Project Title: Directing plant-microbe relations toward resiliency post-fumigation

Report Type: Final Project Report

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Item	2021-2022	2022-2023
Salaries*	NA	NA
Benefits	NA	NA
Wages*	18,359	18,359
Benefits	6,922	6,922
Sequencing Costs	12,800	4000
Lab Supplies	19,125	10,500
Soil Analysis Tests	2,974	NA
Travel	NA	NA
Miscellaneous	NA	NA
Plot Fees	NA	NA
Total	60,180	39,781

*Biological technician with benefits (0.5 FTE as needed, to be hired at beginning of grant)

Brief Overview of Project Goals:

As a replant disease control strategy, pre-plant soil fumigation is the industry standard. Although fumigation significantly reduces pathogen activity and improves tree growth, this benefit is limited to approximately 1 year. Post-fumigation, orchard soil rapidly re-establishes a microbial community indistinguishable from that found in the corresponding non-fumigated replant soil and the proliferation of soilborne pathogens infecting apple, including *Pratylenchus* and *Pythium* spp., is commonly observed (i.e., a chronic disease state). The application of soil amendments following fumigation may be an opportune time to improve the ability of the soil to defend against pathogen reinvasion and improve orchard productivity for an extended period.

The primary objective of this study was to evaluate select soil amendments (listed in Table 1) for the ability to recruit and maintain rhizosphere microbiomes in fumigated soil, that are suppressive to pathogen re-invasion. In addition, this study evaluated the capacity of selected soil amendments to improve other characteristics of soil productivity (nutrient availability, water holding capacity, etc.).

Table 1. Application rates of organic amendments used

Post-fumigation Soil Amendment	Application Rate	Notes
Fumigated alone (block 14b)	NA	Control to "steer" away from
Unfumigated alone (block 12b)	NA	Control to "steer" away from
Unfumigated + 2t Bj/Sa SM	2 tons per acre	Control to "steer" towards
Fum + 1t Bj/Sa SM	1 ton per acre	
Fum + 2t Bj/Sa SM	2 tons per acre	
Fum + CCM (composted chicken manure)	0.7 tons per acre	Rate estimated based on E.C. value
Fum + SMC (shitake mushroom compost)	2% v/v	
Fum + LC (liquid chitin)	2 gal per acre	Rate recommended by manufacturer
Fum + IF (insect frass) *	1.5 cups per ft^{-3} soil	Rate recommended by manufacturer
Fum + 2t *B.napus* *	2 tons per acre	

*Amendment not included in original proposal

Collection of soil: WSU Sunrise Orchard block 14b was fumigated on April 01, 2021 (pers comm Cameron Burt). The old orchard block, previously planted to apple, had been removed in 2017. Telone II (1,3-dichloropropene) was applied at a rate of 122 lbs. per acre; injected at 18 inches depth. Sectagon K-54 (metam potassium) was also applied at a rate of 318 lbs. per acre; injected into the top 6 inches. Fumigated soil was collected on April 26th, 2021 (orchard manager advised a 2-3 week waiting period). Approximately 90 gallons of fumigated soil was removed from the plot by shoveling soil from the top 12" into 5 gallon buckets, which were placed throughout the plot area at regular intervals. Buckets and shovels were disinfected with 10% bleach prior to use to minimize cross-contamination. In addition, approximately 30 gallons of unfumigated replant soil was collected from a nearby block containing apple trees (SRO block 12b). The top stubble within tree rows was removed using a shovel; soil was then *carefully* collected from the top 12", so as not to damage existing tree roots. Lids were placed on all buckets to minimize moisture loss and soil was transported back to the lab. Soil was then stored in 30 gallon bins with lids in a cool, dry place until use.

Soil Amendments: Seed meal formulations (*B.juncea* + *S.alba* and *B.napus*) were ground and passed through a 1 mm^2 sieve, prior to addition to fumigated or unfumigated SR orchard soil according to the application rates listed in Table 1. The *B.juncea* + *S.alba* SM formulation was prepared by blending *B. juncea* and *S. alba* at a ratio of 1:1. Pre-weighed packets of seed meal were added and thoroughly incorporated into the soil by hand. 2.5L of seed meal-amended soil was placed into each pot, moistened with 300 ml autoclaved water and sealed in gas impermeable bags (Bitran) to retain seed meal-generated volatile compounds (e.g. allyl isothiocyanate). The bags were removed after 1 week, and pots were

maintained in the greenhouse for an additional 6-weeks prior to planting to allow for degradation of potentially phytotoxic compounds. All other soil amendments were thoroughly mixed into the fumigated soil 2-weeks prior to planting, according to the application rates listed in Table 1. The amount of material added to each pot was calculated according to an "applied" volume of 6340 cubic feet of soil per acre, based on a high density orchard system with a layout of 12' between tree rows, 3.5 ft wide weed-free strips, and tilling 6" deep. The amendment rate for the composted chicken manure treatment was determined based on a soil EC threshold value of 1.6 mmho/cm (DuPont and Granatstein, 2020). Fruit trees are relatively salt sensitive, suffering decreased growth and yield when EC values in the root zone > 2 mmhos/cm (https://www.bctfpg.ca/horticulture/fruit-tree-nutrition).

