# Developing Improved Strategies for Management of Replant Problems

**Project No.:** **15-PATH1-Browne**

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**Objectives:**

1) Determine the causes of Prunus replant disease (PRD).
2) Support the development of non-fumigant-based approaches for management of RD and other soilborne diseases

**Interpretive Summary:**

Preplant soil fumigation can economically manage most biological replant problems of almond. However, due to increasing regulatory restrictions on the practice, there are needs to: (i) improve integrated pest management approaches for orchard replant problems and (ii) develop economical alternatives to soil fumigation.

This project focuses on these needs. As a rule, integrated pest management (IPM) approaches include, among other steps: identification and monitoring of the pest, assessing losses that may be caused by the pest, and use of sound guidelines and combinations of treatments for managing the pests. The first objective of the project (i.e., determining causes of Prunus replant disease [PRD]) addresses IPM needs for pest identification, monitoring, and assessment, while the second objective (i.e., developing non-fumigant based approaches for replant problem management) answers to IPM needs for sound and economical treatment alternatives for preplant soil fumigation. Soil fumigation will remain as a treatment of choice for the foreseeable future, but development of alternatives to it may help to preserve its availability for essential uses. This project is testing anaerobic soil disinfestation (ASD) as an alternative to soil fumigation. ASD, developed initially in Japan and the Netherlands, is implemented by mixing readily available carbon source(s) with soil, covering with a clear tarp, and maintaining

high soil moisture content for several weeks. The treatment can generate anaerobic conditions, organic acids, pH reduction, toxic metal ions, and microbial community shifts that suppress many soilborne diseases.

In 2015-16, we: (i) completed a greenhouse bioassay trial examining PRD incidence and severity among diverse replant soils in the Sacramento and San Joaquin Valleys, (ii) continued four orchard replant trials testing anaerobic soil disinfestation (ASD) for control of PRD and (iii) examined microbial community responses in bioassay and ASD experiments. The activities were designed to support both objectives 1 and 2.

Soils for the bioassay were collected in 2015 from depths of 0.3 to 2.0 ft. below the soil surface at 26 locations throughout the Central Valley, representing 24 soils with *Prunus* cropping history, two with grape history, and all with various and biological, chemical, and physical soil properties (details of the soils were presented in 2014-15 report to the Almond Board of California [ABC], Browne et al.). Each soil was given three alternative preplant treatments--fumigation, pasteurization, and a non-treated control - - and subjected to a greenhouse-based bioassay for PRD using Nemaguard peach seedlings. The plants were grown in the test soils for 2 months, and then resulting plant top and root fresh weights were used as a measure of PRD potential (i.e., soils in which top fresh weights were suppressed in the non-treated control treatment, compared to weights in fumigation and pasteurization treatments, were considered to have high PRD potential; conversely, soils in which neither fumigation or pasteurization improved plant growth were considered to have low PRD potential). At the end of the bioassay, root and soil samples were collected for high throughput sequencing (HTS) of microbial community DNA in the soils and plant roots. By the end of the bioassay, mean increases in top plant weight resulting from fumigation or pasteurization ranged from -20 to 611% of the control, depending on the soil. The weight increases resulting from fumigation were significantly correlated with those from pasteurization ($r=0.95$, $P<0.0001$). Among six of 26 soils tested in 2015, plant growth was relatively good with or without preplant soil treatments, and no significant increase in plant fresh weights resulted from preplant fumigation or pasteurization (the two vineyard soils and four *Prunus* history soils were in this category). In another five of the soils (all from Prunus history), plant growth was still relatively good without a preplant treatment, but small-to-moderate, statistically significant increases in plant top fresh weights (avg. 23 to 46%) resulted from preplant fumigation or pasteurization. In the 15 remaining Prunus soils, plant growth was poor without treatment, and preplant soil fumigation or pasteurization increased plant top fresh weights moderately to greatly (avg. of 52 to 524%). Growth responses of Nemaguard peach rootstock seedlings to the preplant bioassay treatments (soil pasteurization and fumigation) showed a relatively low, positive correlation with orchard growth responses of almond or peach trees to preplant soil fumigation ($r= 0.59$; $P= 0.02$). It was concluded that the bioassay testing offers useful insights into the PRD potential among orchard soils, but temporal and environmental differences between greenhouse and orchard test settings can “blur” the insights.

Four ASD trials were established at the Kearney Research and Education Center (KREC) near Parlier, CA. As described previously, two trials were treated and planted in 2013-14, and another two were treated and planted in 2014-15 (Browne et al., 2013-14 and 2014-15 Annual Reports to the ABC). The trials, conducted in replant soil impacted by PRD but not plant parasitic nematodes, were designed to compare the costs and tree growth benefits of preplant

ASD to those of preplant strip fumigation with Telone C35. ASD was implemented by incorporating rice bran in 6- to 10-ft wide strips centered over future tree rows at 5.4 to 9.0 tons per treated acre (1.6 to 4.5 tons per orchard acre). In 2015-16, tree growth was assessed at the end of second and first seasons for the trials established in 2014-15 and 2015-16, respectively. Both ASD and soil fumigation significantly improved tree growth in all four trials.

Our data suggested that ASD using rice bran applied to a 6- to 10-ft-wide row strip at 9 tons per treated acre under TIF tarp (approximate cost of $1500 to 2500 per orchard acre) can match the benefit of preplant soil fumigation with Telone C35 (50% shank fumigation in strip, no tarp, full rate; cost approx. $1200/acre). Our current research is testing reduced-cost ASD treatments using economical carbon sources and streamlined application approaches.

To gain insight into microbial roles in mediating PRD and its management, HTS of rDNA was used with: (i) Nemaguard root samples from control, fumigated, and steam pasteurized treatments from 10 of the bioassay soils (five that induced PRD and five that did not) and (ii) Nemaguard root samples from control, fumigated, and ASD treatments in the 2013-14 and 2014-15 ASD trials. For each soil-treatment combination, rDNA fragments (16S for bacteria and archaea; ITS for true fungi and oomycetes) were amplified using three separate primer pairs (799f and 1193r for bacteria and archaea; BITSf and B58S3r for true fungi and oomycetes; and ITS6 and ITS7 for oomycetes) and reactions. The PCR-generated rDNA amplicons were “barcoded” into 378 “libraries”, with each library comprised of labeled amplicons generated from a single PCR primer set, a soil-treatment combination, and DNA from the fine roots ($\leq$ 1 mm diameter) of a single plant (in the case of the Bioassay) or pooled fine roots from two trees (in the case of the ASD trials). The libraries were sequenced by the Core Sequencing Facility at UC Davis using the Illumina Miseq platform in three different sequencing runs, one for amplicons of each primer pair. After quality control to remove sequences with errors, a total of 3 to 5 million sequences per experiment per primer pair were obtained. Amplicon sequences generated with the same PCR primer set were clustered into OTU groups that shared 97% DNA base sequence homology (this level of sequence homology in rDNA generally represents biological species). Taxonomic names were assigned to the OTUs to the extent possible using existing sequence databases. Bioinformatics analyses of the sequenced libraries are still underway, but previews of the fungal (and partial oomycete) microbial communities are presented in this report.

**Materials and Methods:**

**Greenhouse bioassay.** Soil samples for bioassays were collected in spring 2015 from 26 orchards and vineyards in northern, central, and southern portions of the Central Valley. The samples were collected from soil depths of 0.3 to 2.0 ft. [10 to 61 cm] at four random spots in each orchard using 3-inch [8-cm]-diameter hand augers. The 26 locations were chosen to represent: (i) soils with a recent history of almond or other stone fruit production and no other treatments (22 soils); (ii) soils that had been given fumigation or ASD treatment after removal of stone fruit (3 soils); and (iii) soils from vineyards (2 soils) (**Table 1**, repeated in this report from 2014-15 report for convenience). The latter soils were of interest because many vineyards are being transitioned to almond production. Vineyard soils are not known to induce PRD, although they often harbor nematodes that parasitize *Prunus*.

Each soil was mixed with sand 2:1 (soil:sand, to facilitate adequate water drainage in pots) and given preplant treatments of: non-treated control, preplant fumigation with chloropicrin, or preplant pasteurization as described previously (Browne et al., 2014-15 report to the Almond Board of California [ABC]). On 15 July 2015, soil from each of the orchard locations and soil treatments was distributed to 12 32-oz [0.9-liter] pots and planted with recently sprouted Nemaguard peach seedlings in a greenhouse that typically maintained air temperatures between 60 and 85 °F [16 and 30 °C]. The treatments were arranged in a randomized complete block design with six blocks. Each block had two potted Nemaguard seedlings (i.e., subplots) per combination of soil number and soil treatment. The plants were watered daily/as-needed with a modified Hoagland's solution. Final plant top and root fresh weight and root cortex necrosis were measured 21-29 September 2015, and isolations were conducted in early October 2015 to assess root incidence of *Cylindrocarpon* and *Pythium* species (PRD contributors) in each treatment.

Where possible, we tested the degree of correlation between bioassay and orchard responses to preplant soil treatments. A simple correlation coefficient was calculated for the variables of: (i) average percentage of plant top weight increase resulting from preplant fumigation and pasteurization (compared to the control) in the bioassays and (ii) average percentage of trunk circumference increase resulting from preplant soil fumigation (compared to the control) in the corresponding orchard trials. For the correlation calculation, there were 15 bioassay data points available (nine points from a bioassay conducted in 2014, six from to bioassay conducted in 2015-16) and 11 orchard data points, four of which represented orchard soils tested in both 2014 and 2015-16. The tree trunk circumference data used for correlation were obtained at the end of the orchards' first year of growth after planting in replicate plots that received fumigation treatments (shank or hand probe soil fumigation treatments with chloropicrin or 1,3-dichloropropene plus chloropicrin) or a non-treated control treatment. Plant growth data were analyzed using version 9.4 software of SAS. The SAS procedure PROC MIXED was used to conduct analysis of variance of plant top weight and root necrosis, and PROC CORR of the software was used to examine correlations among plant response and environmental variables.

**Anaerobic soil disinfestation.** We continued monitoring almond tree growth responses to anaerobic soil disinfestation (ASD), fumigation, and control treatments in four replant trials impacted by PRD. The trials were established, two in 2013 and two in 2014, at the Kearney Research and Education Center (KREC), as detailed previously (Browne et al., reports to the ABC for 2013-14 and 2014-15) (**Table 2**). Before the trials, the land had been used for >12 years to grow nectarine and peach trees on Nemaguard rootstock. Soil sampled from the trial areas induced PRD in our greenhouse bioassays but did not contain growth-suppressing plant parasitic nematodes.

The four trials are comparing costs and benefits of preplant ASD to those of preplant strip fumigation with Telone C35, which is considered to provide optimal control of PRD and nematodes. Several variations of ASD treatments are being tested in the trials, including ASD with and without a sudan grass rotation, wide (10-ft) vs. narrow (6-ft) ASD strips, and high (9 tons/treated acre; 4.5 tons per orchard acre) vs. low (5.4 tons per treated acre; 1.6 tons per orchard acre) substrate rates (**Table 2**).

Tree growth variables measured in 2016 were trunk circumferences at 20" [51 cm] above the soil line (determined at end of each growing season) and percent photosynthetically active radiation absorbed by tree canopies (% PAR, determined mid-summer in second and third growing seasons). The 2016 PAR measurements will not be available until 2017. Root and soil samples were collected from the trials and subjected to mist chamber and sugar centrifugation to determine whether plant parasitic nematodes were present.

**Microbial community analyses.** Root and soil samples were collected to examine underlying shifts in the root and soil microbial communities that had occurred in: (i) the greenhouse bioassay experiment with different Central Valley replant soils and (ii) the orchard replant trial testing ASD and standard preplant treatments (those described above).

For the bioassay microbial analyses, root and soil samples were collected at the end of the experiment. Six replicate Nemaguard root systems and their surrounding soil were sampled for each of the 96 combinations of 26 bioassay soils and three soil treatments (control, fumigated, and steam pasteurized). Approximately 2 to 10 g of roots (depending on amount of roots available) and 50 g of soil from a depth of 2 to 10 cm were sampled per plant.

For the ASD trial analyses, root and soil samples were collected during the first growing season after orchard planting. The samples were collected in May and August 2014 from experiment 2 and in July and November 2015 from experiment 4. At least 5 g of roots and 50 g of soil were collected per tree, and two trees were sampled for each combination soil treatment (control, ASD, and fumigated) and sampling date (May and August in experiment 2; July and November in experiment 4). Root and soil samples from the same plot (subsamples) were pooled, leaving three replicate samples per treatment per sample date. In each sampling, collection of fine roots ($\leq$ 1 mm diameter) and elongating root ends was emphasized. Most roots were collected from 1 to 3 ft. [0.3 to 1 m] from the trunk of the tree being sampled and from a soil depth of 0.5 to 2 ft. [0.2 to 0.8 m]. All samples were frozen on dry ice within a few minutes after collection, and then transported within 24 h to long-term storage at -80 C until use for microbial analyses.

