

## Optimization and Implementation of Biologically Active Soil Amendments as a Fumigation Alternative for Soilborne Disease Control in California Strawberry



**Principal Investigator**

**Dr. Mark Mazzola**

USDA-ARS  
1104 N. Western Ave  
Wenatchee, WA 98801  
(509) 664-2280  
[mark.mazzola@ars.usda.gov](mailto:mark.mazzola@ars.usda.gov)

**Collaborators**

**Dr. Joji Muramoto**

Department of Environmental Studies  
University of California, Santa Cruz  
1156 High Street  
Santa Cruz, CA 95064  
(831) 247-3804  
[joji@ucsc.edu](mailto:joji@ucsc.edu)

**Dr. Dan Legard**

Vice President, Research & Education  
California Strawberry Commission

**Dr. Carol Shennan**

Department of Environmental Studies  
University of California, Santa Cruz  
1156 High Street  
Santa Cruz, CA 95064  
(831) 459-4181  
[cshennan@ucsc.edu](mailto:cshennan@ucsc.edu)

## SUMMARY

A series of controlled environment and field-based studies were conducted that examined modification of anaerobic soil disinfestation (ASD) and mustard seed meal (MSM) application protocols to optimize disease control efficacy. Fusarium wilt, incited by *Fusarium oxysporum* f. sp. *fragariae*, demonstrated the greatest challenge in the use of ASD or MSM as a soilborne disease control management tactic in strawberry. Emphasis was placed upon modification of application protocols in a manner that would most effectively target this disease. Several field studies were established at the Monterey Bay Academy field site in Watsonville, CA. Over the three-year duration of this program, the MBA study site possessed excessively elevated populations of *F. oxysporum* f. sp. *fragariae*. Multiple carbon sources were evaluated in ASD trials conducted in 2015-2016. Anaerobic soil disinfestation bed treatments failed to improve yield and failed to suppress *F. oxysporum* f. sp. *fragariae* at the MBA site. Reduced fumigant rates in concert with MSM amendment had an additive effect on strawberry yields. Although in different, but adjacent plots, PicChlor-60 at 150 lbs per acre/MSM treatment provided yields that were equivalent to full rate fumigation (300 lb·ac<sup>-1</sup>). In the 2016-2017 season, efficacy of reduced fumigation rates, reduced rate fumigation in conjunction with biologically active soil amendments, and host resistance were evaluated for control of Fusarium wilt. Full rate PicChlor 60 (300 lb·ac<sup>-1</sup>) soil fumigation was the only treatment that significantly reduced soil density of *F. oxysporum*. Both full rate and half rate (150 lb·ac<sup>-1</sup>) soil fumigation effectively limited *F. oxysporum* f. sp. *fragariae* strawberry crown infection. As observed in the 2015-2016 season, MSM amendment in concert with low rate PicChlor 60 fumigation significantly increased early season strawberry yields; however no significant effect of MSM on strawberry yields in fumigated soil was evident at the end of the harvest period. Autumn application of rice bran (RB) alone or autumn ASD with RB resulted in elevated populations of *F. oxysporum* and a corresponding increase in Fusarium wilt incidence and diminished 'Monterey' strawberry yields. When utilized in concert with the Fusarium wilt resistant cultivar 'San Andreas', but not the susceptible cultivar 'Albion', both MSM and RB soil amendments enhanced overall strawberry yields and reduced incidence of other soilborne pathogens resident to the site. Studies conducted in the 2017-18 season, focused on the use of a summer season cover crop in concert with RB as a carbon input for summer flat ASD as a means to reduce overall input cost and improve efficacy for control of Fusarium wilt. All ASD treatments significantly reduced soil density of *F. oxysporum* f. sp. *fragariae* as determined by quantitative PCR. Changes in pathogen density were not correlated with the production of organic acids but was associated with shifts in the soil microbiome, in particular significant changes in fungal community composition. The summer flat ASD treatment achieved the previously reported threshold of >300 hrs with soil temperature above 86°F necessary to attain Fusarium wilt control.

## INTRODUCTION

Anaerobic soil disinfestation (ASD) techniques and mustard seed meal (MSM) amendments have demonstrated capacity to suppress diverse soilborne pests across a diversity of cropping systems and locations (Shennan et al., 2014) and have emerged as promising alternatives to soil fumigation. Initially two anaerobic methods, biological soil disinfestation for open fields in the Netherlands and soil reductive sterilization for greenhouses in Japan, were developed independently. Anaerobic soil disinfestation involves addition of a labile carbon source (to stimulate microbial growth and respiration), tarping with plastic to limit gas exchange, and irrigation to fill soil pore space with water. Anaerobic conditions are created due to initial rapid growth of aerobic microorganisms which depletes remaining soil oxygen and subsequently the microbial community shifts to facultative and obligate anaerobes. Anaerobic conditions are maintained for a period that varies with soil temperature and C-sources used (Shennan et al., 2018), before the tarp is either removed or planting holes punched through the tarp to allow oxygen back into the soil and stimulate the degradation of remaining by-products of anaerobic decomposition. In a series of field-based trials established in California, ASD achieved strawberry yields that were comparable to pre-plant soil fumigation at multiple sites across production districts (Shennan et al., 2018).

