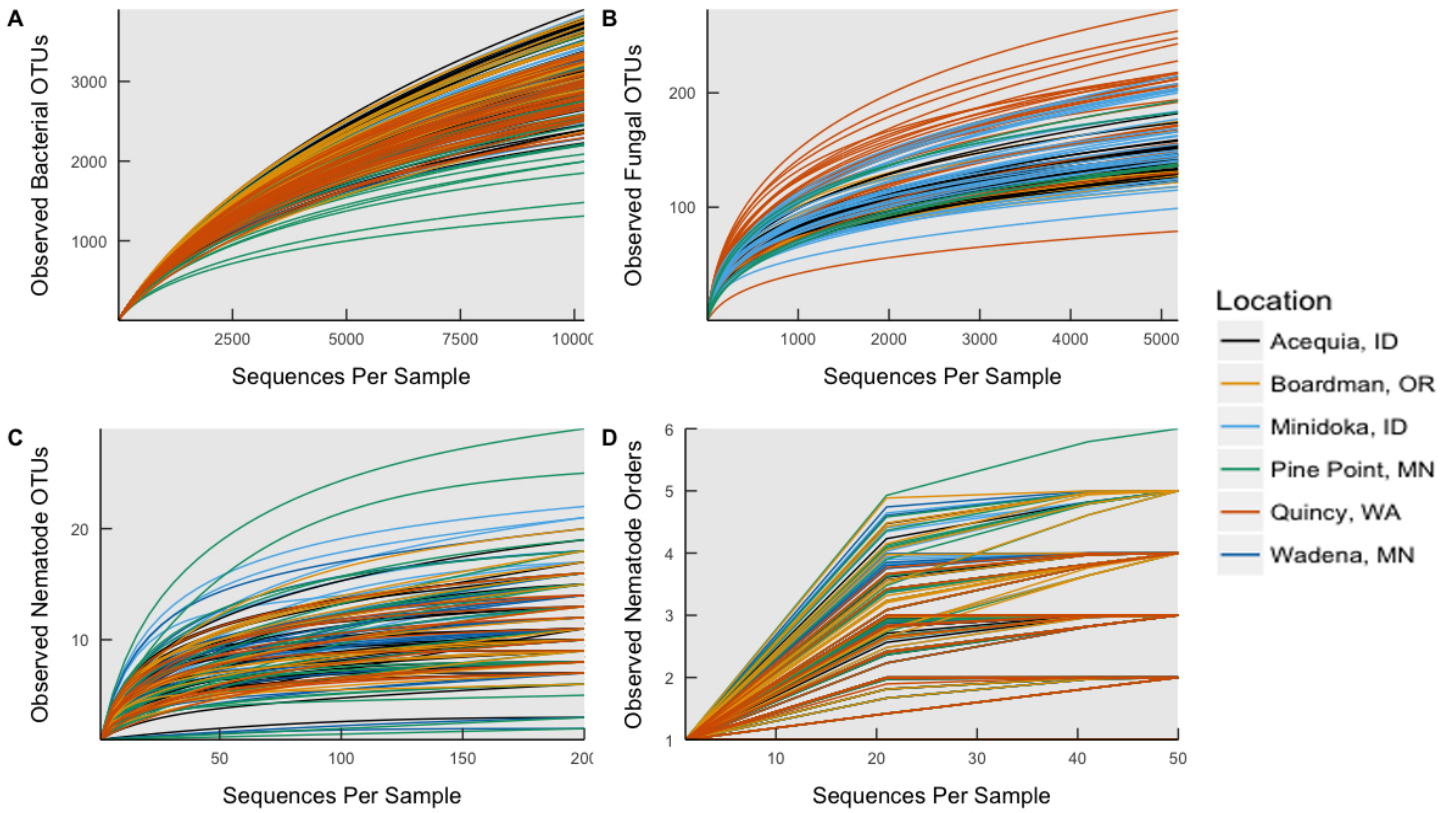

Figure 2. Alpha rarefaction plots
Alpha rarefaction plots for bacterial, fungal and nematode OTUs. A) Observed bacterial OTUs at a rarefaction of 10,248 reads B) Observed fungal OTUs are a rarefaction of 5,185 reads C) Observed nematode OTUs are a rarefaction of 200 reads and D) Observed nematode orders at a rarefaction of 50 reads