In 2015-16 a subset of the bioassay samples and all of the ASD root samples were subjected to high-throughput sequencing of rDNA amplicons from their bacterial, archaeal, fungal, and oomycete microbial communities. Briefly, all of the selected root samples were ground into powder, while frozen, using a Retsch homogenizer. Total DNA was extracted from the samples using the MoBio PowerPlant Pro kit and further purified with MoBio PowerClean Pro kit (Cat # 13400-50 and 12997-50, Mo Bio Laboratories, Inc.). Alternate kits were tried with less satisfactory results. DNA quality and quantity was measured using Nanodrop spectrophotometer and Qubit fluorimeter, respectively. Before attempting PCR amplification, $A_{260/280}$ ratios (a general indication of DNA purity with respect to contaminating proteins) of >1.7 were achieved. 100ng of purified DNA from each sample was subjected to PCR using modifications of previously described primers and protocols. The PCR primer pairs used were 799f and 1193r; BITSf and B58S3r; and ITS6 and ITS7; which, respectively, amplified: 394bp fragments of DNA from 16S rRNA genes of bacteria and archaea; 140-400bp fragments from ITS regions of rRNA genes of fungi and oomycetes; and 200-400bp fragments from ITS regions of rRNA genes of oomycetes. These primers were chosen to maximize the representation of microbial diversity in the root samples. The PCR-generated rDNA amplicons

were “barcoded” into 378 “libraries”, with each library comprised of amplicons generated from a single PCR primer set, a soil-treatment combination, and DNA from the fine roots of a single plant (bioassay) or from two trees (ASD trials). The libraries were sequenced in three different runs by the Core Sequencing Facility at UC Davis using the Illumina Miseq platform. After quality control to remove sequences with errors, about 3 to 5 million sequences per experiment per primer pair were obtained.

To date, bioinformatics analyses of the sequences have been initiated for amplicons from the BITSf and B58S3r primer set, which generates amplicons from fungi and oomycetes. These amplicons were clustered into OTU groups that shared 97% DNA base sequence homology (this level of sequence homology in rDNA generally represents biological species). Taxonomic names were assigned to the OTUs to the extent possible using existing the UNITE fungal sequence database, and additional taxonomic notations were added to some OTUs using the NCBI sequence database. After processing, the bioassay experiment yielded 85 libraries with 3,151 to 106,952 sequences per library constituting 1146 fungal and oomycete OTUs, and the ASD trials yielded 36 libraries with 6,305 to 306,676 sequences per library constituting 1,116 fungal and oomycete OTUs. A Bray-Curtis dissimilarity matrix was generated for each experiment using libraries subsampled to equal number of sequences without replacement. This distance matrix was visualized using non-metric multidimensional scaling (NMDS), and significant differences in community structure were tested using permutational ANOVA (999 permutations). To determine if any OTUs changed significantly with treatment, we implemented a negative binomial test using DESeq2 using non-subsampled libraries.

**Results and Discussion:**

**Greenhouse bioassay.** By the completion of the experiment in late September 2015, root and shoot fresh weights exhibited highly significant soil × treatment interaction ($P<0.0001$) (**Figure 1 A, B**). Mean increases in top plant weight resulting from fumigation and pasteurization ranged from -20 to 611% of the control, depending on the soil and pretreatment (**Figure 1 A**); root fresh weights exhibited similar trends (**Figure 1 B**). The weight increases resulting from fumigation were highly correlated with those from pasteurization ($r=0.95$, $P<0.001$). Among several of the soils, plant growth was relatively good with or without preplant soil treatments, and no significant increase in top plant fresh weight resulted from preplant fumigation or pasteurization; these soils included: two from almond orchard locations near Arbuckle, CA (soils 6 and 7, **Figure1 A**); one from almond replant plots that had been fumigated before collection near Delhi, CA (soil 9); two from vineyard locations near Parlier, CA (soils 11 and 12); and one from peach replant plots that had been fumigated before collection near Parlier (soil 14, fumigated plots of the 2014 ASD trial). In another group of soils tested in the bioassay, plant growth was still relatively good without preplant fumigation or pasteurization, but relatively small and statistically significant increases in plant top fresh weights (avg. 23 to 46%) resulted from preplant fumigation or pasteurization; these soils included: one from non-fumigated almond replant plots near Delhi (soil 8); one from ASD-treated peach replant soil near Parlier, CA (soil 15); and three from peach and almond replant settings near Sanger and Reedley (soils 16, 17, and 20). In the remaining soils, which were all from standing or recently replanted almond or peach orchards, bioassay plant growth was relatively poor without a preplant soil treatment, and preplant soil fumigation and pasteurization increased plant top fresh weights moderately to greatly (avg. 52 to 524 %). Significant levels of root cortex

necrosis occurred in the control treatment for all soils, including several soils that did not induce plant top or root growth suppression without fumigation or pasteurization (**Figure 2**).

Based on culture-based isolations, root incidences of *Pythium* and *Cylindrocarpon* species were negatively correlated with plant top fresh weights (r= -0.32, *P*=0.004; and r= -0.63, *P*<0.0001, respectively). Significant negative correlations between root incidences of *Pythium* and *Cylindrocarpon* species and plant top fresh weights had also occurred in a previous (2014) bioassay (r= -0.71, *P*<0.0001; r= -0.67, *P*<0.0001, respectively). In previous trials, some isolates of these organisms were found to be pathogenic on 'Nemaguard'. After removal of the data from the vineyard soils and the orchard-fumigated soils, the plant top fresh weight data showed no significant correlation between soil pH and percentage of increase in plant top fresh weights in response to preplant soil treatment (average values from fumigation and pasteurization treatments) (*P*=0.22). In contrast, in a 2014 bioassay, a significant positive correlation had resulted between pH and mean percentage of growth increase from preplant fumigation/pasteurization (r= 0.44, *P*=0.05). Apparently, soil pH values are not consistently associated with the degree of response to preplant soil fumigation/pasteurization.

The plant top fresh weight responses to bucket pasteurization and fumigation in 2014 and 2015 bioassays correlated significantly but at a relatively low level with tree trunk circumference responses to fumigation in corresponding orchard trials (**Figure 3**; r=0.59; *P*=0.02).

Overall, it was concluded that the bioassay offers useful insights into the PRD potential among orchard soils, but temporal and environmental differences between greenhouse and orchard test settings can "blur" the insights. We will continue to use the bioassay, yet it will continue to be important to "cross-check" bioassay findings for orchard environment relevance.

**Anaerobic soil disinfestation.** In ASD experiments 1 and 2, in which tree trunk circumferences were measured after completion of the second growing season, all preplant ASD and fumigation treatments supported significantly greater and similar tree trunk circumference increases, compared to the non-treated controls (**Figure 4 A, B**). The preplant rotation with sudan grass alone ("control, with sudan; detailed in **Table 2**) significantly improved tree growth, but not as much as ASD or soil fumigation (**Figure 4 A**). There was no clear impact of whether the fumigation occurred in October or November or whether it occurred following sudan rotation or not, but it should be considered that it was an exceptionally dry, warm December (2013) during which the fumigation was applied; it is likely that in relatively cool wet winters fumigation in December would achieve poor fumigation results.

In ASD experiments 3 and 4, in which tree trunk circumferences were measured after the end of the first growing season, the high rate ASD treatments applied in wide row strips and both fumigation treatments (October, with vs. without sudan rotation) performed similarly as in experiments 1 and 2; i.e., they all supported significantly greater and similar tree trunk circumference increases, compared to the non-treated controls (**Figure 5 A, B**). Reducing the strip width from 10 ft. to 6 ft. while retaining the rice bran rate at 9 tons per treated acre resulted in only a small reduction in ASD efficacy, whereas reducing the strip width and also reducing the rice bran rate (from 9 to 5.4 tons per treated acre) significantly reduced three growth, compared to the other ASD and fumigation treatments (**Figure 5 A**).

Our data suggest that ASD using rice bran applied to a 6- to 10-ft-wide row strip at 9 tons per acre under TIF tarp (approximate cost of $1500 to 2500 per orchard acre) can match the benefit of preplant soil fumigation with Telone C35 (50% shank fumigation in strip, no tarp, full rate; cost approx. $1200/acre). Our current research is testing reduced-cost ASD treatments using economical carbon sources and streamlined application approaches.

**Fungal and oomycete community analyses.** In all 10 of the bioassay soils, regardless of whether or not they induced PRD growth suppression, the fumigation and pasteurization treatments induced changes fungal and oomycete community structure that developed in and on roots, as evidenced by significant NMDS ordinations of species dissimilarity gradients (e.g., as shown for soil "1.Durham-Mea.Tri.St", **Figure 6**). In NMDS ordinations, the distance between the points is proportional to the degree of genetic dissimilarity between their OTU community compositions.

When NMDS ordination was used to examine differences in fungal/oomycete root community structure among non-treated soils from the 10 bioassay soil locations submitted to HTS analysis, there was a tendency for sample communities from different soils to cluster separately (**Figure 7**). However, the root fungal/oomycete communities associated with PRD (i.e., from soils 1, 3, 10, 13, and 23) did not, as a group, cluster distinctly from those that did not induce PRD (i.e., from soils 6, 11, 12, 14, and 15) (**Figure 7**). When the communities ordinated by NMDS were limited to include only those from the KAC soils near Parlier, the communities clustered distinctly by soil, except that one of the "15.Parlier-KAC2014.Tri.ASD.CL" samples differed from its two partners as much or more than from samples from some of the other soils (**Figure 8**).

In the bioassay, significant changes in root fungal/oomycete OTU abundance in response to the fumigation and pasteurization treatments are tabulated, listed according to OTU identity and overall mean abundance (**Tables 3-6**). The changes are listed separately for soils that did induce PRD (**Tables 3 and 4**) and soils that did not do so (**Tables 5 and 6**). It can be noted, in each of the tables (**Tables 3-6**), that many fungal/oomycete OTUs decreased in abundance in response to fumigation or pasteurization treatments (as indicated by negative values in the "$Log_2$ fold change" column), while others increased in abundance (as indicated by positive values in the "$Log_2$ fold change" column).

In the 2013 ASD experiment, NMDS ordination of the fungal/oomycete root microbial communities revealed relatively distinct clustering by preplant soil treatments (control, fumigation, ASD) and sampling dates (May, August) (**Figure 9**). NMDS ordination of the fungal/oomycete root microbial communities from the 2014 ASD experiment (**Figure 10**) revealed less-distinct and less-consistent clustering by soil treatments and sampling dates, compared to the ordination of the 2013 fungal/oomycete communities (**Figure 9**). Significant changes in 2013 ASD trial's root fungal/oomycete OTU abundance in response to the fumigation and pasteurization treatments are tabulated, listed according to specific OTU identity and overall mean abundance (**Tables 7-10**). The changes are listed separately for ASD and fumigation treatments in 2013 (**Tables 7 and 8, respectively**) and 2014 (**Tables 9 and 10, respectively**). It can be noted, in each of the tables (**Tables 7-10**), that many fungal/oomycete OTUs decreased in abundance in response to ASD or fumigation treatments

(as indicated by negative values in the "$Log_2$ fold change" column), while others increased in abundance (as indicated by positive values in the "$Log_2$ fold change" column).

NMDS ordination was applied to compare the fungal/oomycete communities in roots of the greenhouse bioassay compared those in roots of ASD trial samples. The ordination indicated that, overall, fungal/oomycete root communities in the bioassay, which clustered on the positive (right) side of axis 1, differed from those of the field ASD trial, which clustered on the negative (left) side of axis 1 (**Figure 11**).

Further bioinformatics work is needed and underway for significant insight into the fungal, oomycete, bacterial, and archaeal microbial communities we accessed in 2015-16. The bioinformatics and microbiological expertise in our USDA-ARS unit at Davis has been strengthened significantly by the hire of a new scientist, Dr. Amisha Poret-Peterson, and she has contributed greatly to the bioinformatics analyses in this report, which should be considered as a partial summary of the work in progress. In the coming project year, among other bioinformatics tasks, we will: i) add our bacteria/archaea and oomycete sequence data sets to the fungal/oomycete datasets introduced in this report, ii) conduct multiple ordination and regression analyses designed to identify individual OTUs and consortia of OTUs that associate with PRD incidence and control in greenhouse and field replant soil environments; and iii) initiate metatranscriptomic analyses (which access actively expressed genes) of the communities we seek to understand.

**Research Effort Recent Publications:**

Browne, G.T. 2016. Resistance to *Phytophthora* species among rootstocks for cultivated *Prunus* species. HortScience: (in press)

Browne, G.T., Bhat, R.G., and Schmidt, L.S. 2016. Growth of diverse almond and stone fruit rootstocks in soil impacted by Prunus replant disease (submitted).