Although ASD has consistently reduced soil densities of *Verticillium dahliae* (Mazzola et al., 2018) causal agent of Verticillium wilt, capacity of ASD to control Fusarium wilt has varied across geographic locations. In field trials conducted in central California, ASD has often failed to suppress populations of the causal pathogen, *Fusarium oxysporum* f. sp. *fragariae*, resulting in high Fusarium wilt incidence and crop failure. Despite this limitation, demonstrated efficacy of ASD has led to adoption of this method as a viable disease control practice primarily by organic strawberry growers and was used to treat nearly 1,500 acres in 2015 (Farm Fuels Inc., personal communication). Implementation of ASD for soilborne disease control in conventional CA strawberry production systems will require greater consistency and efficacy in order to replace soil fumigation as the primary control option.

Research has shown that reduced rates of fumigants as low as 50 lb·ac<sup>-1</sup> are partially effective in controlling *V. dahliae*. Our previous field trials examined whether use of reduced fumigation rates or reduced rates in concert with application of the biologically active mustard seed meal soil amendment or ASD, could be substituted for full rate soil fumigation for the control of *F. oxysporum* f. sp. *fragariae*. When confronted with excessively high populations of the causal pathogen, ASD using RB as the carbon input had no significant effect on yields and resulted in higher early season Fusarium wilt incidence when used in concert with low rate chloropicrin fumigation. In contrast, MSM in concert with reduced rate fumigation reduced early season wilt score and in 2015-2016 field season at MBA, MSM in concert with low rate soil fumigation enhanced marketable strawberry yields. The MBA site possessed significantly elevated populations of *F. oxysporum* f. sp. *fragariae*, and it was suggested that lack of efficacy from these alternative methods, in part, may have resulted from unrealistically high populations of the pathogen. During the 2016-17 field season, a low rate fumigation treatment was applied at MBA to reduce overall levels of the pathogen prior to establishment of treatments to be evaluated for control of Fusarium wilt.

Results from previous field trials indicated that rice bran amendment without addition of water could provide enhanced strawberry yields (Mazzola et al., 2018). Field trials conducted as part of this program demonstrated that rice bran amendment in the absence of summer ASD resulted in elevated populations of saprophytic *F. oxysporum* and the highest level of plant mortality when trials were conducted with the susceptible cultivar 'Monterey'. During the 2016-2017 field season, *F. oxysporum* soil populations were amplified in response to autumn RB application irrespective of whether ASD treatment was conducted using this amendment. When RB or MSM soil treatments were followed by planting with the Fusarium wilt resistant cultivar 'San Andreas', but not the susceptible cultivar 'Albion', strawberry yields were improved relative to the no treatment control. However, autumn application of either soil amendment at the rates employed resulted in increased soil density of *F. oxysporum*. These findings demonstrated that ASD has to be installed during the summer to control *F. oxysporum* and there exists a need to evaluate alternative carbon inputs for use in application of ASD to attain effective control of the spectrum of soilborne plant pathogens that limit strawberry production systems.

In the 2017 season, experiments were conducted to evaluate the suitability of various summer season cover crops as an ASD carbon source input when used in conjunction with rice bran for the suppression of *F. oxysporum* f. sp. *fragariae*. The goal of this research was to determine whether cover crop biomass could be substituted as a partial replacement for rice bran as the ASD carbon input thus reducing overall treatment costs. The trial also sought to confirm a previous study conducted at the MBA site demonstrating that a summer flat ASD treatment could provide control of Fusarium wilt, a disease for which autumn bed ASD treatment has been ineffective as a control option. In the conduct of these trials, assessments were performed to determine the effect of such treatments on anaerobicity, temperature, composition of the soil microbiome and on the generation of organic acids, factors that have been identified as possible mechanisms contributing to ASD-induced soilborne disease control.