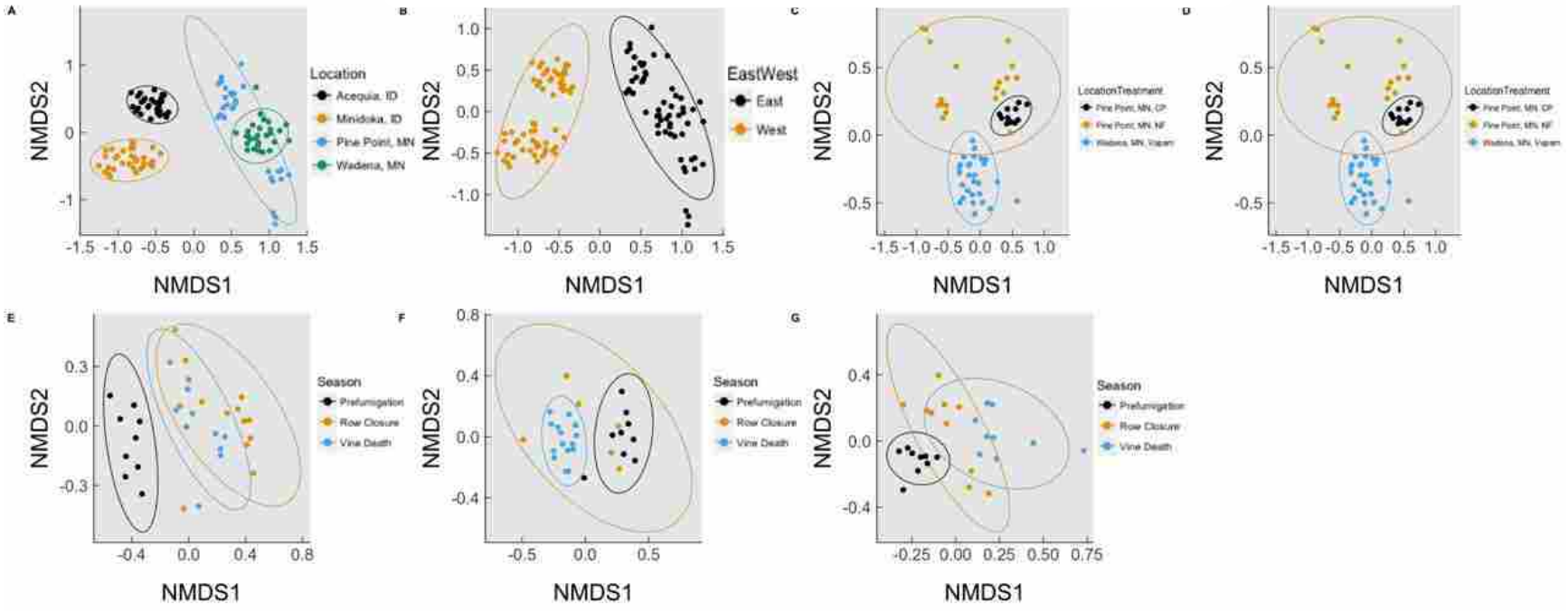

Figure 3. NMDS plots for fungi
NMDS plot (A) represents the differences in populations between all samples included in this analysis for fungi while plot (B) represents differences between samples in the Western and Midwest United States (West and East). NMDS plots (C) and (D) display population differences between the Minnesota samples. Plots (E), (F) and (G) depict differences in fungal populations at prefumigation, row closure and vine death at, from left to right, the Acequia, ID, Minidoka, ID and Pine Point, MN locations.

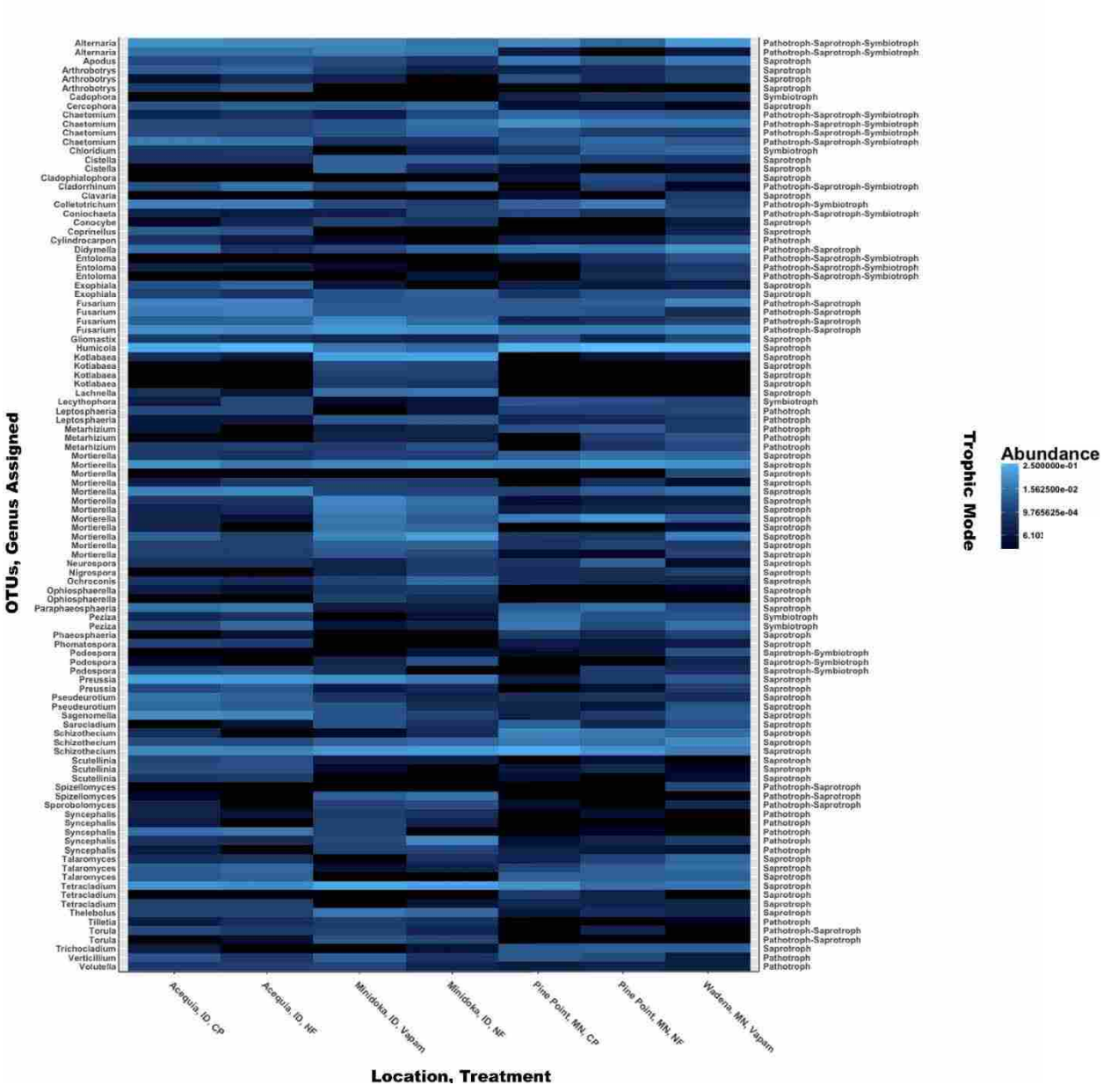

Figure 4. Heatmap displaying ANCOM-calculated differentially abundant taxa which were assigned a trophic mode by FunGuild