**Table 1.** Soils used in 2015 greenhouse bioassay that was completed in 2015-16

| 2015 soil number and code[a] | Crop history[b] | Nematode count (per 250 cc)[c] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ring | Lesion | RKN | Dagger | Pin | Free living |
| 1.Durham-Mea.Tri.CK.St | Almond/Lovell, 11 yr | 0 | 0 | 0 | 0 | 62 | 92 |
| 2.Durham-Mtz.Tri.CK.St | Almond/Lovell, 11 yr | 0 | 0 | 0 | 2 | 112 | 134 |
| 3.Durham-Mtz.S.St | Almond/Lovell, >20 yr | 0 | 0 | 0 | 0 | 360 | 54 |
| 4.Durham-Gilb.N.St | Almond/Lovell, >20 yr | 0 | 0 | 0 | 0 | 104 | 8 |
| 5.Durham-Gil.S.St | Almond/Lovell, >20 yr | 0 | 0 | 0 | 0 | 26 | 22 |
| 6.Arbuckle-Nic.Tri.CK.St | Almond/Nemaguard, 6 yr | 0 | 0 | 0 | 0 | 646 | 64 |
| 7.Arbuckle-Hen.St | Almond/Lovell, >20 yr | 0 | 0 | 0 | 36 | 318 | 6 |
| 8.Delhi-Lit.Tri.CK.Cl | Almond/Nemaguard, >20 yr | 30 | 0 | 0 | 0 | 0 | 54 |
| 9.Delhi-Lit.Tri.C35.Cl | Almond/Nemaguard, >20 yr | 14 | 0 | 0 | 0 | 0 | 132 |
| 10.Firebaugh-WO.Tri.CK.St | Almond/Nemaguard, 8 yr | 0 | 0 | 0 | 0 | 883 | 29 |
| 11.Parlier-KAC.Vin.S.St | Vineyard, >20 yr | 808 | 0 | 15 | 7 | 317 | 149 |
| 12.Parlier-KAC.Vin.N.St | Vineyard, >20 yr | 56 | 0 | 0 | 22 | 544 | 336 |
| 13.Parlier-KAC2014.Tri.CK.Cl | Peach/Nemaguard, ca. 12 yr | 0 | 0 | 0 | 0 | 4 | 248 |
| 14.Parlier-KAC2014.Tri.C35.Cl | Peach/Nemaguard, ca. 12 yr | 0 | 0 | 0 | 0 | 0 | 178 |
| 15.Parlier-KAC2014.Tri.ASD.Cl | Peach/Nemaguard, ca. 12 yr | 0 | 0 | 0 | 0 | 0 | 586 |
| 16.Reedley-Klas.N.St | Nectarine/Nemaguard, ca. 12 yr | 37 | 4 | 0 | 0 | 900 | 35 |
| 17.Reedley-Klas.S.St | Peach/Nemaguard, ca 15 yr | 0 | 13 | 0 | 0 | 538 | 134 |
| 18.Sanger-MG.Rep.St | Plum/Nemaguard, 1 yr | 0 | 38 | 0 | 0 | 45 | 70 |
| 19.Sanger-LTB.Hc.Cl | Almond/Nemaguard, >20 yr | 0 | 0 | 0 | 0 | 186 | 146 |
| 20.Sanger-LTB.Rc.Cl | Almond/Nemaguard, >20yr | 29 | 0 | 0 | 1 | 941 | 80 |
| 21.Traver-Famt.St | Nectarine/Nemaguard, ca. 15 yr | 0 | 0 | 0 | 27 | 662 | 92 |
| 22.Shafter-3901.K&B.St | Almond/Nemaguard, >20 yr | 892 | 184 | 3 | 38 | 179 | 42 |
| 23.Shafter-WO.3010.S.St | Almond/Nemaguard, >20 yr | 0 | 0 | 0 | 0 | 268 | 34 |
| 24.Shafter-WO.3010.N.Stb | Almond/Nemaguard, >20 yr | 0 | 0 | 0 | 0 | 184 | 33 |
| 25.Belridge-WO.3540.196.St | Almond/Nemaguard, >20 yr | 0 | 0 | 0 | 0 | 824 | 58 |
| 26.Belridge-WO.3580.211.St | Almond/Nemaguard, >20 yr | 0 | 4 | 0 | 45 | 500 | 89 |

[a] Soil location number is followed by nearest city or landmark and additional coded information. In code text, “Vin” indicates soil was from vineyard (all other soils were from almond or stone fruit orchards) “Tri” indicates that location had hosted or is hosting fumigation trial; “C35” indicates that soil was treated with Telone C35 before collection from the field; “ASD” indicates that soil was treated with anaerobic soil disinfestation before collection from the field; “CK” indicates soil was from control plots that did not receive C35 or ASD; “St” indicates standing orchard or vineyard; “Cl” indicates cleared orchard.

[b] Years are estimates

[c] Based on sugar flotation method. “RKN” indicates root knot nematode.

**Table 2.** Overview of trials testing anaerobic soil disinfestation and other preplant treatments near Parlier at Kearney Agricultural Center

| Year | Expt. | Trt. no. | Treatment name | Month of old orchard tree removal | Month of sudan rotation | Fall/winter soil disinfestation treatment |
|---|---|---|---|---|---|---|
| 2013 | 1 | 1 | Control, no sudan | Sep | None | None |
| | | 2 | Control, with sudan | May | May-Oct | None |
| | | 3 | ASD, high bran rate, wide strip, with sudan | May | May-Oct | ASD, 20 metric tons /treated ha, 3.0-m-wide strips |
| | | 4 | Fumigation in Oct, no sudan | Sep | No | Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips |
| | | 5 | Fumigation in Oct, with sudan | May | May-Oct | Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips |
| | | 6 | Fumigation in Dec, no sudan | Sep | None | Telone C35, 600 kg/treated ha in Dec, 3.4-m-wide strips |
| | 2 | 1 | Control, no sudan | May | None | None |
| | | 2 | ASD, high bran rate, wide strip, no sudan | May | None | ASD, 20 metric tons /treated ha, 3.0-m-wide strips |
| | | 3 | Fumigation in Oct, no sudan | May | None | Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips |
| 2014 | 3 | 1 | Control, no sudan | Sep | None | None |
| | | 2 | Control, with sudan | May | May-Oct | None |
| | | 3 | ASD, high bran rate, wide strip, with sudan | May | May-Oct | ASD, 20 metric tons /treated ha, 3.0-m-wide strips |
| | | 4 | ASD, high bran rate, narrow strip, no sudan | Sep | None | ASD, 20 metric tons /treated ha, 1.8-m-wide strips |
| | | 5 | ASD, low bran rate, narrow strip, no sudan | Sep | None | ASD, 12 metric tons /treated ha, 1.8-m-wide strips |
| | | 6 | Fumigation in Oct, no sudan | Sep | None | Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips |
| | | 7 | Fumigation in Oct, with sudan | May | May-Oct | Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips |
| | 4 | 1 | Control, no sudan | May | None | None |
| | | 2 | ASD, high bran rate, wide strip, no sudan | May | None | ASD, 20 metric tons /treated ha, 3.0-m-wide strips |
| | | 3 | Fumigation in Oct, no sudan | May | None | Telone C35, 600 kg/treated ha in Oct, 3.4-m-wide strips |

**Table 3.** Significant changes in abundance of rDNA ITS amplicons resulting from preplant fumigation of bioassay soils that DID induce PRD, as compared to the non-fumigated controls for the same soils.

| OTU[z] | Base mn. amplicon count[y] | $Log_2$ fold change[x] | Value of *P*[w] | Taxon level and identity using RDP classifier and UNITE fungal sequence database[v] | Additional identity notes, based on BLAST of NCBI database[u] |
|---|---|---|---|---|---|
| OTU117 | 1112 | -13.25 | 0.0000 | p__Ascomycota_unclassified | -- |
| OTU54 | 459 | -11.18 | 0.0000 | p__Ascomycota_unclassified | -- |
| OTU148 | 354 | -10.83 | 0.0000 | p__Ascomycota_unclassified | -- |
| OTU131 | 296 | -12.24 | 0.0000 | p__Ascomycota_unclassified | -- |
| OTU33 | 171 | 3.12 | 0.0252 | k__Fungi_unclassified | No significant similarity found |
| OTU31 | 99 | -7.80 | 0.0001 | k__Fungi_unclassified | Pythium spinosum |
| OTU17 | 88 | -8.62 | 0.0000 | g__unclassified_Ascomycota | -- |
| OTU278 | 71 | -9.41 | 0.0006 | o__Sebacinales_unclassified | -- |
| OTU43 | 64 | -6.80 | 0.0000 | k__Fungi_unclassified | Pythium debaryanum |
| OTU362 | 41 | -2.57 | 0.0382 | g__unclassified_Fungi | -- |
| OTU30 | 40 | -7.19 | 0.0000 | k__Fungi_unclassified | Uncultured Sebacinaceae |
| OTU134 | 37 | -8.72 | 0.0001 | k__Fungi_unclassified | No significant similarity found |
| OTU696 | 30 | -8.45 | 0.0003 | k__Fungi_unclassified | Fungal sp |
| OTU18 | 29 | -5.28 | 0.0012 | k__Fungi_unclassified | Pythium vexans |
| OTU1911 | 28 | 3.77 | 0.0114 | k__Fungi_unclassified | Fusarium sp. |
| OTU1657 | 28 | -8.27 | 0.0008 | k__Fungi_unclassified | No significant similarity found |
| OTU70 | 27 | -7.32 | 0.0021 | p__Ascomycota_unclassified | -- |
| OTU416 | 25 | -4.28 | 0.0252 | g__Fusarium | -- |
| OTU231 | 24 | -7.82 | 0.0017 | g__Exophiala | -- |
| OTU160 | 23 | -7.63 | 0.0017 | g__unclassified_Ascomycota | -- |
| OTU1209 | 19 | -5.53 | 0.0021 | g__Fusarium | -- |
| OTU1836 | 12 | -7.10 | 0.0044 | p__Ascomycota_unclassified | -- |
| OTU1659 | 12 | -5.54 | 0.0240 | k__Fungi_unclassified | Cladosporium sp. |
| OTU335 | 9 | -8.54 | 0.0022 | k__Fungi_unclassified | No significant similarity found |
| OTU42 | 8 | -6.79 | 0.0015 | g__Didymosphaeria | -- |
| OTU1215 | 8 | -5.52 | 0.0003 | f__Pezizaceae_unclassified | -- |
| OTU1435 | 7 | -8.34 | 0.0026 | g__unclassified_Orbiliaceae | -- |
| OTU199 | 6 | -5.74 | 0.0189 | k__Fungi_unclassified | No significant similarity found |
| OTU1094 | 5 | -6.15 | 0.0114 | k__Fungi_unclassified | Pythium irregulare |
| OTU1161 | 4 | -3.76 | 0.0095 | g__Cladosporium | -- |
| OTU99 | 3 | -5.60 | 0.0451 | g__Flagelloscypha | -- |

[z] Individual numbered OTUs indicate "operational taxonomic units" that share 97% DNA sequence identity
yBase mean amplicon counts indicate mean numbers of amplicons falling within the designated OTU for all soil treatments

[x] Log2 fold change values indicate the mean change in amplicon count going from the control treatment to the preplant fumigation treatment.

[w] The value of P indicates the likelihood that the log2 fold change resulted from chance alone (values are adjusted to keep overall probability of an error to less than .05).

[v] Taxonomic identification to kingdom (k), phylum (p), class (c), order (o), family (f), and genus (g) using the RDP classifier method and the UNITE fungal sequence database.

[u] Additional taxonomic identity notes added to kingdom-level id's, using BLAST searches on NCBI sequence database.

**Table 4.** Significant changes in abundance of rDNA ITS amplicons resulting from preplant pasteurization of bioassay soils that DID induce PRD, as compared to the non-pasteurized controls for the same soils.

| OTU[z] | Base mn. amplicon count[y] | $Log_2$ fold change[x] | Value of *P*[w] | Taxon level and identity using RDP classifier and UNITE fungal sequence database[v] | Additional identity notes, based on BLAST of NCBI database[u] |
|---|---|---|---|---|---|
| OTU117 | 1112 | -12.85 | 0.0000 | p__Ascomycota_unclassified | |
| OTU54 | 459 | -11.54 | 0.0000 | p__Ascomycota_unclassified | |
| OTU148 | 354 | -10.46 | 0.0000 | p__Ascomycota_unclassified | |
| OTU80 | 328 | 4.38 | 0.0012 | k__Fungi_unclassified | Uncult. Endophyt. /Alternaria sp. |
| OTU120 | 310 | 4.14 | 0.0014 | k__Fungi_unclassified | Uncult. Endophyt. /Alternaria sp. |
| OTU131 | 296 | -11.87 | 0.0000 | p__Ascomycota_unclassified | |
| OTU33 | 171 | 3.16 | 0.0238 | k__Fungi_unclassified | No significant similarity found |
| OTU31 | 99 | -8.77 | 0.0000 | k__Fungi_unclassified | Pythium spinosum |
| OTU17 | 88 | -7.13 | 0.0000 | g__unclassified_Ascomycota | |
| OTU278 | 71 | -10.20 | 0.0001 | o__Sebacinales_unclassified | |
| OTU890 | 69 | 5.63 | 0.0002 | f__Pleosporaceae_unclassified | |
| OTU43 | 64 | -7.39 | 0.0000 | k__Fungi_unclassified | Pythium debaryanum |
| OTU30 | 40 | -7.26 | 0.0000 | k__Fungi_unclassified | Uncultured Sebacinaceae |
| OTU134 | 37 | -8.62 | 0.0001 | k__Fungi_unclassified | No significant similarity found |
| OTU696 | 30 | -8.29 | 0.0003 | k__Fungi_unclassified | Annulohypoxylon bovei |
| OTU18 | 29 | -6.45 | 0.0001 | k__Fungi_unclassified | Pythium vexans |
| OTU1911 | 28 | 3.76 | 0.0123 | k__Fungi_unclassified | Fusarium sp |
| OTU1657 | 28 | -7.98 | 0.0012 | k__Fungi_unclassified | No significant similarity found |
| OTU70 | 27 | -7.60 | 0.0014 | p__Ascomycota_unclassified | |
| OTU231 | 24 | -7.74 | 0.0018 | g__Exophiala | |
| OTU160 | 23 | -7.30 | 0.0026 | g__unclassified_Ascomycota | |
| OTU1836 | 12 | -7.01 | 0.0050 | p__Ascomycota_unclassified | |
| OTU1659 | 12 | -5.31 | 0.0323 | k__Fungi_unclassified | Cladosporium sp |
| OTU37 | 10 | -5.87 | 0.0198 | g__unclassified_Tricholomataceae | |
| OTU335 | 9 | -8.40 | 0.0026 | k__Fungi_unclassified | No significant similarity found |
| OTU42 | 8 | -6.09 | 0.0042 | g__Didymosphaeria | |
| OTU1215 | 8 | -5.28 | 0.0005 | f__Pezizaceae_unclassified | |
| OTU1435 | 7 | -8.19 | 0.0032 | g__unclassified_Orbiliaceae | |
| OTU199 | 6 | -5.43 | 0.0286 | k__Fungi_unclassified | No significant similarity found |
| OTU81 | 5 | -4.16 | 0.0235 | k__Fungi_unclassified | Chlorella saccharophila |
| OTU1094 | 5 | -5.84 | 0.0185 | k__Fungi_unclassified | Pythium irregulare |
| OTU51 | 3 | -5.26 | 0.0156 | k__Fungi_unclassified | Pythium irregulare |

[z] Individual numbered OTUs indicate "operational taxonomic units" that share 97% DNA sequence identity
yBase mean amplicon counts indicate mean numbers of amplicons falling within the designated OTU for all soil treatments

[x] Log2 fold change values indicate the mean change in amplicon count going from the control treatment to the preplant fumigation treatment.