## MATERIALS AND METHODS

The 2017 field studies were conducted at the MBA site in Watsonville, CA. The trial was established to determine the effect of cover crop residues used in concert with rice bran as the ASD carbon source for the suppression of *F. oxysporum* f. sp. *fragariae* soil densities. The trial employed a randomized complete block design with four replicates of each treatment. The six different cover crops employed in the trial were sudan grass (cv. Piper), triticale, FL104 rye grass, Italian rye grass, white mustard (*Sinapis alba* cv. IdaGold) and open pollinated broccoli. The trial included a no cover crop/ no carbon input control and a rice bran only ASD treatment resulting in a total of 32 experimental plots. The cover crops were established in May 2017 and plants were grown for a period of two months at the study site. The cover crops were mowed and incorporated into the soil profile in July. For the no cover crop control, weed biomass that developed on the site was also incorporated into soil on the same date. For the RB only treatment, weed biomass that developed in the ASD-RB assigned plots was physically removed from the site prior to application and incorporation of rice bran at 9 tons per acre. For the cover crop treatments, cover crop dry biomass was determined, and a sufficient quantity of RB was added to the plot to attain a total (CC + RB) biomass input of 9 tons per acre. Thus, for all soil treatments there existed a uniform input of 9 ton per acre dry biomass with the exception of the no cover crop control which possessed only the weed biomass as the carbon input. Biomass was incorporated into soil using an initial process of chiseling followed by rototilling of the plots. All plots were covered with a clear totally impermeable film and drip tapes were installed. Drip irrigation was applied (1.5 ac-in of water) and the summer flat ASD treatment was conducted from July 19 until late August 28, 2017.

Prior to establishment of the ASD treatments, inoculum of *F. oxysporum* f. sp. *fragariae*, in the form of naturally infested soil from an adjacent site, was buried into the trial plots with three replicates of the inoculum per plot. The *F. oxysporum* f. sp. *fragariae* infested soil was mixed with the cover crop plus RB at the equivalent rate for each treatment and packed into a nylon mesh bag for use as an inoculum. Inoculum were recovered from the trial at four, five and six weeks after ASD treatment, with one packet of the inoculum extracted from each plot on each sampling date. An un-treated inoculum control consisted of an air-dried inoculum packet that was not buried in soil but was maintained in storage until the appropriate sampling period. This protocol was employed due to the fact that the trial site did not possess a sufficient population of *F. oxysporum* f. sp. *fragariae* to conduct the analysis in an environment equivalent to that known to incite significant incidence of Fusarium wilt. Data were log transformed prior to conduct of one-way ANOVA and mean separation using the Tukey all pairwise comparisons test. During the ASD treatment period, soil Eh at the 6-inch depth and temperature at the 4.5-inch soil depth were monitored to assess the time course development and duration of anaerobic conditions across the soil treatments.

Microbial analyses were conducted using molecular methods to assess changes in *F. oxysporum* f. sp. *fragariae* population density over time, and changes in composition of the soil microbiome. For each sample, DNA was extracted from 5 g of soil from an individual inoculum packet using the Qiagen DNeasy PowerMax Soil Kit. DNA extracts were used in qPCR to determine relative effect of soil treatments on soil density of *F. oxysporum*. Amplification was conducted with the primer pair PFO2 and PFO3 (Edel et al., 2000) using a StepOne Plus real-time PCR system. Assessment of treatment effects on soil bacterial and fungal community composition was conducted using terminal restriction fragment polymorphism (T-RFLP) analysis. Fungal DNA was amplified using fluorescently labeled ITS1F and ITS4 primer pair. Bacterial DNA was amplified using the labeled 8f and 907r primer pair. Thermocycling was performed using a StepOne Plus Real Time PCR System with reaction mixtures and cycling conditions as previously described for amplification of fungal (Weerakoon et al., 2012) and bacterial (Strauss et al., 2014) DNA. Restriction digestion of resulting amplicons, purification and fragment separation were conducted essentially as described previously. Fragment separation was conducted using the CEQ 8000 Genetic Analysis System and T-RFLP profiles generated using the Fragment Analysis Module of the CEQ system.

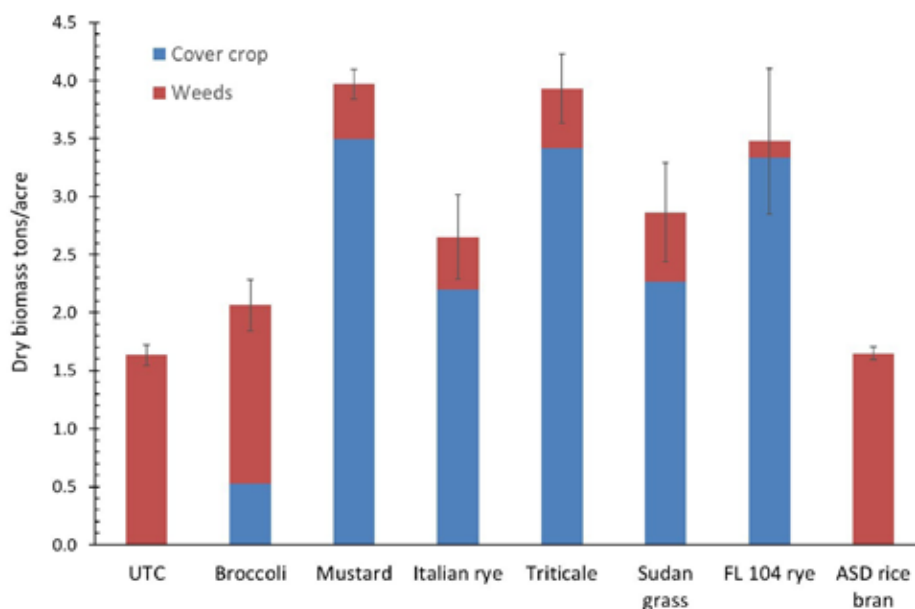
Assessment of similarity in microbial community composition among soil treatment groups was conducted by non-metric multidimensional scaling (NMDS) of bacterial and fungal T-RFLP data using PAST software package ver 3.16 (Hammer et al., 2001). Dice similarity coefficient was calculated among groups of samples and used to perform ordination and one-way analysis of similarity (ANOSIM). Results with  $P \leq 0.05$  were regarded as significant, with a large positive  $R$  (up to 1) value signifying dissimilarity among groups (Hammer et al., 2001).