Planting/harvest: G.11 rootstocks were used in Experiment 1/Year 1 and Experiment 2/Year 2. Prior to planting, root volume, trunk diameter (16-18 cm above soil line), and total biomass were recorded. For each treatment type (including fumigated, unfumigated, and unfumigated + seed meal controls), there were 7 replicate pots. Pots were set up in a completely randomized block design in the greenhouse and maintained under standard light and temperature regimes (Somera et al., 2021). At the end of each experiment (3 months post planting), the effect of soil treatments on rootstock growth was assessed by measuring increases in trunk diameter, total rootstock weight, root mass, and leader-shoot length. Upon harvest of Experiment 1/Year 1, a variety of chemical and physical properties were measured to assess the influence of the above soil amendments on overall soil health. The measured properties are listed in Table 2.

Significant Findings:

- High nitrate levels in composted chicken manure and liquid chitin led to high salinity as measured by electrical conductivity (EC) when incorporated into fumigated soil. Most notably, composted chicken manure, when used as a post-fumigation soil amendment (even when applied at a relatively low rate, 2 week prior to planting), resulted in the death of all trees.

- Seed meal, shitake mushroom compost (SMC), and insect frass (IF) soil amendments all altered the chemical and physical properties of fumigated replant soil in similar ways including increased water holding capacity, increased pH and increased C:N ratio.

- All amendments, with the exception of insect frass and BjSa SM (1t), were relatively successful in their ability to significantly alter the ***bacterial*** composition of the rhizosphere microbiome and "steer" the community in a positive direction post-fumigation. *B. napus* SM (2t) did not, however, counteract the adverse effects of fumigation on the bacterial rhizobiome as effectively as SMC, LC, or BjSa SM (2t).

- All amendments, with the exception of BjSa SM (1t), successfully altered ***fungal*** community composition and directed the community in a positive manner post-fumigation. In all treatments, a handful of potentially beneficial fungi with activity against specific apple replant disease (ARD) pathogens were significantly enriched relative to the fumigated control. Notably, BjSa SM (2t) increased the potential for fungal-based nematode control in the apple rhizosphere post-fumigation.

- The Fum + LC treatment resulted in ***bacterial*** communities with a high degree of degradative/bioremediation potential. However, liquid chitin was less effective than other treatments at stimulating potentially beneficial ***fungi***.

- Insect frass did *not* effectively shift the ***bacterial*** community away from fumigation-alone or replant control treatments (i.e., chronic disease states). However, with respect to changes in the ***fungal*** community, this treatment moved the community closest to that of the "target" treatment (which is *unfumigated* orchard replant soil amended with BjSa SM at a rate of 2 t per acre).

- Insect frass resulted in a significant increase in trunk diameter relative to the fumigated control.

Results and Discussion:

Experiment 1/Year 1

Table 2. Effect of the different soil amendments on the chemical and physical properties of fumigated replant soil. These metrics are for bulk soil collected 3 months post planting.

	pH	Electrical Conductivity	Cation Exchange Capacity	Na	Ca	Mg	K	Water Holding Capacity	OM	Total N	Total C	C:N	NO_3	NH_4	SO_4^{2-}	P	K	B	Zn	Mn	Cu	Fe
Experimental Treatment		*mmhos/cm*	*meq/100g #*	*percent (%) of CEC*				*in/ft*	*percent (%)*			*ratio*	*mg/kg*									
Fumigated alone control	**6**	**0.3**	**9.1**	**1.3**	**57**	**16**	**11**	**1.28**	**1.4**	**0.08**	**0.65**	**8.7**	**7.9**	**1.1**	**28**	**16**	**393**	**0.2**	**6**	**1.7**	**0.6**	**24**
Fum + 1t Bj/Sa SM	7	0.45	8	2.7	72	22	17	1.4	1.4	0.10	0.89	9.3	35	14	38	40	531	0.2	7.1	2.2	1.1	14
Fum + 2t Bj/Sa SM	6.6	0.74	7.8	4.9	78	26	23	1.95	1.5	0.11	1.12	10.2	70	14	55	40	706	0.3	7	3.7	1.4	17
Fum + CCM (composted chicken manure)	7.8	3.29	9.5	30	84	40	88	1.46	2.6	0.23	1.96	8.7	277	267	271	157	3262	3.4	25	30	15	43
Fum + SMC (shitake mushroom compost)	7.5	0.36	8.5	1.2	72	21	14	1.41	1.9	0.09	0.89	9.7	2.5	4.5	16	23	471	0.2	5.9	1.8	0.5	13
Fum + LC (liquid chitin)	5.2	1.56	9.3	2.7	80	21	13	1.57	1.9	0.12	0.97	8.3	148	8.5	168	44	474	0.2	7.8	22	1	28
Fum + IF (insect frass) *	7.1	0.05	8.7	1.2	66	21	14	1.5	1.6	0.08	0.78	9.3	16	1.5	23	40	489	0.2	6.9	1.2	0.6	13
Fum + 2t *B.napus* *	6.6	0.53	8.2	1.5	71	21	15	1.38	1.3	0.08	0.79	10.1	80	1.4	32	21	477	0.3	6.6	2.6	0.7	27

All analyses were conducted by Soiltest Farm Consultants (Moses Lake, WA).
*Additional treatments not included in original proposal
millequivalents per 100 grams of soil

Effect of amendments on chemical and physical properties of fumigated orchard soil:

Shitake mushroom compost (SMC): The results of the compost-specific analyses indicate that, although neither material is fully composted, "fresh" SMC is more stable and mature than composted chicken manure. Therefore, SMC is likely to benefit soil health by building soil organic matter.