[w] The value of P indicates the likelihood that the log2 fold change resulted from chance alone (values are adjusted to keep overall probability of an error to less than .05).

[v] Taxonomic identification to kingdom (k), phylum (p), class (c), order (o), family (f), and genus (g) using the RDP classifier method and the UNITE fungal sequence database.

[u] Additional taxonomic identity notes added to kingdom-level id's, using BLAST searches on NCBI sequence database.

**Table 5.** Significant changes in abundance of rDNA ITS amplicons resulting from preplant fumigation of bioassay soils that did NOT induce PRD, as compared to the non-fumigated controls for the same soils.

| OTU[z] | Base mn. amplicon count[y] | $Log_2$ fold change[x] | Value of *P*[w] | Taxon level and identity using RDP classifier and UNITE fungal sequence database[v] | Additional identity notes, based on BLAST of NCBI database[u] |
|---|---|---|---|---|---|
| OTU17 | 1162 | -11.44 | 0.0000 | g__unclassified_Ascomycota | |
| OTU8 | 430 | -7.88 | 0.0004 | g__Gymnopus | |
| OTU33 | 122 | 3.23 | 0.0029 | k__Fungi_unclassified | No significant similarity |
| OTU57 | 118 | -9.49 | 0.0000 | g__unclassified_Diaporthales | |
| OTU45 | 111 | 5.98 | 0.0016 | g__Phoma | |
| OTU25 | 102 | 3.31 | 0.0009 | k__Fungi_unclassified | No significant similarity |
| OTU97 | 77 | -9.07 | 0.0000 | p__Ascomycota_unclassified | |
| OTU18 | 74 | -5.08 | 0.0003 | k__Fungi_unclassified | Pythium vexans |
| OTU1020 | 50 | 3.36 | 0.0075 | g__Fusarium | |
| OTU117 | 42 | -8.12 | 0.0007 | p__Ascomycota_unclassified | |
| OTU51 | 42 | -8.67 | 0.0000 | k__Fungi_unclassified | Pythium irregulare |
| OTU34 | 35 | -7.72 | 0.0008 | o__Sebacinales_unclassified | |
| OTU42 | 30 | -8.76 | 0.0000 | g__Didymosphaeria | |
| OTU31 | 30 | -6.54 | 0.0004 | k__Fungi_unclassified | Pythium spinosum |
| OTU148 | 24 | -7.59 | 0.0004 | p__Ascomycota_unclassified | |
| OTU54 | 22 | -6.72 | 0.0113 | p__Ascomycota_unclassified | |
| OTU74 | 19 | -6.78 | 0.0051 | g__unclassified_Sebacinales | |
| OTU1911 | 18 | 3.94 | 0.0022 | k__Fungi_unclassified | Fusarium sp. |
| OTU32 | 17 | -6.93 | 0.0016 | g__Piriformospora | |
| OTU38 | 17 | -6.89 | 0.0033 | p__Ascomycota_unclassified | |
| OTU37 | 17 | -5.35 | 0.0312 | g__unclassified_Tricholomataceae | |
| OTU105 | 17 | -6.12 | 0.0150 | f__Auriculariales_family_Incertae_sedis_unclassified | |
| OTU43 | 13 | -5.06 | 0.0025 | k__Fungi_unclassified | Pythium debaryanum |
| OTU147 | 11 | -5.63 | 0.0433 | f__Auriculariales_family_Incertae_sedis_unclassified | |
| OTU30 | 11 | -6.72 | 0.0002 | k__Fungi_unclassified | Uncultured Sebacinaceae |
| OTU55 | 11 | -6.23 | 0.0002 | k__Fungi_unclassified | Eustigmatos magnus |
| OTU863 | 10 | -5.14 | 0.0133 | k__Fungi_unclassified | Enterobacteria phage phiX |
| OTU1094 | 9 | -6.63 | 0.0003 | k__Fungi_unclassified | Pythium irregulare |
| OTU1657 | 9 | -6.07 | 0.0212 | k__Fungi_unclassified | No significant similarity |
| OTU701 | 7 | -5.58 | 0.0476 | g__Pyrenochaeta | |
| OTU864 | 6 | -6.28 | 0.0012 | k__Fungi_unclassified | Uncultured fungus clone |
| OTU365 | 5 | -5.49 | 0.0420 | k__Fungi_unclassified | No significant similarity |
| OTU128 | 5 | -6.24 | 0.0007 | k__Fungi_unclassified | Pythium paroecandrum |
| OTU121 | 5 | -4.30 | 0.0312 | k__Fungi_unclassified | Uncultured organism clone |
| OTU160 | 5 | -5.97 | 0.0025 | g__unclassified_Ascomycota | |
| OTU166 | 4 | -5.40 | 0.0184 | k__Fungi_unclassified | Paracercomonas sp |

[z] Individual numbered OTUs indicate "operational taxonomic units" that share 97% DNA sequence identity
yBase mean amplicon counts indicate mean numbers of amplicons falling within the designated OTU for all soil treatments

[x] Log2 fold change values indicate the mean change in amplicon count going from the control treatment to the preplant fumigation treatment.

[w] The value of P indicates the likelihood that the log2 fold change resulted from chance alone (values are adjusted to keep overall probability of an error to less than .05).

[v] Taxonomic identification to kingdom (k), phylum (p), class (c), order (o), family (f), and genus (g) using the RDP classifier method and the UNITE fungal sequence database.

[u] Additional taxonomic identity notes added to kingdom-level id's, using BLAST searches on NCBI sequence database.

**Table 6.** Significant changes in abundance of rDNA ITS amplicons resulting from preplant pasteurization of bioassay soils that did NOT induce PRD, compared to the non-pasteurized controls for the same soils.

| OTU[z] | Base mn. amplicon count[y] | $Log_2$ fold change[x] | Value of *P*[w] | Taxon level and identity using RDP classifier and UNITE fungal sequence database[v] | Additional identity notes, based on BLAST of NCBI database[u] |
|---|---|---|---|---|---|
| OTU17 | 1162 | -10.64 | 0.0000 | g__unclassified_Ascomycota | |
| OTU8 | 430 | -10.07 | 0.0000 | g__Gymnopus | |
| OTU20 | 143 | 7.31 | 0.0012 | f__Lyophyllaceae_unclassified | |
| OTU33 | 122 | 4.13 | 0.0001 | k__Fungi_unclassified | No significant similarity |
| OTU57 | 118 | -8.65 | 0.0001 | g__unclassified_Diaporthales | |
| OTU25 | 102 | 4.28 | 0.0000 | k__Fungi_unclassified | No significant similarity |
| OTU97 | 77 | -8.71 | 0.0000 | p__Ascomycota_unclassified | |
| OTU18 | 74 | -7.23 | 0.0000 | k__Fungi_unclassified | Pythium vexans |
| OTU1020 | 50 | 3.74 | 0.0032 | g__Fusarium | |
| OTU117 | 42 | -7.85 | 0.0010 | p__Ascomycota_unclassified | |
| OTU51 | 42 | -8.86 | 0.0000 | k__Fungi_unclassified | Pythium irregulare |
| OTU34 | 35 | -7.96 | 0.0006 | o__Sebacinales_unclassified | |
| OTU245 | 31 | 2.95 | 0.0117 | g__unclassified_Fungi | |
| OTU525 | 30 | 2.77 | 0.0134 | g__unclassified_Fungi | |
| OTU42 | 30 | -8.03 | 0.0000 | g__Didymosphaeria | |
| OTU31 | 30 | -7.90 | 0.0000 | k__Fungi_unclassified | Pythium spinosum |
| OTU94 | 24 | 5.75 | 0.0184 | g__Torula | |
| OTU35 | 24 | 3.13 | 0.0393 | g__Mucor | |
| OTU148 | 24 | -7.26 | 0.0008 | p__Ascomycota_unclassified | |
| OTU54 | 22 | -6.49 | 0.0156 | p__Ascomycota_unclassified | |
| OTU74 | 19 | -6.83 | 0.0056 | g__unclassified_Sebacinales | |
| OTU362 | 19 | 2.87 | 0.0189 | g__unclassified_Fungi | |
| OTU1911 | 18 | 4.89 | 0.0001 | k__Fungi_unclassified | Fusarium sp |
| OTU32 | 17 | -6.51 | 0.0039 | g__Piriformospora | |
| OTU38 | 17 | -6.83 | 0.0046 | p__Ascomycota_unclassified | |
| OTU37 | 17 | -6.63 | 0.0060 | g__unclassified_Tricholomataceae | |
| OTU105 | 17 | -6.51 | 0.0102 | f__Auriculariales_family_unclassified | |
| OTU43 | 13 | -5.63 | 0.0009 | k__Fungi_unclassified | Pythium debaryanum |
| OTU30 | 11 | -6.60 | 0.0001 | k__Fungi_unclassified | Uncultured Sebacinaceae |
| OTU55 | 11 | -7.13 | 0.0000 | k__Fungi_unclassified | Eustigmatos magnus |
| OTU1094 | 9 | -6.87 | 0.0001 | k__Fungi_unclassified | Pythium irregulare |
| OTU1657 | 9 | -5.73 | 0.0327 | k__Fungi_unclassified | No significant similarity |
| OTU416 | 7 | 2.64 | 0.0477 | g__Fusarium | |
| OTU426 | 6 | 3.73 | 0.0210 | g__Fusarium | |
| OTU864 | 6 | -6.19 | 0.0016 | k__Fungi_unclassified | Uncultured fungus clone |
| OTU365 | 5 | -5.44 | 0.0438 | k__Fungi_unclassified | No significant similarity |
| OTU128 | 5 | -6.30 | 0.0007 | k__Fungi_unclassified | Pythium paroecandrum |
| OTU121 | 5 | -5.24 | 0.0073 | k__Fungi_unclassified | Uncultured organism clone |
| OTU160 | 5 | -5.04 | 0.0144 | g__unclassified_Ascomycota | |
| OTU166 | 4 | -5.19 | 0.0251 | k__Fungi_unclassified | Paracercomonas sp. |
| OTU1647 | 2 | -4.75 | 0.0197 | o__Hypocreales_unclassified | |

[z] Individual numbered OTUs indicate "operational taxonomic units" that share 97% DNA sequence identity
yBase mean amplicon counts indicate mean numbers of amplicons falling within the designated OTU for all soil treatments

[x] Log2 fold change values indicate the mean change in amplicon count going from the control treatment to the preplant fumigation treatment.

[w] The value of P indicates the likelihood that the log2 fold change resulted from chance alone (values are adjusted to keep overall probability of an error to less than .05).

[v] Taxonomic identification to kingdom (k), phylum (p), class (c), order (o), family (f), and genus (g) using the RDP classifier method and the UNITE fungal sequence database.

[u] Additional taxonomic identity notes added to kingdom-level id's, using BLAST searches on NCBI sequence database.