Organic acids were extracted from the soil (20 g) using a 2M KCl solution (20 ml) and continuous shaking for 30 min, followed by gravity filtration of the supernatant through a Whatman #1 filter paper. The resulting filtrate was then filtered through a 0.45  $\mu\text{m}$  pore size cellulose acetate filter. Concentrations of acetic, butyric, propionic and lactic acids in the filtered extracts were determined by high performance liquid chromatography using an Agilent 1050 HPLC system. UV detection was at 210 nm. A Restek Ultra AQ C18, 5u, 150 x 4.6 cm column was used for the separation. The mobile phase was 50 mM potassium phosphate monobasic, adjusted to pH 2.5 with phosphoric acid, plus 1% acetonitrile. Injection volume was 10  $\mu\text{l}$  and conducted via the 1050 autosampler.

## RESULTS

### Cover Crop Biomass Production

Cover crop dry biomass produced at the trial site was variable across the individual crop species (Figure 1). The control and rice bran plots yielded total weed biomass of approximately  $1.4 \text{ t} \cdot \text{ac}^{-1}$ . Weed biomass was incorporated into soil for the control plots in the same manner as cover crop incorporation but was removed from the ASD-RB plots prior to application of the TIF. For the cover crop plots, weed biomass was added to the cover crop biomass prior to determination of the rice bran input to yield a total carbon input of  $9 \text{ t} \cdot \text{ac}^{-1}$ . As such, triticale ( $3.31 \text{ t} \cdot \text{ac}^{-1}$ ) and FL 104 rye ( $3.18 \text{ t} \cdot \text{ac}^{-1}$ ) yielded the highest biomass over the two-month cover crop growth period.



**Figure 1.** Cover crop and weed dry biomass produced at the MBA trial site during the two-month growth period prior to incorporation into soil and application of ASD in July 2017. Weed biomass was incorporated into soil for the untreated control and cover crop plots, but was removed from the RB plot, prior to application of TIF and application of ASD.

### ASD Effects on Soil Physical/Chemical Properties

Strong anaerobic conditions were obtained for all cover crop plus RB and the weed only ASD treatments. Cumulative hours of temperature above 86°F at the 4.5 in soil depth was 350, 467 and 556 hours at the four, five and six-week interval during the conduct of ASD.

The generation of organic acids in ASD treated soil was assessed at the four-week sampling time point. Organic acids that were monitored included acetic acid, butyric acid, lactic acid and propionic acid. Among soil treatments, pairwise comparison of means indicated that a significant difference in organic acid concentration existed between the no amendment ASD and the Italian rye + RB ASD treatment (Table 1). All other ASD treatments yielded a statistically similar organic acid concentration in the MBA field soil.

**Table 1.** Effect of anaerobic soil disinfestation conducted using different carbon inputs on organic acid concentration detected in treated soils.

ASD treatment <sup>z</sup>	log <sub>10</sub> organic acid concentration in soil
Weeds without RB	0.876 b <sup>y</sup>
Broccoli + RB	1.249 b
Fl 104 rye + RB	1.784 ab
Italian rye + RB	2.467 a
Mustard + RB	1.545 ab
Rice bran (RB)	1.867 ab
Sudan + RB	1.577 ab
Triticale + RB	1.911 ab

<sup>z</sup> Biomass generated from a specific cover crop was determined after two months growth and the total ASD biomass input was adjusted to 9 t ac<sup>-1</sup> by the addition of rice bran. The weeds without RB treatment was conducted using weed biomass resident to the designated plots at the end of the cover crop cultivation period. Weed biomass was removed from the rice bran plots prior to application of this carbon source and application of ASD.