Composted chicken manure (CCM): CCM was moderately alkaline (pH = 8.5), which is typical of manure-based composts. In general, high pH compost should be avoided on soils which are already above the optimum pH for tree fruit (optimum pH = 6-6.5) (Dupont and Granatstein, 2020). The Fum + CCM treatment resulted in an electrical conductivity (EC) value of 3.3 mmho/cm, which exceeds the damage threshold for apple/pear (1.7 mmho/cm). Nitrates (277 mg/kg) made up most of the soluble salts in the EC reading (Table 2). In this experiment, the high EC/nitrates and pH of CCM clearly had a negative impact on plant growth as none of the trees survived. Use of this material as a post-fumigation amendment is likely to negatively affect plant root growth even if the amendment rate is considerably low (as in this experiment).

Test results also point to the potential for carbon and nitrogen loss. Relatively high rates of CO_2 evolution (6.3 CO_2-C/g OM/day) during compost stability testing suggest that a portion of the organic carbon in CCM is being lost as CO_2 gas due to microbial respiration. Moreover, because CCM is moderately alkaline (pH > 7.5), a greater proportion of NH_4 may be exuded in the form of NH_3 gas (i.e., ammonia) leading to a loss of N to the atmosphere (Sullivan et al., 2018). Further, a low C:N ratio (10:1) also indicates the potential for N leaching/loss (although the product is marketed as a "slow nitrogen release plant food").

Liquid Chitin (LC): Similar to Fum + CCM, Fum + LC also had high nitrate/EC values. Although the trees were able to tolerate the extremely high nitrate concentrations (148 mg/kg) resulting from the LC amendment, this treatment would be expected to lead to production of nitrous oxide (N_2O) from microbial denitrification. Nitrous oxide is a greenhouse gas which is approximately 300 times more potent than CO_2. Unlike CCM, LC amendments resulted in a strongly acidic soil (pH = 5.2).

Fum + BjSa (2t): This treatment resulted in the greatest increase in soil C:N ratio and water holding capacity (the amount of water that a given soil can physically hold against the force of gravity), with the lowest cation exchange capacity (CEC) value. That is significant because CEC values refer to the relative ability of a soil to store exchangeable cations (many of which are essential nutrients) and buffer against rapid changes in pH. The most dominant soil cations were Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) in all treatments except Fum + CCM (Table 2).

Amendment-based changes to rhizosphere microbial community composition:

The aim of Experiment 1/Year 1 was to identify materials which could be used to "steer" the apple rhizosphere in favor of a more prophylactic or disease-suppressive state, post-fumigation. **Figure 1** shows how the soil amendments (represented by different colors) altered ***bacterial*** community composition in apple rhizospheres. Assessment of bacterial community sequence data indicated that all those except Insect frass and BjSa SM (1t) were relatively successful in terms of their ability to significantly alter the bacterial composition of the rhizosphere and "steer" the community in a positive direction. **Figure 2** shows how the soil amendments (represented by different colors) altered ***fungal*** community composition in apple rhizospheres. Assessment of fungal community sequence data indicated all those except BjSa SM (1t) successfully altered rhizobiome composition relative to the fumigated control and directed the community in a positive direction post-fumigation.

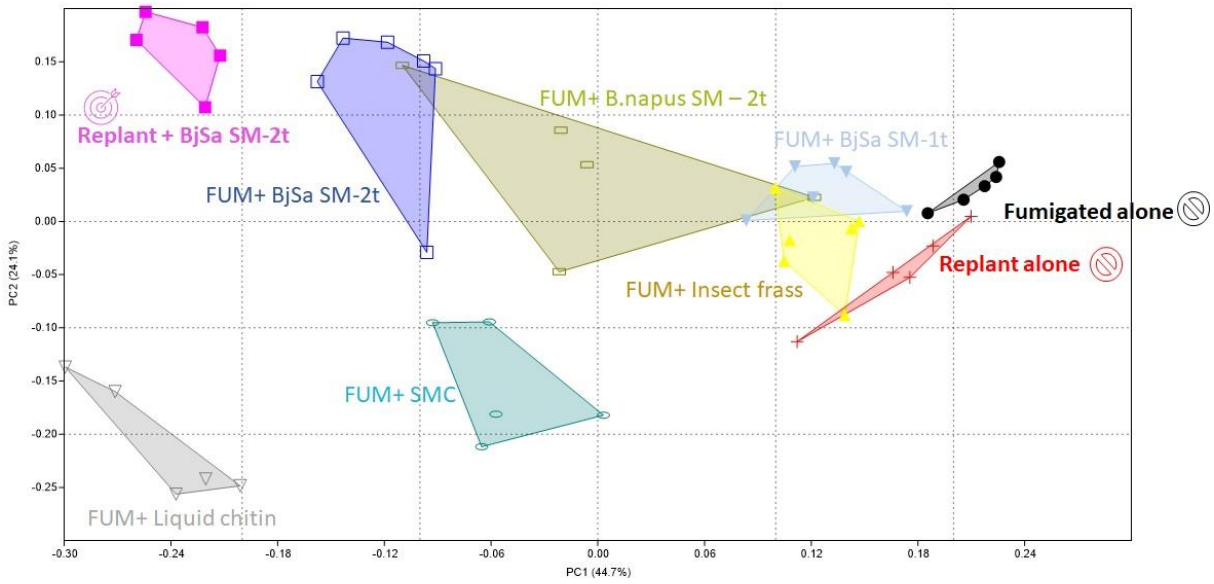


Figure 1. Principal coordinates analysis of bacterial community composition in G.11 apple rhizospheres 4-weeks post-planting at the Order level. Each point represents bacterial community

sequence data generated from an individual soil sample. Filled shapes represent the clustering of replicate samples associated with plants cultivated in the different treatments as labeled.