**Table 7.** Significant changes in abundance of operational taxonomic units (OTUs) of rDNA ITS amplicons resulting from anaerobic soil disinfestation (ASD), as compared to abundance of the OTUs in non-treated control, 2013 trial

| OTU[z] | Base mn. amplicon count[y] | $Log_2$ fold change[x] | Value of *P*[w] | Taxon level and identity using RDP classifier and UNITE fungal sequence database[v] | Additional identity notes, based on BLAST of NCBI database[u] |
|---|---|---|---|---|---|
| OTU11 | 16731 | 8.70 | 0.0010 | g__Conocybe | |
| OTU1 | 10182 | -4.06 | 0.0459 | g__Psathyrella | |
| OTU5 | 7181 | -6.23 | 0.0037 | f__Agaricaceae_unclassified | |
| OTU12 | 4193 | -7.57 | 0.0058 | g__Marasmius | |
| OTU23 | 3982 | -7.95 | 0.0042 | g__Gymnopus | |
| OTU22 | 3775 | 11.33 | 0.0000 | g__Clitopilus | |
| OTU19 | 3494 | -7.72 | 0.0027 | g__Thanatephorus | |
| OTU82 | 2034 | 6.38 | 0.0083 | f__Auriculariales_family_Incertae_sedis_ | |
| OTU15 | 1994 | -5.48 | 0.0344 | g__Lepiota | |
| OTU36 | 1850 | 10.04 | 0.0002 | g__Gilbertella | |
| OTU102 | 1683 | 6.64 | 0.0022 | o__Auriculariales_unclassified | |
| OTU21 | 1071 | -6.30 | 0.0498 | g__Conocybe | |
| OTU24 | 1062 | 4.19 | 0.0102 | f__Hypocreaceae_unclassified | |
| OTU133 | 979 | 8.19 | 0.0017 | f__Auriculariales_family_Incertae_sedis_ | |
| OTU9 | 854 | 8.08 | 0.0000 | g__Leucoagaricus | |
| OTU35 | 692 | 9.11 | 0.0000 | g__Mucor | |
| OTU61 | 459 | 6.01 | 0.0229 | c__Agaricomycetes_unclassified | |
| OTU31 | 416 | 7.47 | 0.0006 | k__Fungi_unclassified | Pythium spinosum |
| OTU32 | 324 | 11.02 | 0.0000 | g__Piriformospora | |
| OTU48 | 274 | -6.69 | 0.0079 | g__Coprinellus | |
| OTU52 | 237 | 8.96 | 0.0014 | g__Panaeolus | |
| OTU1871 | 185 | 8.61 | 0.0022 | g__Panaeolus | |
| OTU1482 | 170 | 6.14 | 0.0229 | g__Oliveonia | |
| OTU448 | 164 | 10.28 | 0.0000 | p__Ascomycota_unclassified | |
| OTU43 | 160 | 6.02 | 0.0065 | k__Fungi_unclassified | Pythium debaryanum |
| OTU62 | 121 | -6.32 | 0.0224 | g__Clitopilus | |
| OTU880 | 116 | -4.72 | 0.0459 | k__Fungi_unclassified | Uncultured fungus |
| OTU1094 | 85 | 8.67 | 0.0002 | k__Fungi_unclassified | Pythium irregulare |
| OTU165 | 80 | 9.37 | 0.0017 | g__Marasmius | |
| OTU92 | 74 | 5.57 | 0.0229 | k__Fungi_unclassified | No sig. similarity |
| OTU105 | 71 | -5.89 | 0.0030 | f__Auriculariales_family_Incertae_sedis | |
| OTU77 | 51 | 8.47 | 0.0002 | k__Fungi_unclassified | No sig. similarity |
| OTU71 | 50 | 7.65 | 0.0063 | g__Parasola | |
| OTU264 | 49 | -5.62 | 0.0133 | f__Auriculariales_family_Incertae_sedis_ | |
| OTU223 | 46 | 9.36 | 0.0014 | g__Leucocoprinus | |
| OTU332 | 46 | -4.57 | 0.0459 | f__Auriculariales_family_Incertae_sedis | |
| OTU66 | 45 | -5.25 | 0.0100 | g__Phanerochaete | |
| OTU87 | 45 | -4.45 | 0.0459 | g__Ramicandelaber | |
| OTU434 | 37 | 8.79 | 0.0037 | g__Pseudogymnoascus | |
| OTU74 | 35 | 5.76 | 0.0102 | g__unclassified_Sebacinales | |
| OTU147 | 30 | -5.07 | 0.0135 | f__Auriculariales_family_Incertae | |
| OTU123 | 30 | -5.35 | 0.0204 | k__Fungi_unclassified | Uncult soil fungus |
| OTU99 | 27 | -4.86 | 0.0461 | g__Flagelloscypha | |
| OTU581 | 19 | 7.83 | 0.0121 | g__Conocybe | |
| OTU890 | 18 | 6.22 | 0.0045 | f__Pleosporaceae_unclassified | |
| OTU130 | 18 | 8.74 | 0.0014 | c__Agaricomycetes_unclassified | |
| OTU137 | 15 | 7.43 | 0.0025 | k__Fungi_unclassified | No sig. similarity |
| OTU204 | 15 | -5.49 | 0.0371 | g__Chlorophyllum | |
| OTU129 | 14 | 5.90 | 0.0226 | k__Fungi_unclassified | No sig. similarity |
| OTU290 | 14 | 8.66 | 0.0009 | k__Fungi_unclassified | No sig. similarity |
| OTU118 | 14 | 7.81 | 0.0098 | g__Coprinus | |

| | | | | | |
|---|---|---|---|---|---|
| OTU162 | 11 | 5.01 | 0.0230 | k__Fungi_unclassified | Chlorococcum novae-angliae |
| OTU101 | 11 | 7.34 | 0.0226 | g__unclassified_Pyronemataceae | |
| OTU378 | 10 | 7.47 | 0.0064 | k__Fungi_unclassified | No sig. similarity |
| OTU213 | 9 | 6.20 | 0.0371 | k__Fungi_unclassified | Pythium sp. |
| OTU324 | 9 | 7.84 | 0.0042 | k__Fungi_unclassified | No sig. similarity |
| OTU297 | 8 | 7.82 | 0.0037 | k__Fungi_unclassified | No sig. similarity |
| OTU816 | 8 | 4.98 | 0.0159 | k__Fungi_unclassified | phage |
| OTU149 | 7 | 7.31 | 0.0125 | g__Piriformospora | |
| OTU266 | 7 | 6.64 | 0.0226 | g__Mortierella | |
| OTU59 | 7 | 7.20 | 0.0141 | k__Fungi_unclassified | Paracercomonas sp |
| OTU825 | 5 | 7.10 | 0.0100 | k__Fungi_unclassified | No sig. similarity |
| OTU386 | 5 | 7.31 | 0.0048 | k__Fungi_unclassified | |
| OTU267 | 4 | -6.42 | 0.0145 | k__Fungi_unclassified | Uncultured fungus |
| OTU347 | 4 | 6.96 | 0.0099 | k__Fungi_unclassified | No sig. similarity |
| OTU508 | 3 | 6.69 | 0.0159 | k__Fungi_unclassified | No sig. similarity |
| OTU406 | 3 | 6.48 | 0.0226 | k__Fungi_unclassified | No sig. similarity |
| OTU453 | 3 | 6.21 | 0.0318 | k__Fungi_unclassified | No sig. similarity |
| OTU1156 | 2 | 5.58 | 0.0461 | k__Fungi_unclassified | No sig. similarity |

[z] Individual numbered OTUs indicate “operational taxonomic units” that share 97% DNA sequence identity
yBase mean amplicon counts indicate mean numbers of amplicons falling within the designated OTU for all soil treatments

[x] Log2 fold change values indicate the mean change in amplicon count going from the control treatment to the preplant fumigation treatment.

[w] The value of P indicates the likelihood that the log2 fold change resulted from chance alone (values are adjusted to keep overall probability of an error to less than .05).

[v] Taxonomic identification to kingdom (k), phylum (p), class (c), order (o), family (f), and genus (g) using the RDP classifier method and the UNITE fungal sequence database.

[u] Additional taxonomic identity notes added to kingdom-level id’s, using BLAST searches on NCBI sequence database.

**Table 8.** Significant changes in abundance of operational taxonomic units (OTUs) of rDNA ITS amplicons resulting from soil fumigation, as compared to abundance of the OTUs in non-treated control, 2013 trial

| OTU | Base mean count | $Log_2$ fold change in count (from control) | Value of *P* | Taxon level and identity using RDP classifier and UNITE fungal sequence database | BLAST result using NCBI database |
|---|---|---|---|---|---|
| OTU3 | 29554 | -8.13 | 0.0001 | g__Leucocoprinus | |
| OTU7 | 17539 | -8.43 | 0.0000 | g__Leucocoprinus | |
| OTU1 | 10182 | -5.26 | 0.0088 | g__Psathyrella | |
| OTU5 | 7181 | -6.15 | 0.0042 | f__Agaricaceae_unclassified | |
| OTU12 | 4193 | -11.00 | 0.0000 | g__Marasmius | |
| OTU23 | 3982 | -9.34 | 0.0011 | g__Gymnopus | |
| OTU26 | 2630 | 5.26 | 0.0032 | g__Conocybe | |
| OTU82 | 2034 | 6.51 | 0.0078 | f__Auriculariales_family_Incertae_sedis_unclassified | |
| OTU15 | 1994 | -9.78 | 0.0001 | g__Lepiota | |
| OTU102 | 1683 | 6.54 | 0.0025 | o__Auriculariales_unclassified | |
| OTU16 | 1447 | 9.74 | 0.0012 | g__Coprinellus | |
| OTU17 | 1380 | -4.28 | 0.0275 | g__unclassified_Ascomycota | |
| OTU21 | 1071 | -7.55 | 0.0198 | g__Conocybe | |
| OTU24 | 1062 | 9.89 | 0.0000 | f__Hypocreaceae_unclassified | |
| OTU27 | 1049 | 6.14 | 0.0088 | g__Conocybe | |
| OTU133 | 979 | 6.04 | 0.0264 | f__Auriculariales_family_Incertae_sedis_unclassified | |
| OTU38 | 625 | -8.45 | 0.0000 | p__Ascomycota_unclassified | |
| OTU160 | 353 | -4.84 | 0.0190 | g__unclassified_Ascomycota | |
| OTU127 | 347 | 6.78 | 0.0016 | f__Pleosporaceae_unclassified | |
| OTU48 | 274 | -8.09 | 0.0016 | g__Coprinellus | |
| OTU4 | 268 | -6.28 | 0.0025 | g__Chlorophyllum | |
| OTU1482 | 170 | 6.98 | 0.0107 | g__Oliveonia | |
| OTU58 | 141 | -8.80 | 0.0016 | o__Agaricales_unclassified | |
| OTU520 | 139 | 4.40 | 0.0498 | g__Trematosphaeria | |
| OTU1006 | 130 | 4.58 | 0.0335 | g__Trematosphaeria | |
| OTU62 | 121 | -7.20 | 0.0098 | g__Clitopilus | |
| OTU100 | 90 | -4.44 | 0.0335 | k__Fungi_unclassified | No Significant similarity |
| OTU105 | 71 | -10.26 | 0.0000 | f__Auriculariales_family_Incertae_sedis_unclassified | |
| OTU126 | 69 | -7.36 | 0.0072 | k__Fungi_unclassified | No Significant similarity |
| OTU152 | 64 | 6.57 | 0.0261 | g__Agaricus | |
| OTU558 | 59 | 9.46 | 0.0016 | p__Ascomycota_unclassified | |
| OTU57 | 58 | -7.10 | 0.0198 | g__unclassified_Diaporthales | |
| OTU86 | 52 | -6.81 | 0.0146 | g__Tubaria | |
| OTU1848 | 49 | 4.94 | 0.0179 | g__Trematosphaeria | |
| OTU264 | 49 | -7.10 | 0.0025 | f__Auriculariales_family_Incertae_sedis_unclassified | |
| OTU332 | 46 | -6.64 | 0.0042 | f__Auriculariales_family_Incertae_sedis_unclassified | |
| OTU66 | 45 | -6.16 | 0.0030 | g__Phanerochaete | |
| OTU87 | 45 | -9.27 | 0.0001 | g__Ramicandelaber | |
| OTU175 | 42 | -7.24 | 0.0116 | c__Agaricomycetes_unclassified | |
| OTU817 | 31 | -6.92 | 0.0020 | f__Auriculariales_family_Incertae_sedis_unclassified | |
| OTU147 | 30 | -5.98 | 0.0042 | f__Auriculariales_family_Incertae_sedis_unclassified | |
| OTU643 | 28 | -5.59 | 0.0422 | c__Sordariomycetes_unclassified | |
| OTU146 | 26 | -8.26 | 0.0021 | f__Agaricaceae_unclassified | |
| OTU106 | 20 | -5.90 | 0.0422 | f__Ceratobasidiaceae_unclassified | |
| OTU167 | 16 | 6.11 | 0.0053 | g__Trichoderma | |
| OTU204 | 15 | -7.76 | 0.0042 | g__Chlorophyllum | |
| OTU138 | 14 | -7.38 | 0.0020 | k__Fungi_unclassified | No Significant similarity |
| OTU111 | 12 | -7.36 | 0.0068 | g__Thanatephorus | |
| OTU221 | 10 | -4.60 | 0.0414 | k__Fungi_unclassified | No Significant similarity |

| | | | | | |
|---|---|---|---|---|---|
| OTU387 | 8 | -6.01 | 0.0422 | k__Fungi_unclassified | No Significant similarity |
| OTU319 | 7 | 7.41 | 0.0082 | g__Trichoderma | |
| OTU199 | 5 | 6.95 | 0.0172 | k__Fungi_unclassified | No Significant similarity |
| OTU267 | 4 | -6.76 | 0.0098 | k__Fungi_unclassified | Uncultured fungus clone |
| OTU804 | 3 | 6.36 | 0.0307 | k__Fungi_unclassified | No Significant similarity |

[z] Individual numbered OTUs indicate "operational taxonomic units" that share 97% DNA sequence identity
yBase mean amplicon counts indicate mean numbers of amplicons falling within the designated OTU for all soil treatments

[x] Log2 fold change values indicate the mean change in amplicon count going from the control treatment to the preplant fumigation treatment.

[w] The value of P indicates the likelihood that the log2 fold change resulted from chance alone (values are adjusted to keep overall probability of an error to less than .05).

[v] Taxonomic identification to kingdom (k), phylum (p), class (c), order (o), family (f), and genus (g) using the RDP classifier method and the UNITE fungal sequence database.

[u] Additional taxonomic identity notes added to kingdom-level id's, using BLAST searches on NCBI sequence database.