<sup>y</sup> Means in the same column followed by the same letter are not significantly ( $P > 0.05$ ) different.

### Response to *F. oxysporum* Soil Density to ASD treatment

Population density of *F. oxysporum* was significantly diminished with time in response to all ASD treatments. At the four-week (August 14, 2017) sampling no significant ( $P = 0.1135$ ) difference was observed among soil treatments in *F. oxysporum* soil density relative to the no treatment (non-buried) control (Table 2). At week five (August 21, 2017) and six-week (August 28, 2017) sampling, a significant ( $P < 0.0001$  and  $P = 0.0005$ , respectively) in the *F. oxysporum* density detected in the buried inoculum bag soils recovered from ASD treated plots relative to the no treatment control. There were no significant differences in *F. oxysporum* soil density among ASD treatments at either of the latter sampling periods including the no amendment ASD treatment. *F. oxysporum* DNA density detected in the Sudan-ASD treatment did not differ significantly from the control in soils sampled at six weeks after ASD treatment.

**Table 2.** Effect of anaerobic soil disinfestation conducted using different carbon inputs on quantity of *Fusarium oxysporum* DNA detected in inocula buried in soil at the MBA field site in Watsonville, CA.

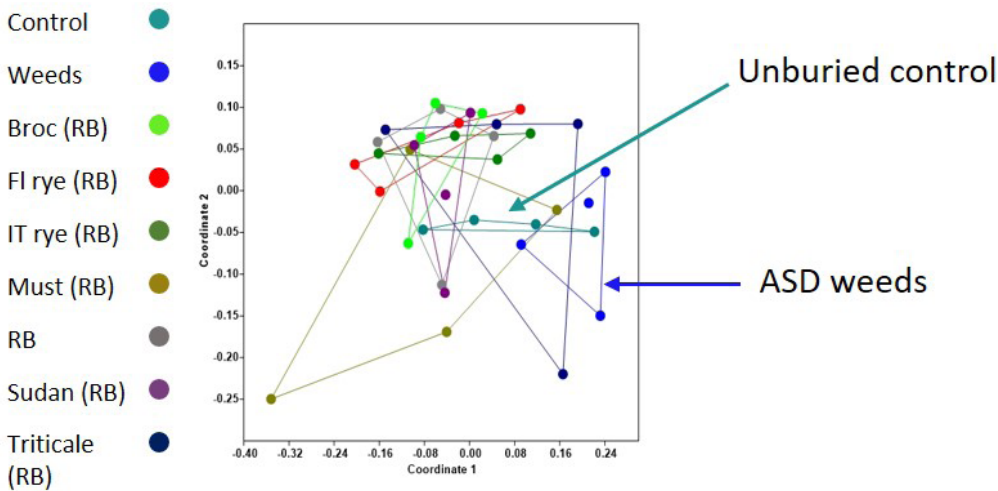
ASD treatment <sup>z</sup>	$\log_{10}$ <i>Fusarium oxysporum</i> DNA quantity		
	14 August 2017	21 August 2017	28 August 2017
Control (unburied)	3.465	2.993 a <sup>y</sup>	2.415 a
Weeds without RB	2.758	0.940 b	1.093 b
Broccoli + RB	2.373	0.390 b	0.638 b
Fl 104 rye + RB	2.757	0.825 b	0.652 b
Italian rye + RB	2.002	0.885 b	0.445 b
Mustard + RB	2.185	0.962 b	0.767 b
Rice bran (RB)	1.785	0.415 b	0.695 b
Sudan + RB	1.753	0.363 b	1.470 ab
Triticale + RB	2.448	0.412 b	0.448 b

<sup>z</sup> Biomass generated from a specific cover crop was determined after two months growth and the total ASD biomass input was adjusted to 9 t·ac<sup>-1</sup> by the addition of rice bran. The weeds without rice bran treatment was conducted using weed biomass resident to the designated plots at the end of the cover crop cultivation period. Weed biomass was removed from the rice bran plots prior to application of this carbon source and application of ASD.