Bacterial communities associated with plants cultivated in **unfumigated orchard replant soil** (See: Replant alone) and those of plants cultivated in **fumigated orchard replant soil** (See: Fumigated alone) represent community configurations which are conducive to future development of replant disease (Fig. 1). In other words, Replant alone and Fumigated alone represent treatments which are susceptible to infection by root pathogens including those that can incite replant disease and limit productivity of the current orchard. By comparison, the filled shape located in the upper left region of the plot, represents rhizosphere samples from plants cultivated in unfumigated orchard replant soil amended with BjSa SM at a rate of 2t per acre. A large body of research has shown that this particular SM formulation promotes disease-suppressive rhizosphere communities (Mazzola, et al., 2009.; Mazzola, et al., 2015., Wang and Mazzola, 2019.; Somera et AL., 2021). Therefore, this treatment represents a good direction to move towards.

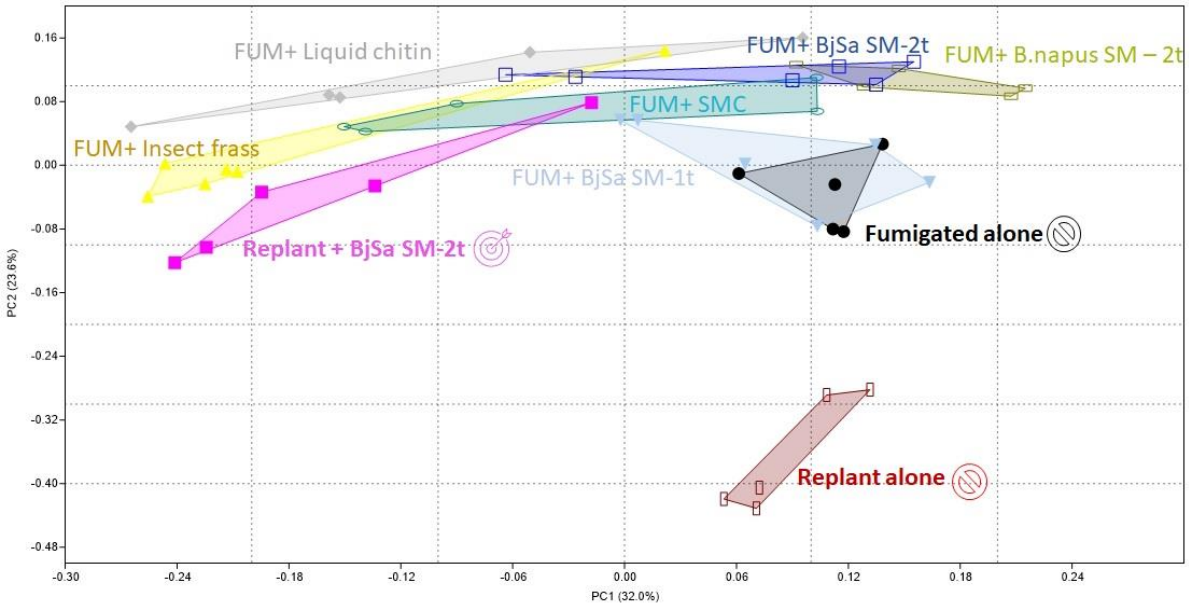


Figure 2. Principal coordinates analysis of fungal community composition in G.11 apple rhizospheres 4-weeks post-planting at the Order level. Each point represents fungal community composition generated from an individual soil sample. Filled shapes represent the clustering of replicate samples associated with plants cultivated in the different treatments as labeled.

Microbial community functional potential varied with amendment-type (Fig. 2-4): Statistical analysis was conducted to identify bacterial and fungal taxa that were significantly enriched in select treatment groups relative to the untreated fumigated control. To explore the functional potential of these systems in more depth, a literature review was conducted on bacterial and fungal species which were identified as being enriched and comparative assessments among treatments were made.

Summary discussion of microbial community steering experiment (in-depth analysis):

Fum + BjSa (2t): This treatment moved the bacterial community closest to that of the "target" treatment (which is *unfumigated* orchard replant soil amended with BjSa SM at a rate of 2 t per acre). A large body