**Table 9.** Significant changes in abundance of operational taxonomic units (OTUs) of rDNA ITS amplicons resulting from anaerobic soil fumigation, as compared to abundance of the OTUs in non-treated control, 2014 trial

| OTU | Base mean count | $Log_2$ fold change in count (from control) | Value of *P* | Taxon level and identity using RDP classifier and UNITE fungal sequence database | BLAST result using NCBI database |
|---|---|---|---|---|---|
| OTU15 | 2654 | -10.57 | 0.0000 | g__Lepiota | |
| OTU3 | 1106 | 9.16 | 0.0000 | g__Leucocoprinus | |
| OTU7 | 1048 | 9.89 | 0.0000 | g__Leucocoprinus | |
| OTU32 | 464 | 9.86 | 0.0001 | g__Piriformospora | |
| OTU40 | 403 | 10.92 | 0.0000 | g__Conocybe | |
| OTU42 | 226 | 5.79 | 0.0004 | g__Didymosphaeria | |
| OTU64 | 196 | 8.65 | 0.0011 | g__Leucocoprinus | |
| OTU44 | 143 | 5.87 | 0.0372 | g__unclassified_Ceratobasidiaceae | |
| OTU330 | 128 | 10.12 | 0.0001 | c__Agaricomycetes_unclassified | |
| OTU66 | 119 | -7.60 | 0.0131 | g__Phanerochaete | |
| OTU31 | 116 | 4.98 | 0.0123 | k__Fungi_unclassified | Pythium spinosum |
| OTU51 | 111 | 7.28 | 0.0011 | k__Fungi_unclassified | Pythium irregulare |
| OTU73 | 54 | 8.89 | 0.0003 | k__Fungi_unclassified | No significant similarity |
| OTU118 | 50 | 9.93 | 0.0006 | g__Coprinus | |
| OTU43 | 48 | 4.70 | 0.0131 | k__Fungi_unclassified | Pythium debaryanum |
| OTU1895 | 47 | 9.41 | 0.0017 | g__Leucoagaricus | |
| OTU592 | 46 | -4.63 | 0.0334 | g__Mortierella | |
| OTU643 | 41 | 6.38 | 0.0002 | c__Sordariomycetes_unclassified | |
| OTU144 | 35 | 6.04 | 0.0123 | g__unclassified_Lycoperdaceae | |
| OTU1848 | 34 | 8.68 | 0.0001 | g__Trematosphaeria | |
| OTU129 | 34 | 8.11 | 0.0002 | k__Fungi_unclassified | No significant similarity |
| OTU41 | 34 | 6.05 | 0.0087 | c__Agaricomycetes_unclassified | |
| OTU694 | 33 | 7.18 | 0.0001 | g__Arnium | |
| OTU520 | 31 | 7.81 | 0.0001 | g__Trematosphaeria | |
| OTU92 | 30 | 8.34 | 0.0011 | k__Fungi_unclassified | No significant similarity |
| OTU85 | 26 | -6.20 | 0.0216 | g__Coprinus | |
| OTU52 | 21 | 7.46 | 0.0110 | g__Panaeolus | |
| OTU104 | 21 | -7.68 | 0.0002 | c__Zygomycota_unclassified | |
| OTU1871 | 18 | 6.44 | 0.0256 | g__Panaeolus | |
| OTU1006 | 18 | 6.32 | 0.0011 | g__Trematosphaeria | |
| OTU169 | 17 | 4.88 | 0.0242 | g__Actinomucor | |
| OTU116 | 15 | -8.66 | 0.0004 | g__Ramicandelaber | |
| OTU77 | 13 | 7.73 | 0.0011 | k__Fungi_unclassified | No significant similarity |
| OTU100 | 13 | -4.59 | 0.0090 | k__Fungi_unclassified | No significant similarity |
| OTU229 | 12 | 8.41 | 0.0048 | k__Fungi_unclassified | No significant similarity |
| OTU1703 | 11 | 7.66 | 0.0183 | c__Sordariomycetes_unclassified | |
| OTU230 | 11 | 8.34 | 0.0048 | k__Fungi_unclassified | No significant similarity |
| OTU240 | 9 | 8.17 | 0.0063 | k__Fungi_unclassified | No significant similarity |
| OTU296 | 9 | 6.12 | 0.0256 | k__Fungi_unclassified | No significant similarity |
| OTU291 | 9 | 8.47 | 0.0018 | k__Fungi_unclassified | No significant similarity |
| OTU137 | 8 | 5.55 | 0.0088 | k__Fungi_unclassified | No significant similarity |
| OTU295 | 8 | 5.63 | 0.0242 | g__unclassified_Lycoperdaceae | |
| OTU327 | 8 | 7.26 | 0.0239 | k__Fungi_unclassified | No significant similarity |
| OTU1094 | 7 | 6.24 | 0.0028 | k__Fungi_unclassified | Pythium irregulare |

| | | | | | |
|---|---|---|---|---|---|
| OTU496 | 7 | -7.13 | 0.0162 | c__Agaricomycetes_unclassified | |
| OTU20 | 7 | 7.21 | 0.0242 | f__Lyophyllaceae_unclassified | |
| OTU74 | 7 | 8.03 | 0.0011 | g__unclassified_Sebacinales | |
| OTU123 | 6 | -5.75 | 0.0131 | k__Fungi_unclassified | Uncultured soil fungus |
| OTU448 | 6 | 6.43 | 0.0117 | p__Ascomycota_unclassified | |
| OTU324 | 5 | 8.17 | 0.0011 | k__Fungi_unclassified | No significant similarity |
| OTU386 | 5 | 5.13 | 0.0162 | k__Fungi_unclassified | No significant similarity |
| OTU1273 | 5 | -6.96 | 0.0212 | c__Agaricomycetes_unclassified | |
| OTU280 | 4 | 4.80 | 0.0216 | k__Fungi_unclassified | No significant similarity |
| OTU384 | 4 | 5.31 | 0.0491 | k__Fungi_unclassified | No significant similarity |
| OTU87 | 4 | -6.42 | 0.0362 | g__Ramicandelaber | |
| OTU648 | 4 | -6.82 | 0.0074 | k__Fungi_unclassified | No significant similarity |
| OTU249 | 4 | -6.42 | 0.0216 | k__Fungi_unclassified | No significant similarity |
| OTU34 | 4 | -6.81 | 0.0154 | o__Sebacinales_unclassified | |
| OTU130 | 3 | 6.95 | 0.0154 | c__Agaricomycetes_unclassified | |
| OTU162 | 3 | 5.79 | 0.0114 | k__Fungi_unclassified | Chlorococcum novae-angliae |
| OTU57 | 2 | 6.64 | 0.0216 | g__unclassified_Diaporthales | |
| OTU389 | 2 | 6.72 | 0.0123 | k__Fungi_unclassified | Uncultured soil fungus clone |
| OTU508 | 2 | 6.62 | 0.0216 | k__Fungi_unclassified | No significant similarity |
| OTU434 | 2 | 6.38 | 0.0226 | g__Pseudogymnoascus | |
| OTU221 | 1 | 6.12 | 0.0404 | k__Fungi_unclassified | No significant similarity |
| OTU825 | 1 | 6.33 | 0.0216 | k__Fungi_unclassified | No significant similarity |
| OTU1255 | 1 | 6.30 | 0.0216 | g__Trematosphaeria | |
| OTU510 | 1 | -5.32 | 0.0346 | k__Fungi_unclassified | No significant similarity |
| OTU665 | 1 | -5.50 | 0.0363 | k__Fungi_unclassified | No significant similarity |
| OTU424 | 1 | 5.95 | 0.0363 | k__Fungi_unclassified | No significant similarity |
| OTU533 | 1 | 5.75 | 0.0456 | k__Fungi_unclassified | No significant similarity |
| OTU1120 | 1 | 5.79 | 0.0362 | g__Conlarium | |

[z] Individual numbered OTUs indicate “operational taxonomic units” that share 97% DNA sequence identity yBase mean amplicon counts indicate mean numbers of amplicons falling within the designated OTU for all soil treatments

[x] Log2 fold change values indicate the mean change in amplicon count going from the control treatment to the preplant fumigation treatment.

[w] The value of P indicates the likelihood that the log2 fold change resulted from chance alone (values are adjusted to keep overall probability of an error to less than .05).

[v] Taxonomic identification to kingdom (k), phylum (p), class (c), order (o), family (f), and genus (g) using the RDP classifier method and the UNITE fungal sequence database.

[u] Additional taxonomic identity notes added to kingdom-level id’s, using BLAST searches on NCBI sequence database.

**Table 10.** Significant changes in abundance of operational taxonomic units (OTUs) of rDNA ITS amplicons resulting from preplant soil fumigation, as compared to abundance of the OTUs in non-treated control, 2014 trial

| OUT | Base mean count | $Log_2$ fold change in count (from control) | Value of *P* | Taxon level and identity using RDP classifier and UNITE fungal sequence database | BLAST result using NCBI database |
|---|---|---|---|---|---|
| OTU4 | 15157 | -8.69 | 0.0002 | g__Chlorophyllum | |
| OTU9 | 7505 | -8.56 | 0.0002 | g__Leucoagaricus | |
| OTU26 | 3662 | 10.38 | 0.0000 | g__Conocybe | |
| OTU15 | 2654 | -10.04 | 0.0001 | g__Lepiota | |
| OTU28 | 1030 | 7.03 | 0.0122 | g__Conocybe | |
| OTU82 | 235 | 7.87 | 0.0015 | f__Auriculariales_family_unclassified | |
| OTU102 | 176 | 6.85 | 0.0019 | o__Auriculariales_unclassified | |
| OTU66 | 119 | -8.97 | 0.0062 | g__Phanerochaete | |
| OTU133 | 88 | 6.81 | 0.0085 | f__Auriculariales_family_unclassified | |
| OTU19 | 59 | 4.85 | 0.0254 | g__Thanatephorus | |
| OTU1482 | 44 | 10.02 | 0.0002 | g__Oliveonia | |
| OTU111 | 38 | 6.69 | 0.0085 | g__Thanatephorus | |
| OTU85 | 26 | -7.19 | 0.0180 | g__Coprinus | |
| OTU104 | 21 | -8.00 | 0.0003 | c__Zygomycota_class unclassified | |
| OTU107 | 20 | -8.11 | 0.0055 | f__Agaricaceae_unclassified | |
| OTU148 | 8 | -5.67 | 0.0187 | p__Ascomycota_unclassified | |
| OTU6 | 2 | 6.29 | 0.0357 | k__Fungi_unclassified | Phytophthora cactorum |

[z] Individual numbered OTUs indicate "operational taxonomic units" that share 97% DNA sequence identity

[y] Base mean amplicon counts indicate mean numbers of amplicons falling within the designated OTU for all soil treatments

[x] Log2 fold change values indicate the mean change in amplicon count going from the control treatment to the preplant fumigation treatment.

[w] The value of P indicates the likelihood that the log2 fold change resulted from chance alone (values are adjusted to keep overall probability of an error to less than .05).

[v] Taxonomic identification to kingdom (k), phylum (p), class (c), order (o), family (f), and genus (g) using the RDP classifier method and the UNITE fungal sequence database.

[u] Additional taxonomic identity notes added to kingdom-level id's, using BLAST searches on NCBI sequence database.

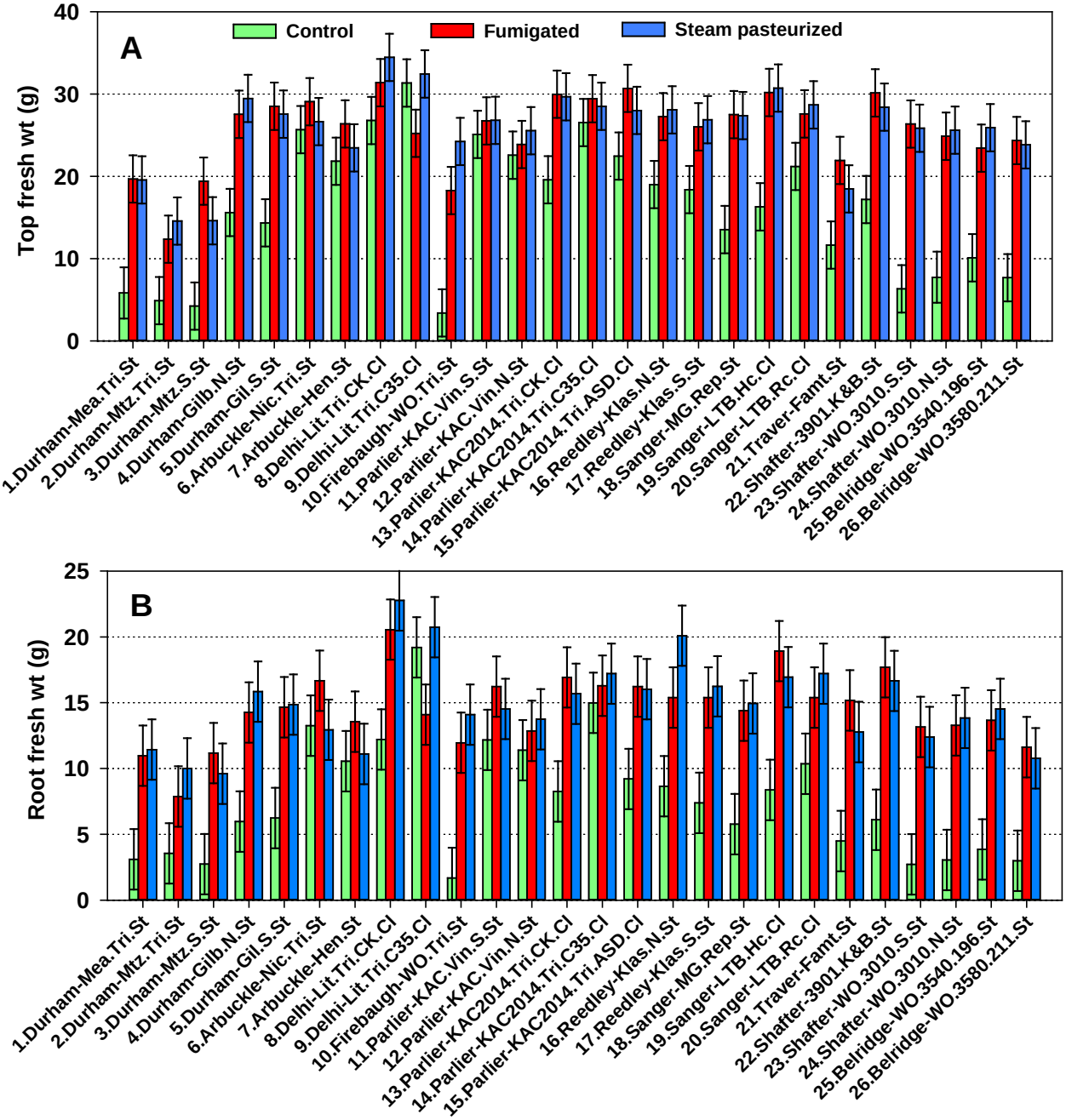


**Figure 1.** Response of Nemaguard rootstock seedlings to preplant soil treatments in 2015 greenhouse bioassay. **A**, plant top fresh weights and **B**, root fresh weights. The soils were collected in spring 2015, mixed with course sand (2:1, soil:sand, v:v) before the preplant treatments (control, fumigation with chloropicrin, and pasteurization with steam) were applied in buckets. The treated soils were distributed to 1-liter plots and planted with sprouted Nemaguard peach seedlings on 15 July, and plant top fresh weights were measured 21-29 September 2015.