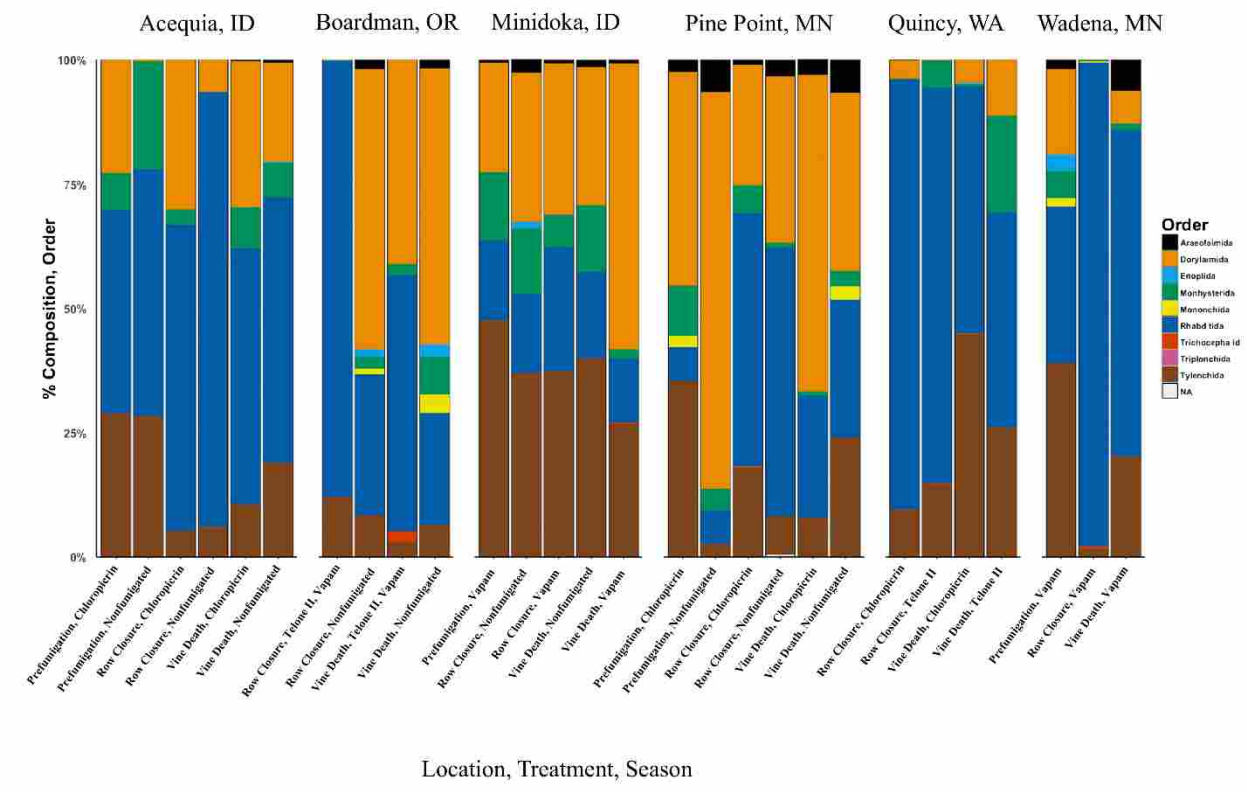

Figure 5. Nematode barplot
A barplot showing the percentage of OTU reads with their respective order at each pair of fields seasonally. The barplot is divided into six facets, representing the six locations. From left to right: Acequia, ID; Boardman, OR; Minidoka, ID; Pine Point, MN; Quincy, WA; Wadena, MN