of research has shown that this particular SM formulation promotes disease-***suppressive*** rhizosphere communities. Therefore, the "target" treatment represents one direction to move towards. The target treatment contained the highest levels of metabolic versatility in terms of biocontrol potential, particularly with regard to secondary metabolite/antibiotic production and nematode control (Fig. 3). In Fum + BjSa (2t) the potential for chitinase/fungal control was largely attributed to multiple chitinolytic bacteria belonging to the family Chitinophagaceae and *Paenibacilus polymyxa*. By comparison, in the Replant + BjSa (2t) treatment this functional characteristic was associated with the family Acidobacteriaceae, *P. polymyxa*, *Clostridium* spp. and *Rhixomonas suberifaciens*. Both fumigated and unfumigated BjSa SM amendments appeared to be enriched with a diversity of bacteria associated with heavy metal tolerance and/or uptake. This capability was attributed to Actinobacteria, Bacillales, and Myxococcales in Fum + BjSa (2t) and Acidobacteria, Bacillales, Sphingomonadales in Replant + BjSa (2t). The bacterial community from the unfumigated BjSa SM-treated soil appeared to have the most functional potential in terms of its ability to acquire/compete for iron (e.g. Actinobacteria and Bacialles). Within the fungal community, Fum + BjSa (2t) stimulated the growth of *Fusarium oxysporum* by 30% (relative to the fumigated control). This is noteworthy because *F. oxysporum* has been shown to suppress infection in apple seedlings by the ARD pathogen *Phytophthora cactorum*. The treatment also led to a 5% increase in the relative abundance of *Mortierella* spp., a group of fungi generally well-known for their ability to provide multiple beneficial functions to a variety of plants, including the production of plant growth promoting compounds. In non-fumigated (replant) soil, however, BjSa SM-structured rhizospheres favored *Humicola* sp. (20% relative abundance). Finally, BjSa (2t) was the only post-fumigation amendment with increased potential for fungal-based nematode control.

Fum + BjSa (1t): This treatment was not as successful as other treatments at "steering" the bacterial community away from the degraded states of fumigation-alone or replant control (Fig. 1). This was also the only soil amendment that did not successfully "push" the fungal community out of the post-fumigation state (Fig. 2).

Fum + Shitake mushroom compost (SMC): This treatment worked well at pushing the bacterial community out of the post-fumigation state, but in a different direction than the BjSa SM treatments. The SMC treatment appeared to have a proliferative effect on chitinolytic bacteria, namely those in the family Chitinophagaceae. This result was not unexpected as SMC, which is largely dominated by shiitake mycelium, contains a high level of chitin. Interestingly, this treatment also appeared to have the greatest potential for oomycete control due to the presence of *B. flexus*, *B.subtilus*, and *Rhizobium* spp. This soil amendment tended to support the growth of bacteria with the ability to fix nitrogen from the atmosphere, namely the Hyphomicrobiales (Rhizobiaceae) (Fig. 5). Although the association between this material and the Rhizobiaceae is not entirely clear, the SMC soil amendment was the only treatment which resulted in reduced levels of plant available NO_3 (relative to the fumigated control) in bulk soil (Table 2). Like BjSa (2t), SMC stimulated the growth of *F. oxysporum* by ~ 10% (relative to the fumigated control). Fum + SMC was also one of the only treatments in which *Hypocrea* (i.e., *Trichoderma*) was enriched. This genus contains multiple members known to be antagonistic towards both ARD fungal and oomycete pathogens. However, this treatment also led to an increase in the relative percentage of the aflatoxigenic fungi *Aspergillus parasiticus* in the rhizosphere (0.02% in fumigated soil vs 1 % in FUM + SMC).

FUM + Liquid Chitin (LC): Similar to SMC, this treatment also worked well at pushing the bacterial community out of the post-fumigation state, but in a different direction than the BjSa SM treatments. Like SMC, LC induced proliferation of chitinolytic bacteria, namely those in the Acidobacteriaceae. Members of this group prefer acidic conditions (3.0–6.5 pH), and the low soil pH of this treatment is likely to have contributed to their enrichment. In terms of the ability of the ***bacteria*** living in the rhizosphere to utilize unique, complex compounds for growth (esp. environmental pollutants), this treatment contained the most metabolic versatility (Fig. 5; Degradative). The Fum + LC treatment also promoted the growth of a variety of ***fungi*** with biocontrol potential (including *Hypocrea/Trichoderma* spp.). The majority of fungi

in this treatment (53%), however, were most similar to *Sordaria tomento-alba*, a species of fungi that is not well described in the literature (although not a known pathogen of apple). Similar to SMC, liquid chitin also led to an increase in the relative percentage of *A. parasiticus* in the rhizosphere (0.02% in fumigated soil vs 2.2% in FUM + LC).

Fum + *B.napus* (2t): Compared to all other treatments, the potential for bacterial-based biocontrol (Fig. 3), bacteria known for their ability to cope with excess heavy metals and/or chemical contaminants (Fig. 4), and bacteria associated with nutrient cycling (Fig. 5) was relatively low. Taken together, these results suggest that *B. napus* SM is not as useful at counteracting the adverse effects of fumigation as other amendment options. With regard to fungal community outcomes, however, this treatment led to a 9% increase in the relative abundance of *Mortierella,* a group associated with healthy orchard soils.

Fum + Insect Frass (IF): Comparatively speaking, this treatment did not effectively shift the ***bacterial*** community away from the fumigation alone or replant control states (Fig. 1). However, in terms of changes to the ***fungi***, this treatment moved the community closest to that of the "target" treatment (Fig. 2). Interestingly, this was the only treatment which did not significantly reduce the relative abundance of the soilborne pathogen *Ilyonectria robusta* in the apple rhizosphere. *Chaetomium* sp. became the dominant fungus in this treatment, increasing from 11% (in the fumigated alone control) to 63% in Fum + IF. This genus includes several metabolically gifted members (e.g. *C. globosum, C. nigricolor*) possessing the potential to control multiple ARD pathogens including *P.ultimum* and *R.solani*.