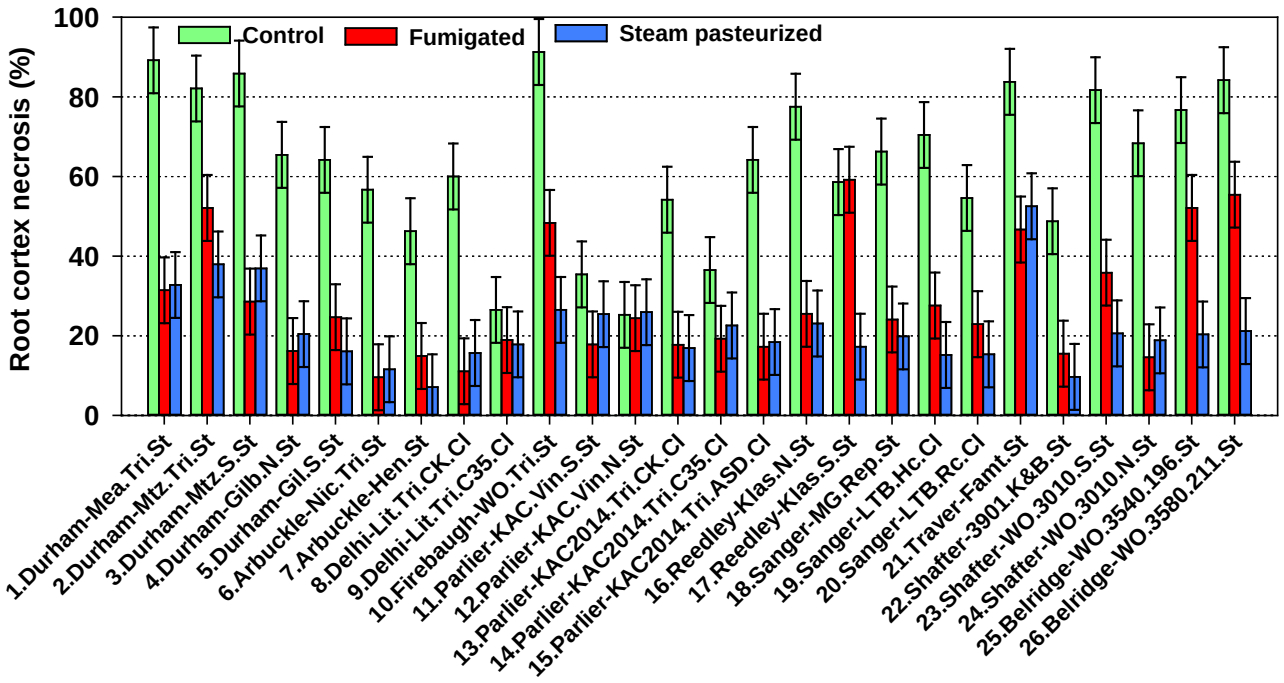


**Figure 2.** Response of root fresh weight of Nemaguard rootstock seedlings to preplant soil treatments in 2015 greenhouse bioassay. The soils were collected in spring 2015, mixed with course sand (2:1, soil:sand, v:v) before the preplant treatments (control, fumigation with chloropicrin, and pasteurization with steam) were applied in buckets. The treated soils were distributed to 1-liter plots and planted with sprouted Nemaguard peach seedlings on 15 July, and root cortex necrosis was estimated visually 21-29 September 2015.

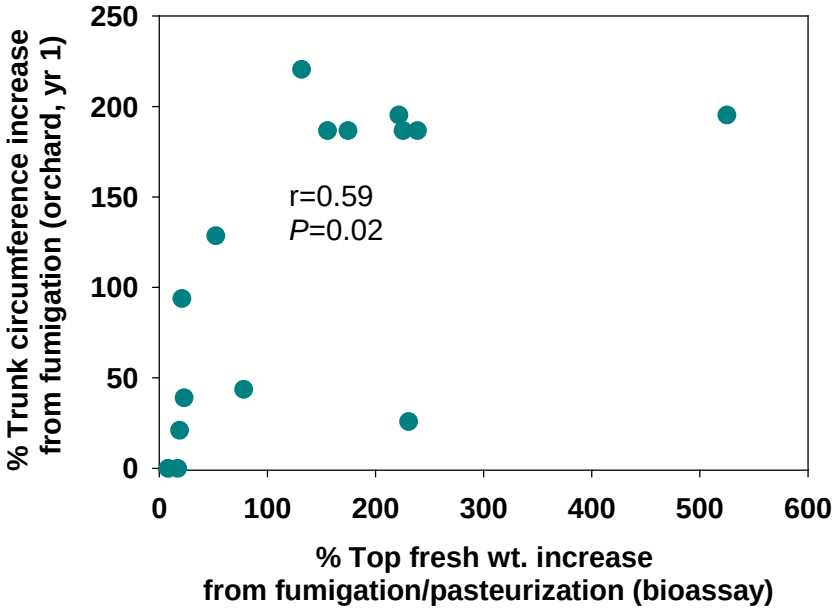


**Figure 3.** Correlation of orchard growth responses to preplant soil fumigation in orchard replant trials and corresponding average plant responses to preplant soil fumigation and pasteurization in greenhouse plant bioassays. Based on data from 11 orchard replant trials conducted from 2006 to 2016 and data from two greenhouse bioassay trials conducted in 2014 and 2015.

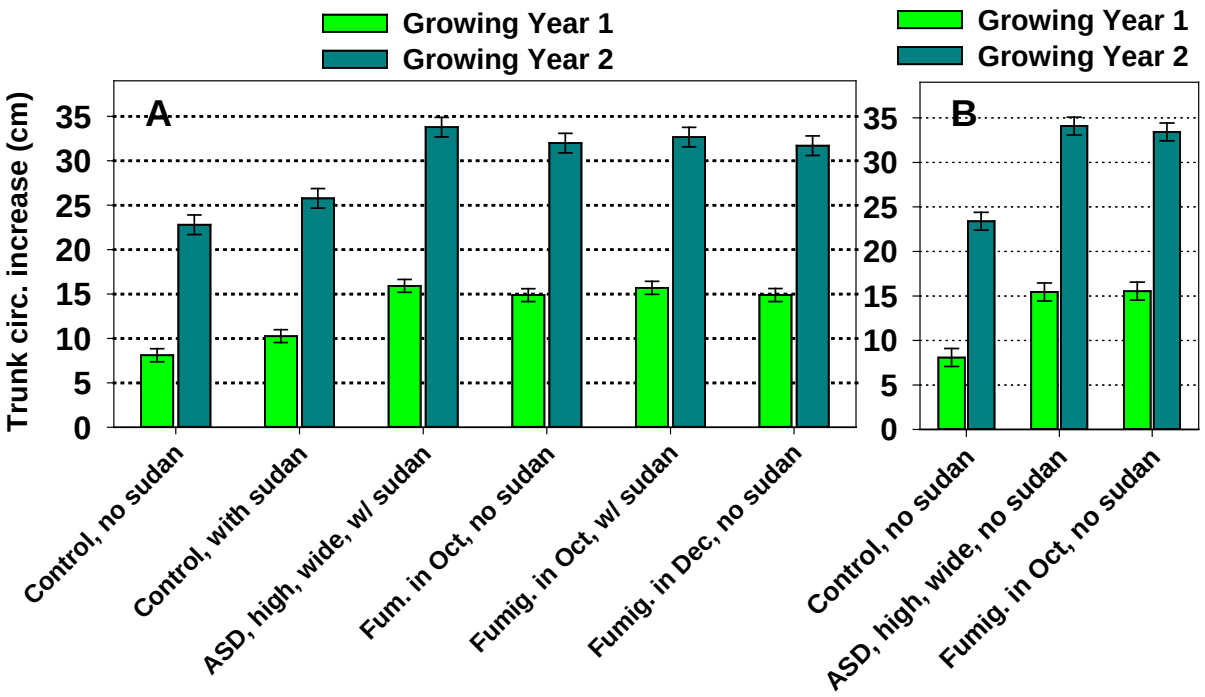


**Figure 4.** Responses of replanted almond orchard growth to preplant treatments with ASD and soil fumigation. **A**, experiment 1, and **B**, experiment 2. Treatment details are given in **Table 2**.

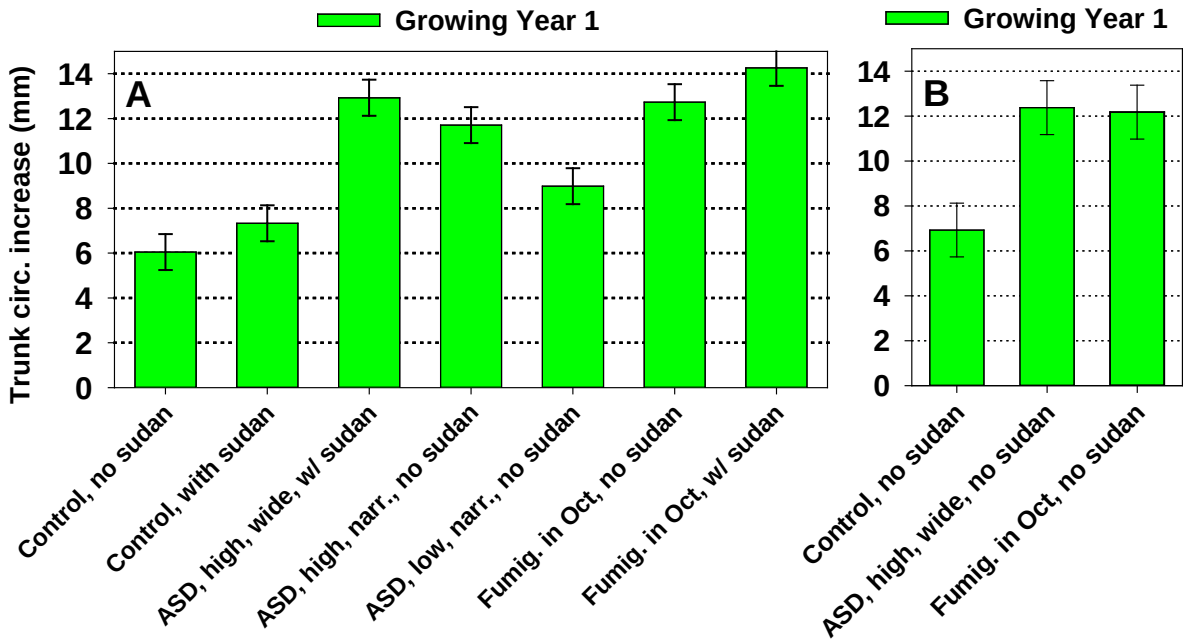


**Figure 5.** Responses of replanted almond orchard growth to preplant treatments with ASD and soil fumigation. **A**, experiment 3, and **B**, experiment 4. Treatment details are given in **Table 2**.