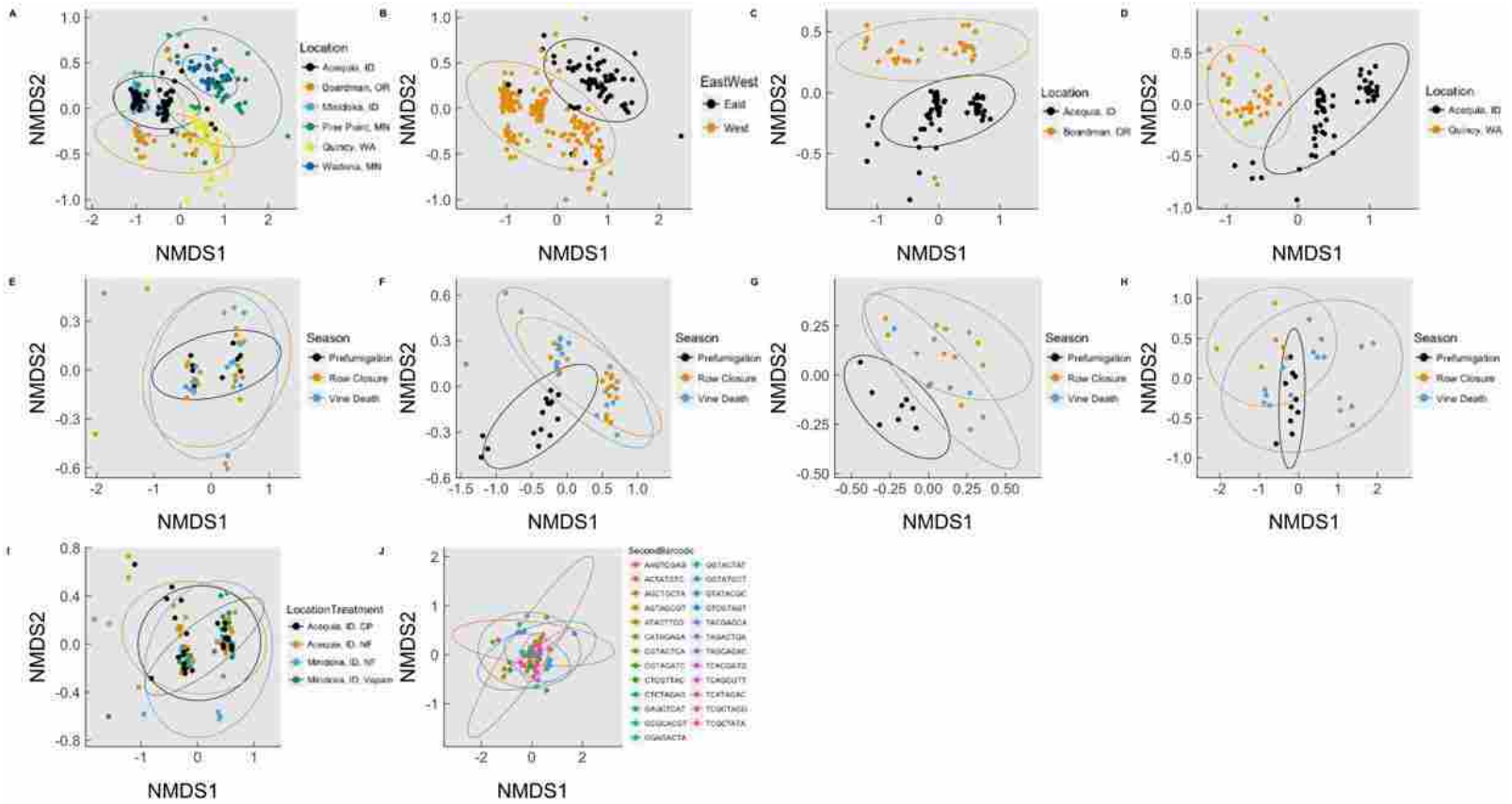

Figure 6. NMDS plots displaying differences in bacterial populations
NMDS plots displaying differences in bacterial populations. (A) Clustering for samples at all locations. (B) Differences in populations of Western and Midwest samples. (C) and (D) display differences between Acequia, ID and Boardman, OR as well as Acequia, ID and Quincy, WA. NMDS plots for seasonal variation of bacterial populations in (E) Acequia ID, (F) Minidoka, ID, (G), Pine Point, MN and (H) Wadena, MN. (I), NMDS plot for paired locations in Minidoka, ID and Acequia, ID. NMDS plot for second barcode for all samples (J).