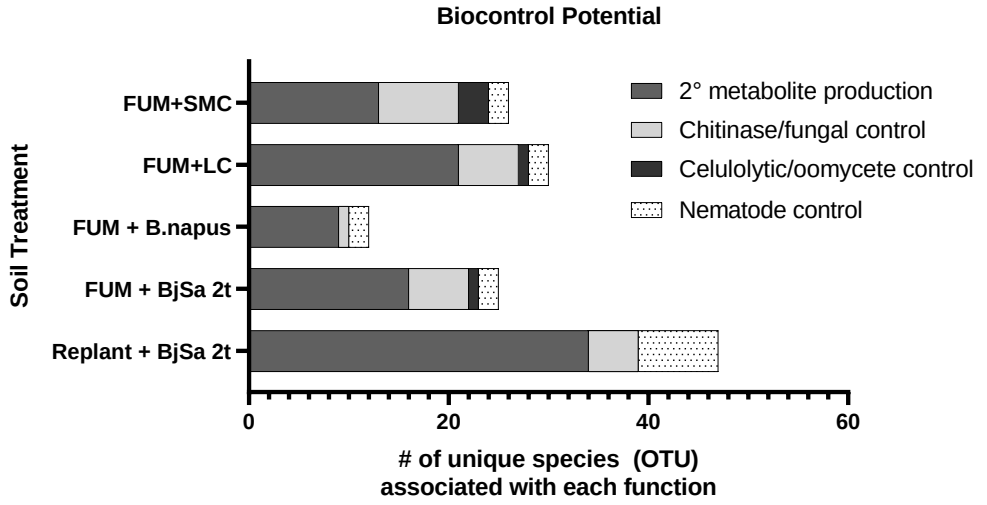


Figure 3. Comparison of the number of unique bacterial species associated with increased biocontrol potential in each treatment (relative to the fumigated control).

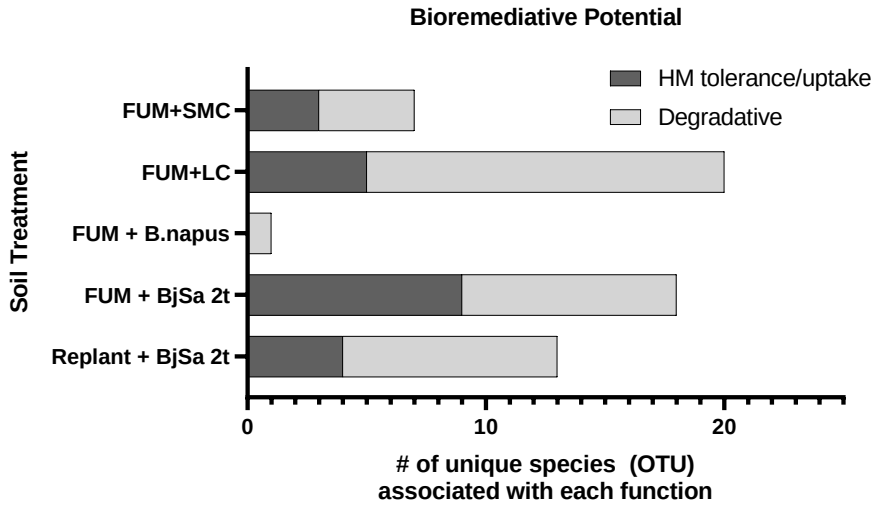


Figure 4. Comparison of the number of unique bacterial species associated with increased heavy metal tolerance/uptake and/or the ability to utilize complex carbon sources in each treatment (relative to the fumigated control).

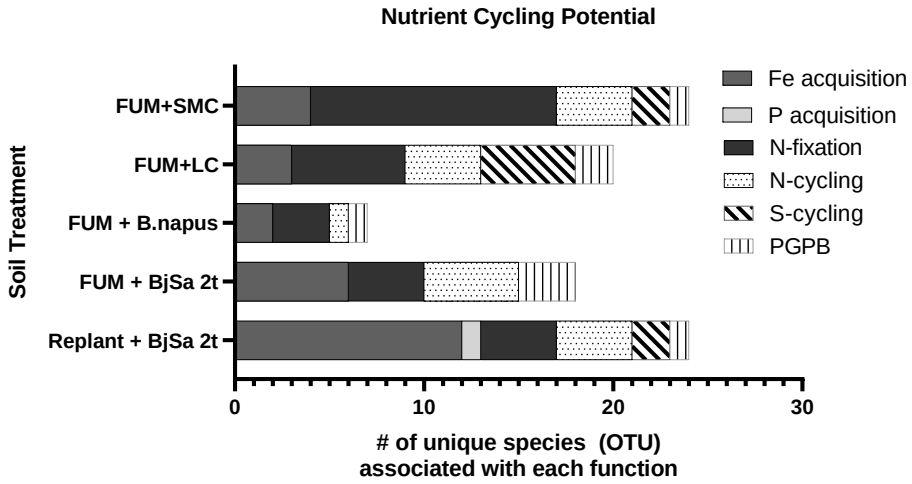
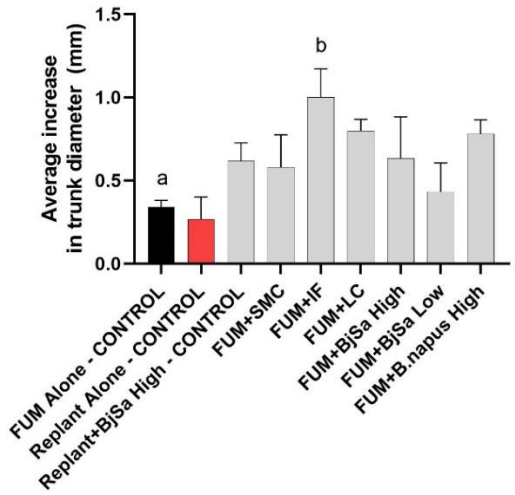


