ASD EFFICACY IS ASSOCIATED WITH ALTERED SOIL MICROBIOME AND METABOLOME

Mark Mazzola*, USDA-ARS, Tree Fruit Research Laboratory, Wenatchee, WA
Shashika S. Hewavitharana, Dept. Plant Pathology Washington State University

Anaerobic soil disinfestation (ASD) has demonstrated potential to control numerous soil-borne pathogens in a diversity of production systems. A variety of environmental, biological and application attributes have potential to determine the overall capacity of ASD to provide effective disease control. In apple, *Rhizoctonia* solani AG-5 and *Pratylenchus penetrans* not only contribute to the phenomenon termed apple replant disease, but also rapidly recolonize fumigated soil systems leading to reduced overall fruit production in established orchards. The current study sought to examine the effect of ASD carbon (C) source input type on control of these two pathogens and to examine the specific attributes of the system that may determine the relative efficacy achieved.

Different C inputs used in the ASD process yielded differential effects on resulting soil chemistry and control of root disease incited by *R. solani* AG-5 and *P. penetrans*. Among five C substrates evaluated, ASD failed to yield effective control of root infection by *R. solani* AG-5 only when compost was utilized as the C input. Orchard grass utilized as the ASD substrate provided the highest level of disease control based upon overall growth of apple seedlings and the amount of *R. solani* AG-5 detected in seedling roots. Similarly, ASD conducted using compost was less effective than ASD conducted with all other carbon sources in suppressing apple root populations of *P. penetrans* in multiple orchard soils. ASD conducted with ethanol, *Brassica juncea* seed meal, orchard grass (*Dactylis glomerata*) or rice bran provided similar levels of nematode suppression. Subsequent C source input rate studies indicated that orchard grass applied at 20 t ha^{-1} provided optimal level of control for both of the targeted root pathogens.

Relative efficacy of ASD conducted with different carbon inputs was associated with differences in both composition and quantity of volatiles produced during ASD, as well as the resulting conformation of the soil microbiome. When exposed to total volatile composition produced, only the ASD-compost system failed to reduce hyphal growth of *R. solani* AG-5 or cause mortality of *P. penetrans*. Among ASD systems, the one that employed compost as the carbon input yielded the fewest volatiles with demonstrated lowest inhibitory activity against *R. solani* AG-5 and *P. penetrans*. Any biologically active volatiles detected in the ASD-compost system were typically at levels that were lower than those recorded when ASD was conducted with other carbon inputs. Active chemistries detected during ASD treated soils included 2- ethyl-1-hexanol, nonanal, decanal, dimethyl disulfide, dimethyl trisulfide and allyl isothiocyanate, among others.

The ASD-compost and ASD-orchard grass systems were used to conduct a comparative analysis of ASD outcomes on the soil microbiome resulting from effective and ineffective disease control systems. The goal of these studies was to identify biological components that may have a functional role in the overall disease control achieved in response to ASD, as well as define potential bio-indicators which may be used in the future to predict ASD disease control efficacy. Pasteurized and non-pasteurized soil was artificially infested with *R. solani* AG-5 and subsequently ASD was conducted using orchard grass or compost as the carbon input. No significant interaction between soil pasteurization status and ASD C input was observed relative to the quantity of *R. solani* AG-5 DNA detected in soil post-treatment. The quantity of *R. solani* AG-5 DNA detected in ASD-grass treatment was significantly lower than the ASD-compost or control treatments. ASD-grass but not ASD-compost significantly improved apple seedling growth irrespective of soil pasteurization status.

Terminal restriction fragment length polymorphism (T-RFLP) analysis was utilized at the outset to examine treatment effects on microbial community composition in both natural and pasteurized soil. T-RFLP data analysis demonstrated that fungal communities were relatively similar to each other in pasteurized and non-pasteurized soil treated with ASD using grass as the carbon input, and dissimilar to the communities detected in the ASD-compost or no treatment control soils. Analysis of NextGen sequence data was confirmatory of the findings from T-RFLP analysis with regard to the effects of pasteurization and carbon input on fungal bacterial and fungal community profiles. Communities in ASD-grass treated soils were highly similar irrespective of soil pasteurization conducted prior to application of ASD, and were distinct from the same communities detected in the control or ASD-compost treated soils. These results suggest that there is potential for grass-based ASD treatment to recruit microbial elements that persist over the anaerobic phase of soil incubation, which may functionally contribute to disease suppression.

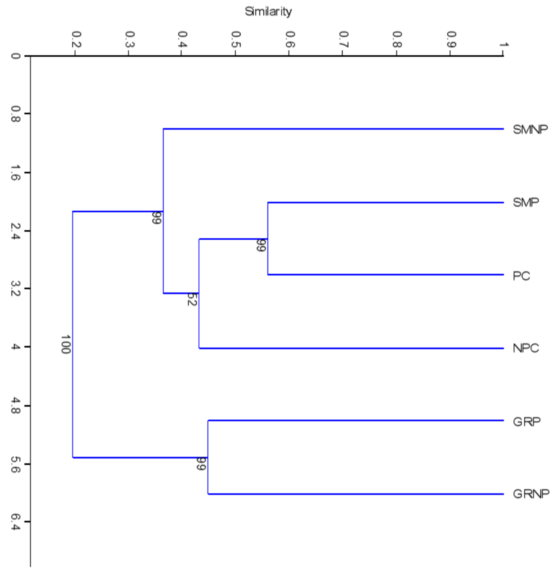

Fig. 1. Effect of pasteurization and different carbon amendments on fungal community similarity in GC orchard soil at completion of a two-week anaerobic incubation period as characterized by cluster analysis of T-RFLP derived data. Soils were pasteurized (P) or non-pasteurized (NP) and amended with composted steer manure (CM), grass clippings (GR) or not amended (C) and inundated to field capacity, bagged and incubated for 2 weeks. The tree was constructed from the Jaccard similarity coefficient of composite profiles from duplicate digestions of a pooled (n=5) DNA sample for each treatment. Bootstrap values are indicated at the tree nodes (based on 10,000 bootstrap replicates).