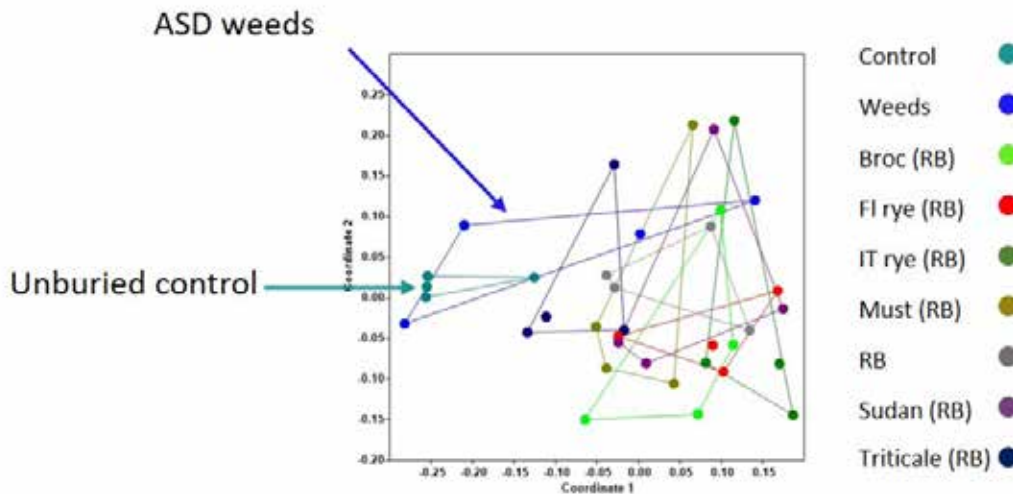
<sup>y</sup> Means in the same column followed by the same letter are not significantly ( $P > 0.05$ ) different.

### Effect of Soil Treatments on Soil Microbial Community

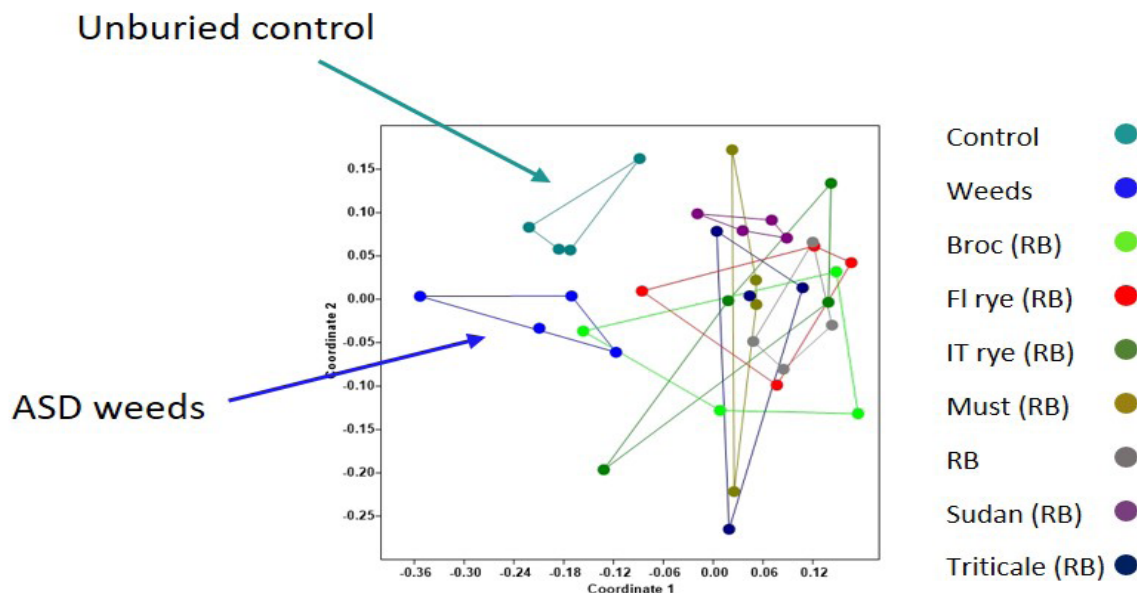
Based on analysis of 16S T-RFLP data, ASD treatments had no significant effect on composition of the soil bacterial community over the three post-ASD sampling periods. Analysis of similarity (ANOSIM) of T-RFLP data indicated that bacterial communities were highly similar among soil treatments, with  $R_{\text{anosim}}$  values of -0.0251, 0.1569 and 0.0996 recorded for the four (Figure 2), five (Figure 3) and six-week (Figure 4) post treatment samples.  $R_{\text{anosim}}$  values less than 0.25 indicates that the communities being compared are considered barely separable (Ramette, 2007). In contrast, analysis of the ITS T-RFLP data indicated that fungal community composition was affected by ASD treatment. At the four-week sampling, non-metric multidimensional scaling of T-RFLP derived fragment data indicated that the fungal community composition was highly similar among all soil treatments (Figure 2). Thereafter, there was an apparent shift in composition of the fungal community in ASD treated soils relative to the control and weeds without RB treatments at the five-week (Figure 3) and six-week (Figure 4) sampling. A comparison of all three ordination plots demonstrates the shift over time with the control emerging as a distinct convex hull from the ASD treatments. At the six-week sampling period, the fungal community from all ASD treatments, with the exception of the Broccoli + RB treatment, differed significantly from the weeds without RB ASD treatment.