Figure 5. Comparison of the number of unique bacterial species enriched in each treatment relative to the fumigated control associated with a variety of functions related to nutrient cycling and/or characterized as plant growth promoting bacteria (PGPB) in the literature.



The effects of the different soil amendments on rootstock growth were also assessed at the end of Experiment 1. None of the rootstocks planted into the fumigated soil amended with chicken manure compost had any signs of new root or shoot growth. Therefore, CCM is not included in this figure.

Figure 6. Increase in trunk diameter at harvest. Different lowercase letters indicate significantly different means and represent statistical comparisons between the fumigation alone control soil (FUM Alone; black) and all other soil treatments. BjSa High and *B.napus* High = 2 tons seed meal per acre, BjSa Low = 1 ton seed meal per acre, SMC = shitake mushroom compost, LC = liquid chitin, IF = insect frass. Bars represent standard error of the mean.

***Impacts on plant fitness*:**

In general, with the exception of the replant alone control (Fig. 6; red), all amendments resulted in an increase (albeit non-significant) in mean trunk diameter relative to the fumigation alone control soil (Fig. 6; black). Insect frass was the only treatment which resulted in a significant increase in trunk diameter (from planting to harvest) relative to the fumigated control ($p=0.027$). The amount of wood produced during the growing period (trunk diameter) is an indicator of overall tree health. These results suggest that insect frass may benefit the growth of young trees in fumigated soil.

The second objective of this study was to determine the role of select amendment-modified soil microbial communities in limiting pathogen re-infestation and reducing potential post-harvest pathogens.

Experiment 2/Year 2

Subsequent to the investigations conducted in year 1, the most promising soil amendments (BjSa SM (2t), SMC, LC, and IF) were selected for use in an experiment designed to determine the ability of the altered microbiome to inhibit pathogen re-infestation of the fumigated orchard soil. This experiment utilized soil from the same orchard location as in Experiment 1; a new batch of unfumigated replant soil was collected in the summer of 2022 for use in Experiment 2. This was because the first batch of unfumigated replant soil lacked *P.penetrans,* a result which was likely related to when the soil was collected (April 2021). During early spring, when soil temperatures generally remain below 70°F, nematode populations in soil may be less active due to overwintering. The new batch of replant soil was used in a secondary (i.e., repeat) pre-requisite bioassay to ensure disease control was in fact obtained in fumigated soil. Total plant biomass (Fig. 7), root biomass and shoot biomass (data not shown) were significantly higher in fumigated

soil (a), providing strong evidence of disease control in the fumigated soil. Upon harvest, fine root tissue was also assessed for *Pratylenchus penetrans* abundance. The average number of *P. penetrans* recovered per gram of root tissue was 183 for plants cultivated in replant soil. In comparison, not a single *P. penetrans* was identified in the roots of apple seedlings cultivated in fumigated or pasteurized replant soil.

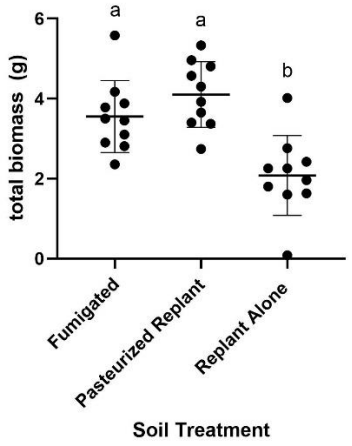


Figure 7. Effect of soil treatment on growth of 4-week old Gala apple seedlings as measured by total plant biomass. Different letters indicate significantly different means and represent comparisons of all three treatments (Kruskal-Wallis followed by Dunn's test; $p<0.05$).

In order to simulate pathogen re-infestation following fumigation and to *directly* test the ability of the apple rhizosphere to limit pathogen re-infestation post-fumigation, a mycelial fragment/spore suspension of the ARD pathogen *Pythium ultimum* was prepared for use as a soil inoculum. In general, *Pythium* spp. populations range from around 60 to 500 propagules in the orchard systems of Central Washington (pers comm. M. Mazzola; Mazzola M., 1998.). The inoculum (300 propagules/g soil) was introduced to pots containing G.11 apple rootstocks cultivated in the select treatments 8 weeks post-planting. Rhizosphere soil samples were collected immediately prior to inoculation (for microbial community sequencing analysis) and bulk soil samples were collected immediately after inoculation (to assess *actual* inoculum density). The experiment was harvested 1 month later (December 2022).