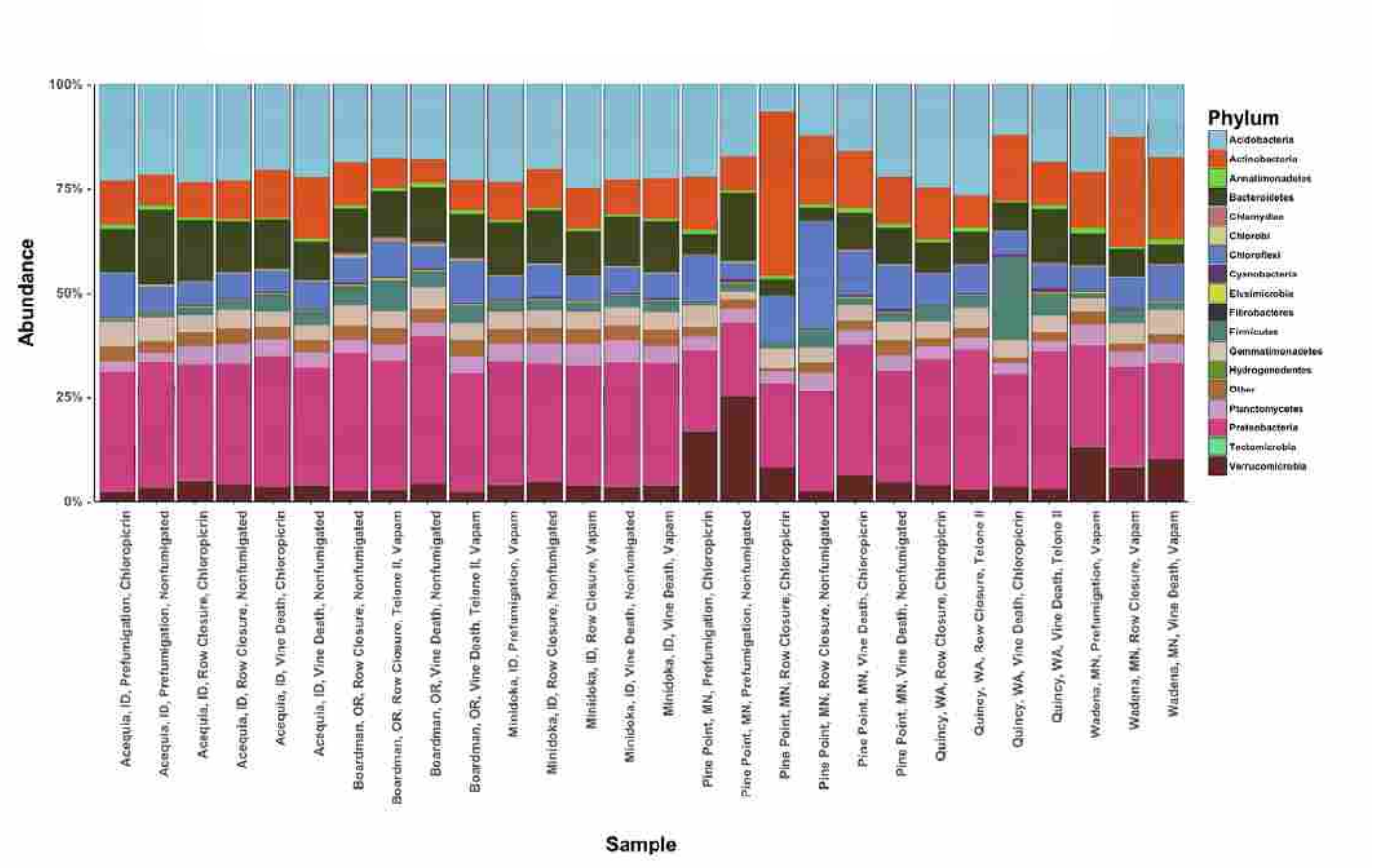

Figure 7. Phylum level barplot displaying bacterial populations at all locations combined with sampling timepoint and treatment
Phylum level barplot displaying bacterial populations across all timepoints at each field location. All samples were rarefied to 10,248 reads, combined by their location, treatment and the season they were sampled. Samples were then graphed as a percentage of the total reads.

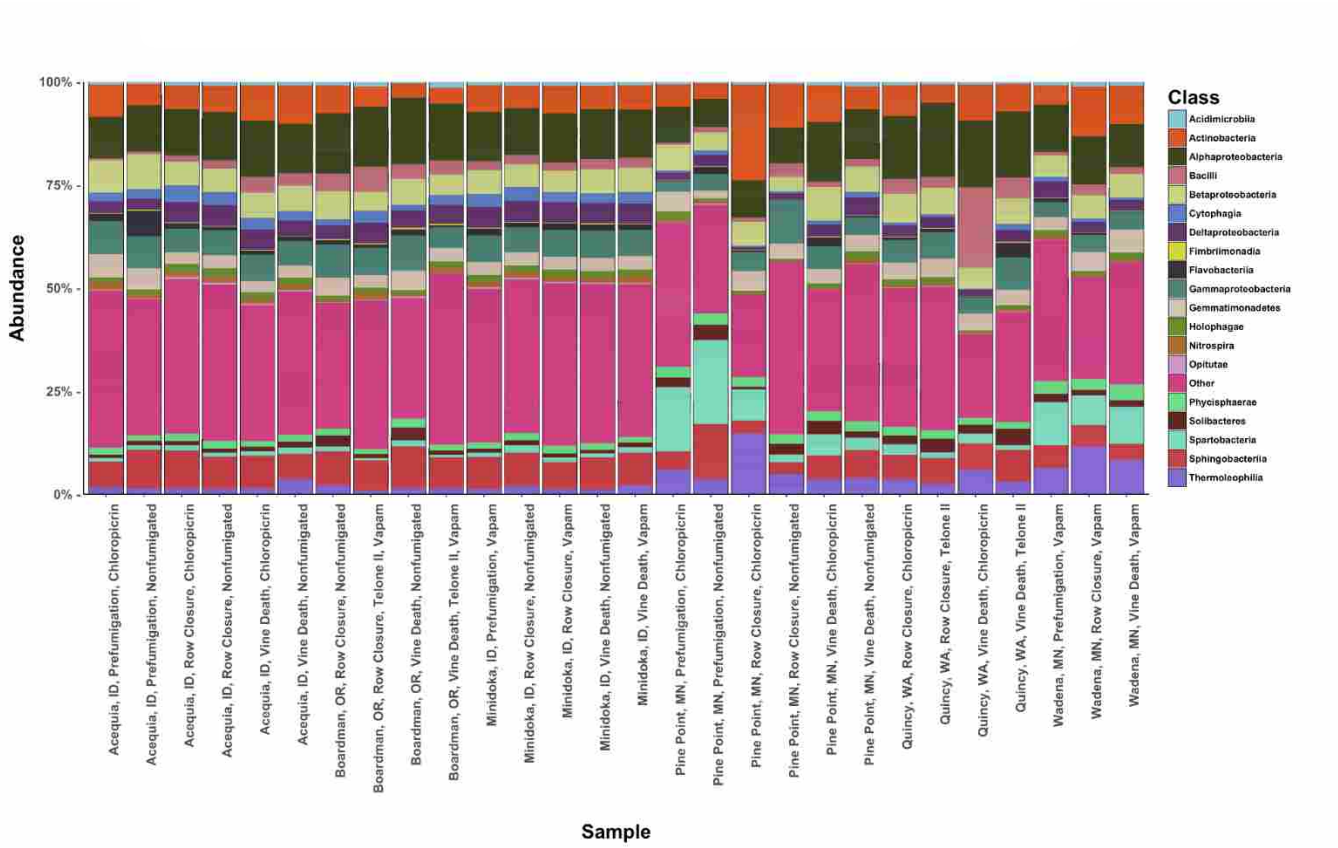

Figure 8. Class level barplot displaying bacterial populations at all locations combined with sampling timepoint and treatment
Class level barplot displaying bacterial populations across all timepoints at each field location. All samples were rarefied to 10,248 reads, combined by their location, treatment and the season they were sampled. Samples were then graphed as a percentage of the total reads