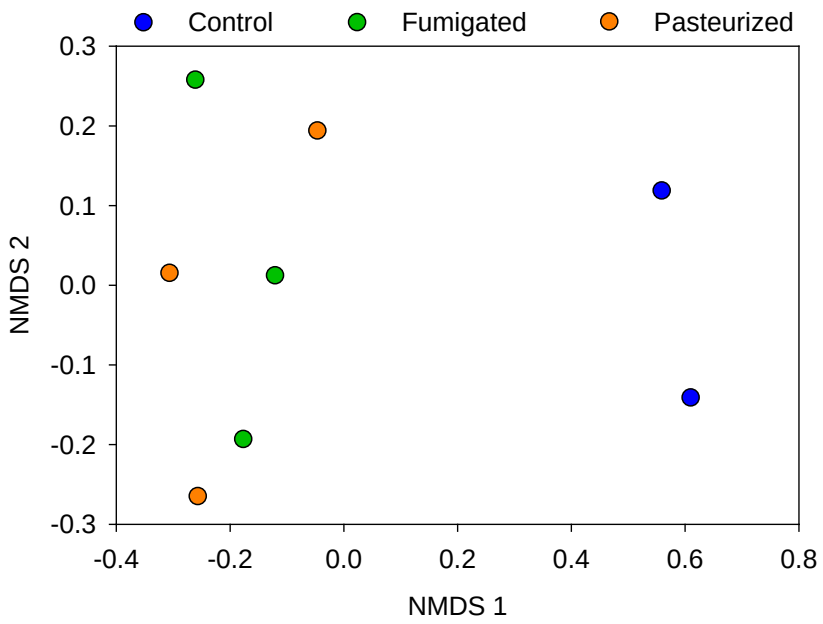


**Figure 6.** Nonmetric multidimensional scaling ordination (NMDS) of root fungal/oomycete community differences among samples and treatments from soil 1 in the greenhouse bioassay.

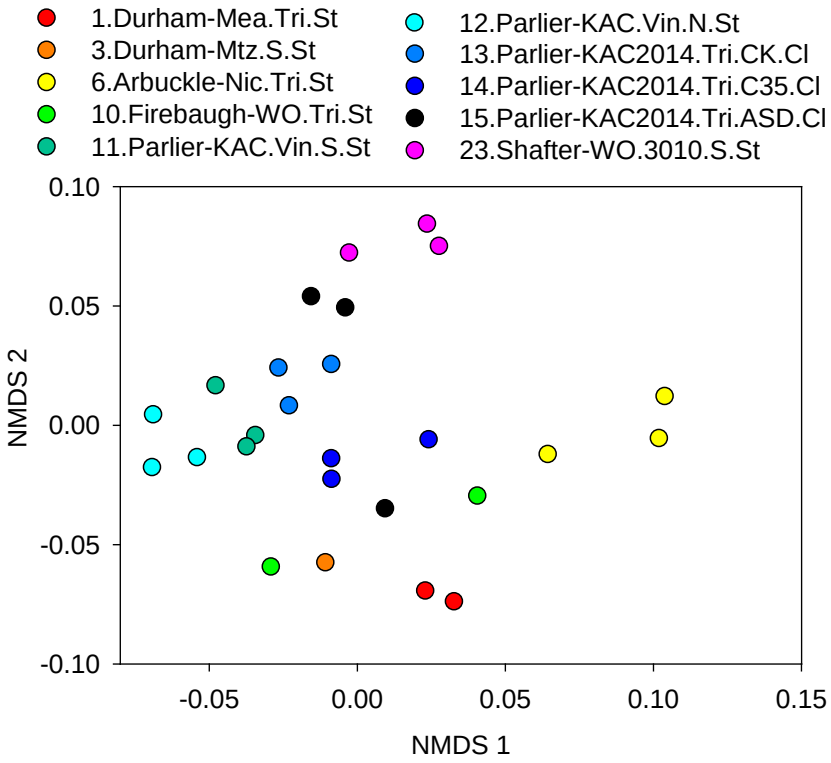


**Figure 7.** NMDS of root fungal/oomycete community differences among samples and treatments from roots from the non-treated treatment of the greenhouse bioassay.

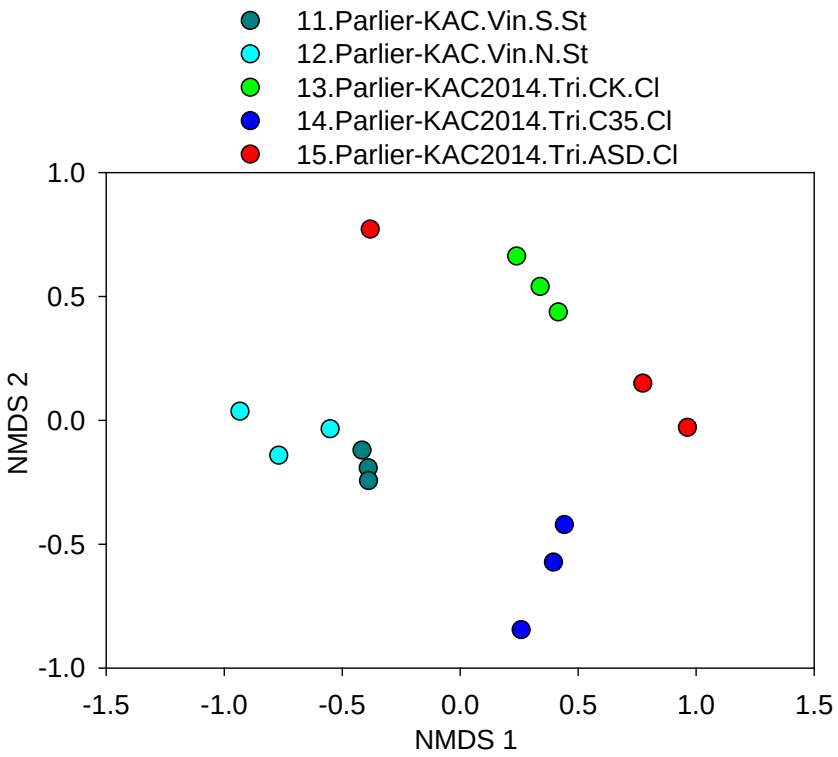


**Figure 8.** NMDS of root fungal/oomycete community differences among samples and treatments from roots from the non-treated treatment of the greenhouse bioassay, with only samples from KAC / Parlier area included.

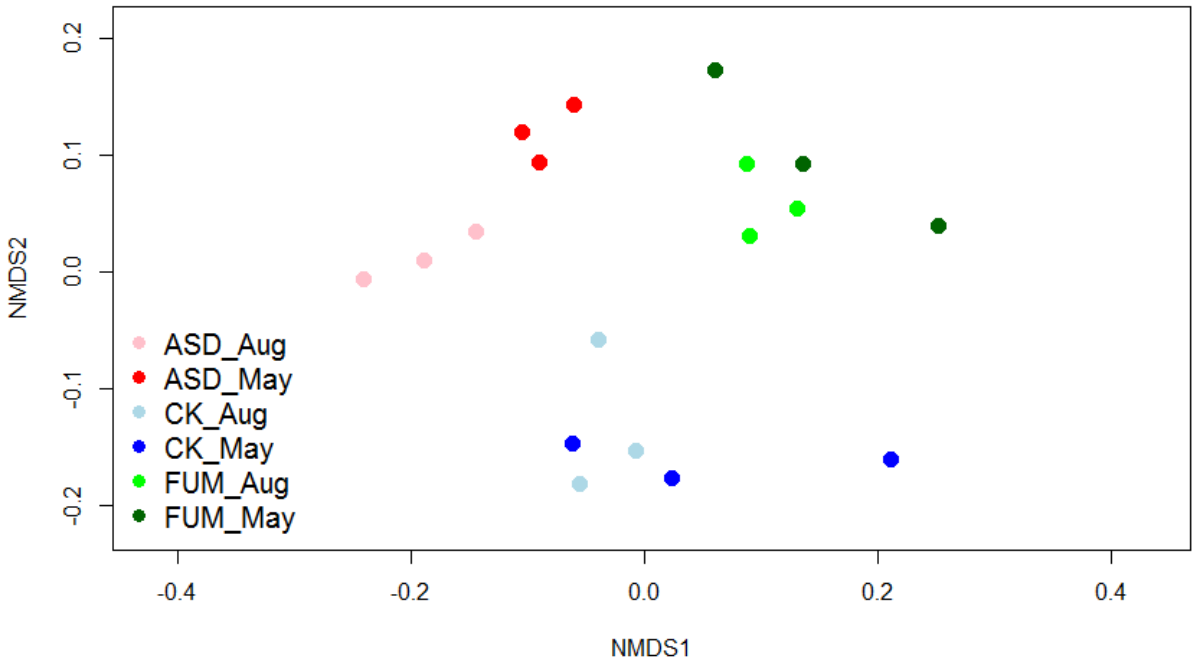


**Figure 9.** NMDS of root fungal/oomycete community differences among samples and treatments from roots from the 2013 ASD trial.

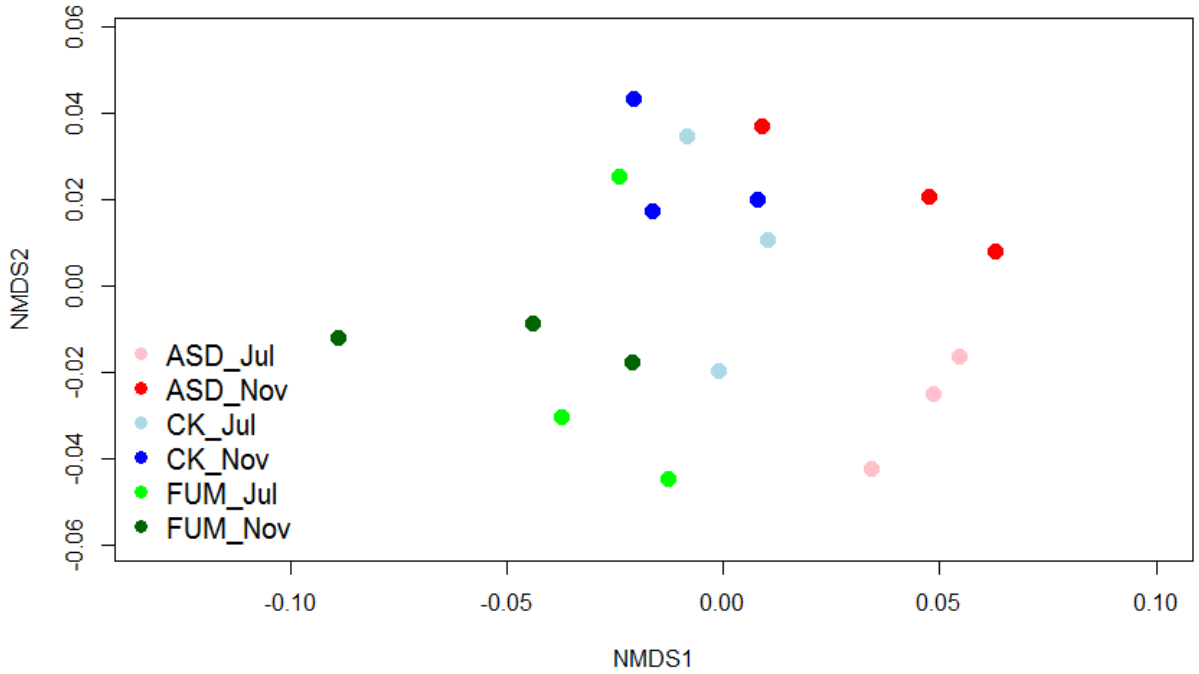


**Figure 10.** NMDS of root fungal/oomycete community differences among samples and treatments from roots from the 2014 ASD trial.

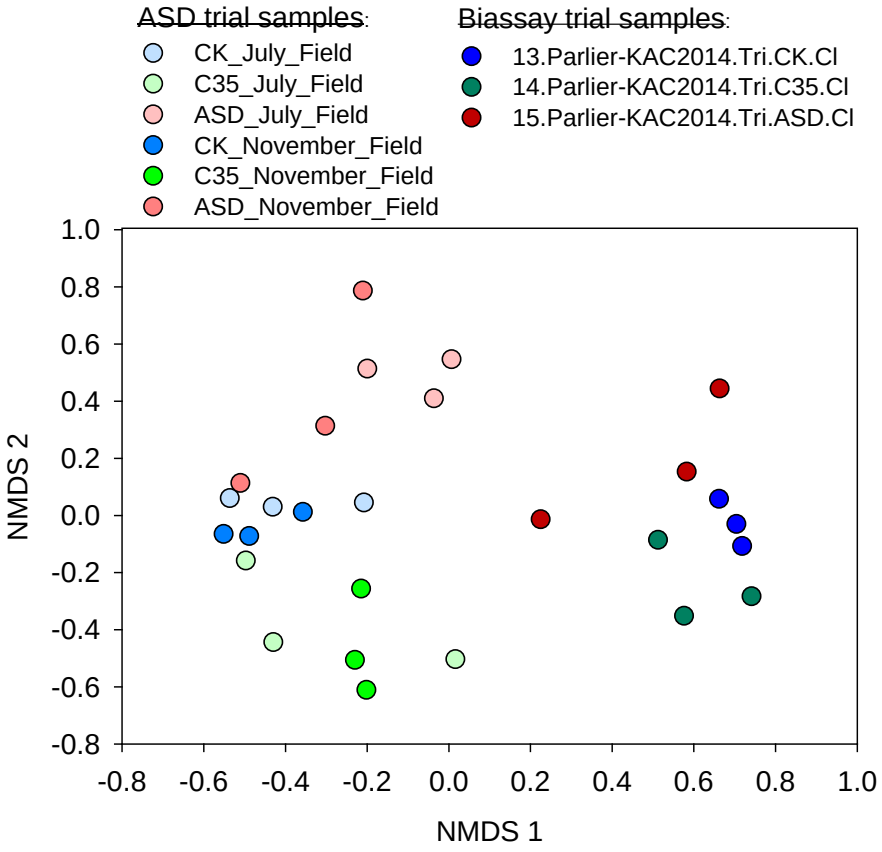


**Figure 11.** NMDS of root fungal/oomycete community differences among samples and treatments from roots from the 2014 ASD trial.