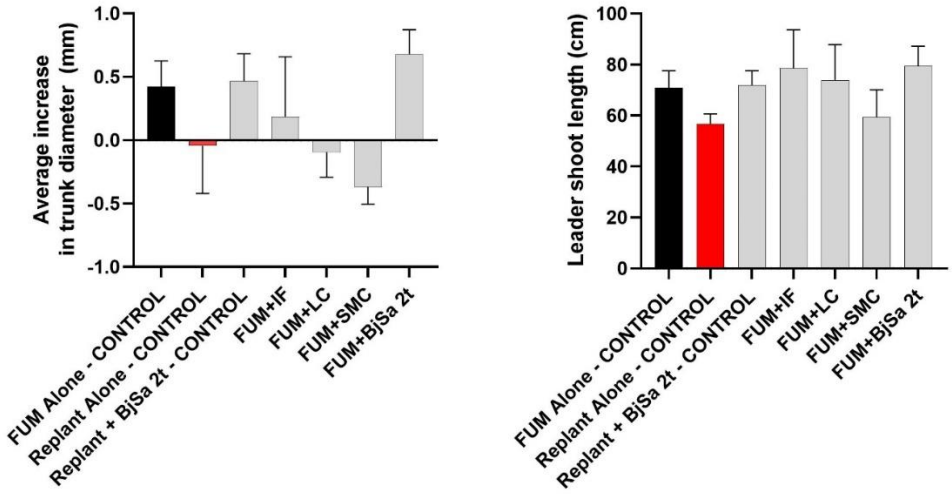


Figure 8. Increase in trunk diameter (left) and leader shoot length (right) at harvest (1 month post inoculation with *P.ultimum*). SMC = shitake mushroom compost, LC = liquid chitin, IF = insect frass. Bars represent standard error of the mean.

In Experiment 2, no treatment resulted in a statistically significant increase in trunk diameter (from planting to harvest) or leader shoot length relative to the fumigated control. However, a positive trend was observed for Fum-BjSa 2t as the treatment resulted in the greatest increase in trunk diameter and leader shoot length. In addition, the SMC amendment resulted in a significant decrease in trunk diameter relative to the fumigated control ($p=0.01$; Kruskal-Wallis followed by Dunn's multiple comparisons test). Three treatments (Replant Alone, FUM + LC and FUM + SMC) resulted in an average trunk diameters that decreased after planting.

Concluding Remarks:

CMC and LC, although relatively inexpensive (~$50 per acre) are likely to be detrimental to plant and soil health when used as a post-fumigation soil amendment. By comparison, the results of these experiments suggest that insect frass and BjSa SM (2t) are both promising treatments for improving multiple aspects of soil health post-fumigation. At this point, however, both of these materials remain costly (~$1,000 -3,000 per acre). The reduced rate (1t per acre) BjSa SM was not enough to effectively "steer" the bacterial community away from that of the fumigation alone or replant control states. The higher amendment rate (2t per acre) is needed to obtain optimal results. When used at this rate, BjSa SM has been shown to consistently provide disease control at levels equivalent to or better than pre-plant soil fumigation. Therefore, use of BjSa SM (2t per acre) as an *alternative* to (rather than in addition to) fumigation is much more cost effective. Future testing of the effects of insect frass on plant/soil health in other soil types and/or at reduced amendment rates is recommended.

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Executive Summary:

Project Title: Directing plant-microbe relations toward resiliency post-fumigation

Key words: Apple, Soil amendment, Soil fumigation, Microbiome, Soil-borne diseases, Replant disease

Abstract:

As a replant disease control strategy, pre-plant soil fumigation is the industry standard. Although fumigation significantly reduces pathogen activity and improves tree growth, this benefit is limited to approximately 1 year. Post-fumigation, orchard soil rapidly re-establishes a microbial community indistinguishable from that found in the corresponding non-fumigated replant soil (i.e., a chronic disease state). The application of soil amendments following fumigation may be an opportune time to improve the ability of the soil to defend against pathogen reinvasion and improve orchard productivity for an extended period. The primary aim of this project was to identify materials which could be used to "steer" the apple rhizosphere in favor of a more prophylactic or disease-suppressive state, post-fumigation. Post-fumigation soil amendments included: *Brassica juncea*/*Sinapis alba* seed meal (2t per acre), BjSa seed meal (1t per acre), *B.napus* seed meal (2t per acre), shitake mushroom compost (SMC, 2% v:v), liquid chitin (LC, 2 gal per acre), composted chicken manure (CCM, 0.7t per acre) and insect frass (IF, 1.5 cups per ft^{-3} soil). In CCM and LC, high nitrate levels led to high salinity as measured by electrical conductivity (EC) and CCM resulted in the death of all trees. Analysis of microbial community sequence data indicated that the remaining amendments, with the exception of BjSa SM (1t), were relatively successful in terms of their ability to significantly alter the microbial community composition of the rhizosphere microbiome and "steer" the community in a positive direction post-fumigation. Insect frass was the only amendment which resulted in a significant increase in trunk diameter relative to the fumigated control. Insect frass and *B. napus* SM (2t), however, did not appear to counteract the adverse effects of fumigation on the bacterial rhizobiome as effectively as SMC, LC, or BjSa SM (2t). By comparison, LC was less effective at stimulating the growth of potentially beneficial fungi. A second experiment designed to *directly* test the ability of the apple rhizosphere to limit pathogen re-infestation post-fumigation was conducted using the ARD pathogen *P. ultimum* as inoculum. At this time, results suggest that insect frass and BjSa SM (2t) are both good candidates for improving soil health post-fumigation; however, *P. ultimum* infection levels in root tissue remain to be determined.