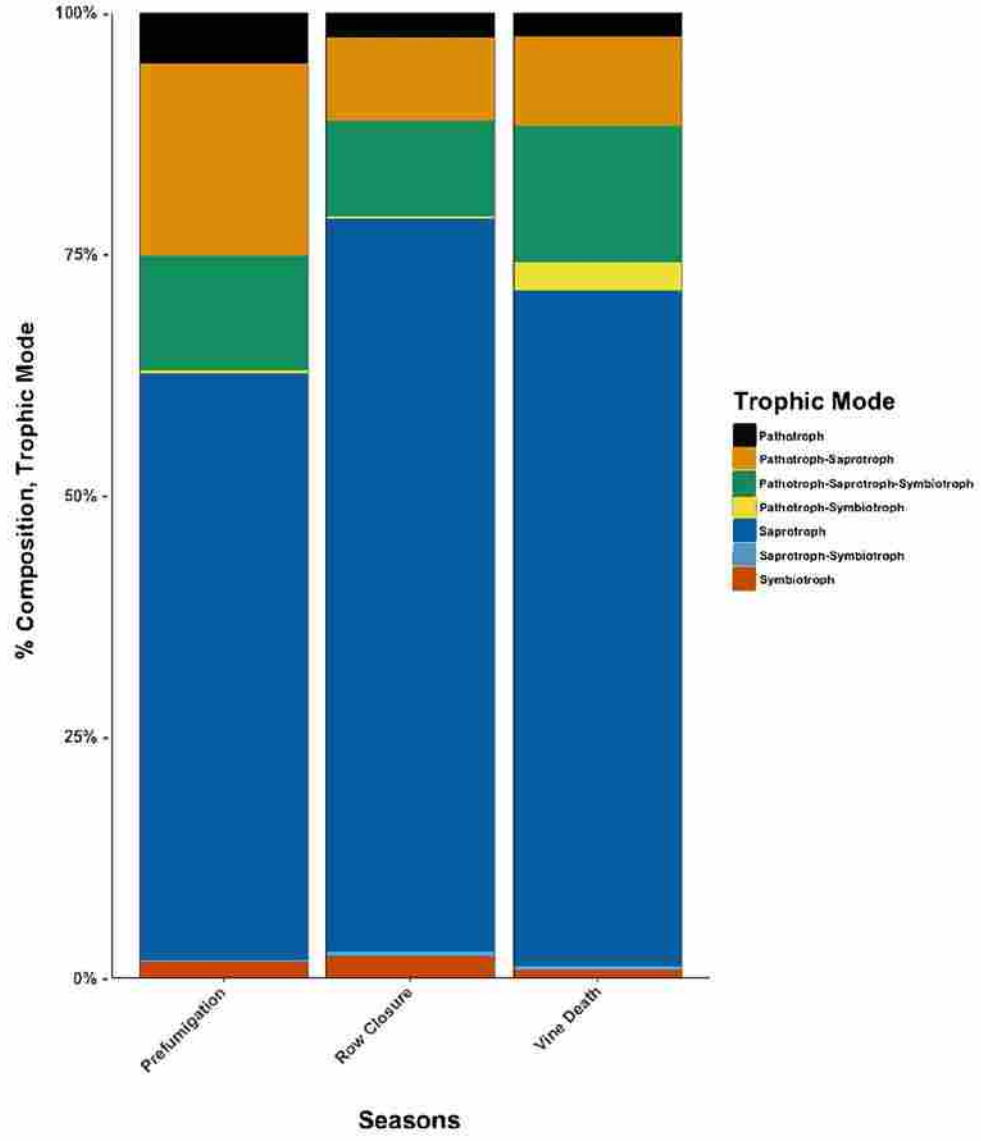

Figure 9. Barplot, temporal variation of fungal trophic modes
Temporal variation of different fungal trophic modes at prefumigation, row closure and vine death. Pathotroph relative abundances are lowest during row closure while, at the same time, saprotroph relative abundances are highest during row closure.

TABLES

Table 1. Primers used, primer sequences, their target organisms and their citations

Primer	Sequence 5'-3'	Specificity	Reference
515f	GTGCCAGCMGCCGCGGTAA	*Bacteria*	(Caporaso, 2011)
806r	GGACTACHVGGGTWTCTAAT		
ITS1F_KYO1	CTHGGTCATTTAGAGGAASTAA	*Fungi*	(Toju, 2016)
ITS2_KYO2	TTYRCTRCGTTCTTCATC		
Euk_1391f	GTACACACCGCCCGTC	*Eukaryotes*	(Amaral-Zettler, 2009)
EukBr	TGATCCTTCTGCAGGTTCACCTAC		

Table 2. Unassigned reads before and after the addition of an oomycete data to the UNITE database

	% Unassigned reads, before oomycetes added		% Unassigned reads, after oomycetes added		Raw % change, before vs. after oomycetes added	
	Ascomycota	Basidiomycota	Ascomycota	Basidiomycota	Ascomycota	Basidiomycota
Class	4.96%	43.48%	5.79%	2.52%	-0.82%	40.96%
Order	20.20%	63.17%	6.86%	2.89%	13.34%	60.28%
Family	24.21%	63.19%	12.37%	2.94%	11.84%	60.26%
Genus	25.63%	63.54%	13.92%	3.34%	11.71%	60.20%
Species	52.40%	64.36%	26.37%	7.26%	26.03%	57.10%

Table 3. Unassigned OTUs before and after the addition of an oomycete data to the UNITE database

	% Unassigned OTUs, before oomycetes added		% Unassigned OTUs, after oomycetes added		Raw % change (before - after)	
	Ascomycota	Basidiomycota	Ascomycota	Basidiomycota	Ascomycota	Basidiomycota
Class	61.82%	13.27%	64.69%	11.48%	-2.87%	1.79%
Order	66.58%	25.07%	68.93%	22.04%	-2.35%	3.03%
Family	70.59%	27.11%	72.76%	24.25%	-2.17%	2.86%
Genus	72.76%	30.05%	74.68%	26.95%	-1.91%	3.11%
Species	77.89%	40.80%	78.73%	39.35%	-0.84%	1.45%

Table 4. Percentages of reads during prefumigation, row closure and vine death for genera with an assignment of over 1,000 reads
The total numbers of reads and the percentage of reads, taken at sampling timepoints prefumigation (Prefum), row closure (RC) and vine death (VD), that have been assigned both a genus and trophic mode. Sampling timepoints are a collection of all sampling locations.