**Figure 2.** Influence of anaerobic soil disinfestation (ASD) using different cover crop carbon inputs on composition of the soil fungal community at four-weeks post-ASD treatment at the Monterey Bay Academy field site Watsonville, CA. With the exception of weed ASD treatment, for all cover crop biomass treatments, total dry cover crop produced was augmented with rice bran to yield a total input of 9 t·ac<sup>-1</sup>. Ordination was conducted by non-metric multidimensional scaling of terminal fragment length polymorphism-derived data and distance was based on the Dice similarity coefficient. Convex hulls enclose all points for a given treatment data set. Legend indicates carbon resource utilized in ASD.



**Figure 3.** Influence of anaerobic soil disinfestation (ASD) using different cover crop carbon inputs on composition of the soil fungal community at five-weeks post-ASD treatment at the Monterey Bay Academy field site Watsonville, CA. With the exception of weed ASD treatment, for all cover crop biomass treatments, total dry cover crop produced was augmented with rice bran to yield a total input of 9 t·ac<sup>-1</sup>. Ordination was conducted by non-metric multidimensional scaling of terminal fragment length polymorphism-derived data and distance was based on the Dice similarity coefficient. Convex hulls enclose all points for a given treatment data set. Legend indicates carbon resource utilized in ASD.



**Figure 4.** Influence of anaerobic soil disinfestation (ASD) using different cover crop carbon inputs on composition of the soil fungal community at six-weeks post-ASD treatment at the Monterey Bay Academy field site Watsonville, CA. With the exception of weed ASD treatment, for all cover crop biomass treatments, total dry cover crop produced was augmented with rice bran to yield a total input of 9 t·ac<sup>-1</sup>.

## DISCUSSION

The flat ASD treatment utilized in this trial resulted in a significant reduction in the quantity of *F. oxysporum* DNA detected in soils between the 4-week and 5-week post-treatment sampling period. This reduction in pathogen density was not correlated with the relative differences in the quantity of organic acids generated among the different treatments over the same time period ( $R^2 = 0.0098$ ). This finding suggests that generation of organic acids may not be the dominant mechanistic driver that led to the reduction of *F. oxysporum* soil density observed in this trial.

Conversely, in the current study the reduction in *F. oxysporum* inoculum density was associated with compositional changes in the total soil fungal community based upon T-RFLP analysis. In previous studies, characterization of the microbiome composition in ASD treated soil through amplicon sequencing resulted in the identification of numerous shifts in fungal community composition relevant to the suppression of plant pathogenic fungi (Hewavitharana and Mazzola, 2016). In particular, when a carbon input consisting of mixed grass species was used in application of ASD, populations of various fungal species possessing capacity to produce fungitoxic metabolites were elevated in the ASD treated soil (Hewavitharana and Mazzola, 2016). Notably, *Coprinellus curtus*, which previously demonstrated activity in controlling diseases incited by *Fusarium* sp. (Nakasaka et al., 2007), was only detected in the ASD treated soil and *F. oxysporum* was reduced from 13.46% of the total fungal sequences in the control to 0.23% in the ASD-grass treated soil (Hewavitharana and Mazzola, 2016).

The T-RFLP method used in the current study provided the ability to document changes in community composition but does not enable taxonomic resolution as to specific elements of the population that were elevated or suppressed. However, contribution of the altered fungal community to disease suppression can be inferred based upon the observed temporal changes in community composition corresponding with reduction in *F. oxysporum* population density and previously reported spectrum of fungitoxic compounds produced by saprophytic fungi that dominate ASD-grass treated soils (Hewavitharana and Mazzola, 2016).

The suppression of *F. oxysporum* soil density in the 2017-2018 trial contrasts with results obtained in previous trials conducted at the MBA site during the course of this three-year research program. In field trials conducted during the 2016-2017 growing season, the autumn RB application/bed ASD treatment resulted in significant increases in populations of *F. oxysporum* and elevated plant mortality when the Fusarium wilt susceptible strawberry cultivar 'Monterey' was employed in the trial. It was hypothesized that failure to control Fusarium wilt was due to the lower temperatures experienced during the autumn ASD application and the use of a bed application rather than an ASD flat application protocol. Average soil temperature at the MBA site during 2016-2017 trials was 68°F with a maximum of 74°F. Previous reports indicate that a soil temperature of 86° F for a minimum of 300 h is necessary for control of Fusarium wilt in response to ASD (Yonemoto et al., 2006); this threshold was attained within four weeks with the use of a summer flat ASD treatment in the current trial. Similar to the current trial, during the 2013-2014 growing season a summer flat ASD trial significantly reduced *F. oxysporum* populations. In total, the findings from these trials, and other reports (Yonemoto et al., 2006; Yossen et al., 2008) indicate that effective control of *F. oxysporum* will require a summer flat ASD treatment in order to realize the elevated soil temperatures necessary to control Fusarium wilt of strawberry in coastal California. While disease control efficacy attained in result of the ASD treatment may result in part through elevated soil temperature it is not the result of a direct temperature effect (Hewavitharana, 2017). Rather, the elevated production of biologically active chemistries, including volatiles such as dimethyl disulfide, likely contributes to control of Fusarium wilt when ASD is conducted at higher soil temperature (Hewavitharana et al., submitted).

## CONCLUSIONS

Anaerobic soil disinfestation and MSM have demonstrated efficacy for the control of various soilborne diseases, including those that affect the economic viability of the California strawberry production system. Anaerobic soil disinfestation (ASD) demonstrated capacity to suppress populations of *Verticillium dahliae* and control *Macrophomina phaseolina* across a diversity of field trials conducted in California (Muramoto et al., 2016; Mazzola et al., 2018; Shennan et al., 2018). In the current program the multitude of field trials conducted over three growing seasons demonstrated that use of autumn bed applied ASD is an unsuitable strategy for the control of Fusarium wilt regardless of the carbon source utilized for ASD. Reduced fumigation rates in concert with MSM may yield an increase in early season strawberry yields but will not provide effective control of Fusarium wilt over the course of the entire growing season. On sites possessing significant *F. oxysporum* f. sp. *fragariae* populations, ASD and MSM will provide benefit to the production system if utilized in concert with a Fusarium wilt resistant cultivar through the control of non-lethal pathogens such as *Rhizoctonia fragariae* and *Meloidogyne hapla*. Although autumn bed ASD was not effective in controlling Fusarium wilt, summer flat ASD has demonstrated potential for suppression of the causal pathogen in both this and previous (2013-2014) trials conducted at MBA.

## REFERENCES

- Hewavitharana, S.S. 2017. Anaerobic soil disinfestation as a sustainable soil-borne disease management practice for apple and strawberry, and mechanisms of disease suppression. PhD dissertation, Washington State University, Pullman. 270 pp.
- Hewavitharana, S.S., Shennan, C., Muramoto, J., Mazzola, M. 2015. Anaerobic soil disinfestation disease control performance in strawberry as influenced by environmental variables. *Phytopathology* 105:S4.59.
- Hewavitharana, S.S. and M. Mazzola. 2016. Carbon source-dependent effects of anaerobic soil disinfestation on soil microbiome and suppression of *Rhizoctonia solani* AG-5 and *Pratylenchus penetrans*. *Phytopathology* 106:1015-1028.
- Mazzola, M., J. Muramoto, and C. Shennan. 2018. Anaerobic disinfestation induced changes to the soil microbiome, disease incidence and strawberry fruit yields in California field trials. *Applied Soil Ecology* 127:74-86.
- Hewavitharana, S.S., C. Shennan, J. Muramoto, and M. Mazzola. 2018. Anaerobic soil disinfestation disease control performance in strawberry as influenced by environmental variables. *Phytopathology* (submitted).
- Muramoto, J., C. Shennan, M. Zavatta, G. Baird, L. Toyama, and M. Mazzola. 2016. Effect of anaerobic soil disinfestation and mustard seed meal for the control of charcoal rot in California strawberries. *Int. J. Fruit Sci.* 16:59-70.
- Nakasaki, K., M. Saito, and N. Suzuki. 2007. *Coprinellus curtus* (Hitoyotake) prevents diseases of vegetables caused by pathogenic fungi. *FEMS Microbiol. Lett.* 275:286-291.
- Shennan, C., J. Muramoto, S. Koike, G. Baird, S. Fennimore, J. Samtani, M. Bolda, S. Dara, O. Daugovich, G. Lazarovits, D. Butler, E. Roskopf, N. Kokalis-Burelle, K. Klonsky and M. Mazzola. 2018. Anaerobic soil disinfestation is a potential alternative to soil fumigation for control of certain soil-borne pathogens in strawberry production. *Plant Pathology* 67:51-66.
- Shennan, C., J. Muramoto, J. Lamers, M. Mazzola, E. Roskopf, N. Kokalis-Burelle, N. Momma, D. Butler, and Y. Kobara. 2014. Anaerobic soil disinfestation for soil borne disease control in strawberries and vegetable systems: Current knowledge and future directions. *Acta Horticulturae* 1044:165-175.
- Yossen, V., G. Zumelzu, L. Gasoni, and K. Kobayashi. 2008. Effect of soil reductive sterilization on Fusarium wilt in greenhouse carnation in Córdoba, Argentina. *Austr. Plant Pathol.* 37:520-522.
- Yonemoto, K., K. Hirota, S. Mizuguchi, and K. Sakaguchi. 2006. Utilization of the sterilization by soil reduction in an open field and its efficacy against Fusarium wilt of strawberry. *Proc. Assoc. Pl. Protection Shikoku* 41:15-24.