Prefum., Reads	Prefum., % reads	RC, reads	RC, % reads	VD, Reads	VD, % Reads	Total Reads	Genus	Trophic Mode
14314	12.9%	41595	30.7%	44839	28.8%	100748	Humicola	Saprotroph
16807	15.2%	15707	11.6%	24142	15.5%	56656	Mortierella	Saprotroph
7465	6.7%	19149	14.1%	14190	9.1%	40804	Schizothecium	Saprotroph
16160	14.6%	7844	5.8%	11847	7.6%	35851	Fusarium	Pathotroph-Saprotroph
10606	9.6%	7004	5.2%	7508	4.8%	25118	Tetracladium	Saprotroph
11140	10.1%	6696	4.9%	7102	4.6%	24938	Alternaria	Pathotroph-Saprotroph-Symbiotroph
3823	3.5%	4100	3.0%	8015	5.1%	15938	Preussia	Saprotroph
1697	1.5%	5832	4.3%	4719	3.0%	12248	Chaetomium	Pathotroph-Saprotroph-Symbiotroph
4620	4.2%	2491	1.8%	4187	2.7%	11298	Kotlabaea	Saprotroph
5434	4.9%	3023	2.2%	1406	0.9%	9863	Didymella	Pathotroph-Saprotroph
338	0.3%	162	0.1%	4983	3.2%	5483	Colletotrichum	Pathotroph-Symbiotroph
1801	1.6%	1326	1.0%	2301	1.5%	5428	Sagenomella	Saprotroph
1107	1.0%	1162	0.9%	3153	2.0%	5422	Talaromyces	Saprotroph
1351	1.2%	2042	1.5%	771	0.5%	4164	Peziza	Symbiotroph
664	0.6%	2006	1.5%	1213	0.8%	3883	Apodus	Saprotroph
1159	1.0%	1520	1.1%	817	0.5%	3496	Syncephalis	Pathotroph
308	0.3%	1264	0.9%	1268	0.8%	2840	Paraphaeosphaeria	Saprotroph
864	0.8%	675	0.5%	1197	0.8%	2736	Pseudeurotium	Saprotroph
653	0.6%	744	0.5%	1228	0.8%	2625	Pythium	Pathotroph
1953	1.8%	78	0.1%	2	0.0%	2033	Typhula	Pathotroph
479	0.4%	565	0.4%	591	0.4%	1635	Cistella	Saprotroph
417	0.4%	727	0.5%	414	0.3%	1558	Chloridium	Symbiotroph
193	0.2%	508	0.4%	832	0.5%	1533	Spizellomyces	Pathotroph-Saprotroph
588	0.5%	607	0.4%	285	0.2%	1480	Metarhizium	Pathotroph
447	0.4%	548	0.4%	470	0.3%	1465	Exophiala	Saprotroph
592	0.5%	259	0.2%	578	0.4%	1429	Thelebolus	Saprotroph
9	0.0%	8	0.0%	1326	0.9%	1343	Psilocybe	Saprotroph
537	0.5%	449	0.3%	316	0.2%	1302	Lachnella	Saprotroph
107	0.1%	358	0.3%	785	0.5%	1250	Cladorrhinum	Pathotroph-Saprotroph-Symbiotroph
267	0.2%	622	0.5%	351	0.2%	1240	Trichocladium	Saprotroph
403	0.4%	336	0.2%	416	0.3%	1155	Arthrobotrys	Saprotroph
629	0.6%	76	0.1%	397	0.3%	1102	Verticillium	Pathotroph
167	0.2%	305	0.2%	624	0.4%	1096	Cercophora	Saprotroph
151	0.1%	574	0.4%	325	0.2%	1050	Podospora	Saprotroph-Symbiotroph

Table 5. Nematode populations at an order level

Fraction of nematodes, at an order level, of all V9 nematode reads within each location by sampling time and treatment

Location	Sampling Time	Treatment	Rhabditida	Dorylaimida	Tylenchida	Monhysterida	Araeolaimida	Mononchida	Enoplida	Trichocephalida	Total Reads
Minidoka, ID	Prefumigation	Vapam	0.18	0.21	0.45	0.15	0.01	0.00	0.00	0.00	2755
Minidoka, ID	Row Closure	Nonfumigated	0.16	0.31	0.38	0.12	0.02	0.00	0.01	0.00	2057
Minidoka, ID	Row Closure	Vapam	0.25	0.31	0.36	0.07	0.01	0.00	0.00	0.00	1975
Minidoka, ID	Vine Death	Nonfumigated	0.18	0.28	0.41	0.13	0.01	0.00	0.00	0.00	1559
Minidoka, ID	Vine Death	Vapam	0.13	0.58	0.27	0.01	0.01	0.00	0.00	0.00	2852
Acequia, ID	Prefumigation	Nonfumigated	0.47	0.00	0.31	0.22	0.00	0.00	0.00	0.00	1072
Acequia, ID	Prefumigation	Chloropicrin	0.41	0.23	0.28	0.08	0.00	0.00	0.00	0.00	2032
Acequia, ID	Row Closure	Nonfumigated	0.88	0.06	0.05	0.00	0.00	0.00	0.00	0.00	5036
Acequia, ID	Row Closure	Chloropicrin	0.64	0.29	0.05	0.03	0.00	0.00	0.00	0.00	6768
Acequia, ID	Vine Death	Nonfumigated	0.55	0.18	0.19	0.07	0.00	0.00	0.01	0.00	1794
Acequia, ID	Vine Death	Chloropicrin	0.52	0.30	0.10	0.08	0.01	0.00	0.00	0.00	2084
Wadena, MN	Prefumigation	Vapam	0.30	0.17	0.40	0.06	0.02	0.02	0.03	0.00	8330
Wadena, MN	Row Closure	Vapam	0.99	0.00	0.01	0.00	0.00	0.00	0.00	0.00	1016
Wadena, MN	Vine Death	Vapam	0.67	0.06	0.21	0.01	0.06	0.00	0.00	0.00	4798
Pine Point, MN	Prefumigation	Nonfumigated	0.06	0.80	0.03	0.05	0.07	0.00	0.00	0.00	2460
Pine Point, MN	Prefumigation	Chloropicrin	0.20	0.41	0.23	0.11	0.03	0.01	0.00	0.00	2407
Pine Point, MN	Row Closure	Nonfumigated	0.53	0.36	0.05	0.01	0.04	0.00	0.00	0.00	755
Pine Point, MN	Row Closure	Chloropicrin	0.65	0.12	0.22	0.00	0.00	0.00	0.00	0.00	2836
Pine Point, MN	Vine Death	Nonfumigated	0.28	0.36	0.23	0.04	0.05	0.03	0.00	0.00	5197
Pine Point, MN	Vine Death	Chloropicrin	0.02	0.88	0.03	0.02	0.05	0.00	0.00	0.00	1849
Boardman, OR	Row Closure	Nonfumigated	0.88	0.00	0.12	0.00	0.00	0.00	0.00	0.01	582
Boardman, OR	Row Closure	Vapam, Telone II	0.25	0.59	0.07	0.03	0.03	0.02	0.01	0.00	5229
Boardman, OR	Vine Death	Nonfumigated	0.52	0.40	0.04	0.02	0.00	0.00	0.00	0.03	2071
Boardman, OR	Vine Death	Vapam, Telone II	0.27	0.52	0.08	0.08	0.01	0.02	0.02	0.00	3357
Quincy, WA	Row Closure	Chloropicrin	0.87	0.03	0.10	0.00	0.00	0.00	0.00	0.00	2744
Quincy, WA	Row Closure	Telone II	0.73	0.00	0.18	0.08	0.00	0.00	0.00	0.00	1629
Quincy, WA	Vine Death	Chloropicrin	0.52	0.04	0.44	0.00	0.00	0.00	0.00	0.00	1082
Quincy, WA	Vine Death	Telone II	0.38	0.14	0.26	0.22	0.00	0.00	0.00	0.00	1747
Percentage of Nematodes Reads			**0.45**	**0.27**	**0.20**	**0.06**	**0.01**	**0.00**	**0.00**	**0.00**	**2788.32**

Table 6. Soil characteristics of all fields
Soil characteristics of all fields. "-" denotes information currently unreported by growers

Location	Treatment	Latitude	Longitude	Elevation	Sand %	Clay %	Silt %	Texture Classification	pH	EC	Na	Mg	Ca	Organic Matter	Cultivar	Tillage
Minidoka, ID	Vapam	42.7293276	-113.5506726	1278 m	56.16	21.12	22.72	Sandy Clay Loam	7.48	1463.00	1.24	2.17	7.71	9.56	Russet Norkotah	Ripper 12"
Minidoka, ID	Nonfumigated	42.7333401	-113.4427048	1301 m	45.55	23.12	31.44	Loam	7.36	1488.89	1.17	4.23	15.13	9.77	Russet Norkotah	Ripper 12"
Acequia, ID	Chloropicrin	42.6509552	-113.589588	1258 m	73.44	15.12	11.44	Sandy Loam	6.56	767.78	-	-	-	10.51	Russet Norkotah	Ripper 12"
Acequia, ID	Nonfumigated	42.7352058	-113.5477704	1279 m	58.16	13.12	28.72	Sandy Loam	6.57	745.90	10.12	1.86	7.39	10.94	Russet Norkotah	Ripper 12"
Pine Point, MN	Chloropicrin	47.00953	-95.3715	472 m	-	-	-	Sandy Loam	5.26	749.17	-	-	-	9.24	Burbank	Chisel Plow
Pine Point, MN	Chloropicrin	47.0062855	-95.37	472 m	64.44	17.12	18.44	Sandy Loam	5.43	740.00	0.92	2.22	7.35	9.91	Burbank	Chisel Plow
Wadena, MN	Vapam	46.45629559	-95.18371668	414 m	-	-	-	Sandy Loam	5.82	646.08	1.05	2.60	11.03	9.12	-	Chisel Plow
Boardman, OR	Vapam, Telone II	45.73888889	-120.0311111	82 m	85.16	9.12	5.72	Loamy Sand	7.20	1695.50	-	-	-	8.51	-	-
Boardman, OR	Nonfumigated	45.742883	-119.9644928	177 m	83.44	11.12	5.44	Loamy Sand	5.84	1149.43	15.86	2.35	12.51	10.17	-	-
Quincy, WA	Chloropicrin, Telone II	47.19029	-119.8414	381 m	65.44	15.12	19.44	Sandy Loam	5.93	745.00	3.76	1.43	5.60	9.92	-	Shank Ripper
Quincy, WA	Chloropicrin, Telone II	47.19899	-119.8409	385 m	66.16	15.12	18.72	Sandy Loam	5.86	968.00	-	-	-	8.96	Wheat	Shank Ripper