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Anaerobic soil disinfestation: A chemical-independent approach to pre-plant control of plant pathogens

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Abstract

Due to increasing regulations and restrictions, there is an urgent need to develop effective alternatives to chemical-dependent fumigation control of soilborne pests and pathogens. Anaerobic soil disinfestation (ASD) is one such alternative showing great promise for use in the control of soilborne pathogens and pests. This method involves the application of a carbon source, irrigation to field capacity, and covering the soil with a plastic tarp. While the mechanisms of ASD are not completely understood, they appear to be a combination of changes in the soil microbial community composition, production of volatile organic compounds, and the generation of lethal anaerobic conditions. The variety of materials and options for ASD application, including carbon sources, soil temperature, and plastic tarp type, influence the efficacy of pathogen suppression and disease control. Currently, both dry (e.g., rice bran) and liquid (e.g., ethanol) carbon sources are commonly used, but with different results depending on environmental conditions. While solarization is not an essential component of ASD, it can enhance efficacy. Understanding the mechanisms that mediate biological changes occurring in the soil during ASD will facilitate our ability to increase ASD efficacy while enhancing its commercial viability.

Keywords: anaerobic soil disinfestation, biological soil disinfestation, soilborne pathogens, fumigation

1. Introduction

Since the mid-20th century, fumigation of agricultural soils has been the primary pre-plant method for controlling soilborne plant pathogens, nematodes, and weeds. Pre-plant fumigation practices suppress a wide variety of plant pathogens, from *Verticillium* spp. which affect crops such as strawberries (Shennan *et al.* 2009) and eggplant (Momma *et al.* 2013), to *Agrobacterium tumefaciens* which affects such perennial woody crops as walnuts, almonds, and roses (Agrios 2005; Yakabe *et al.* 2014). Left unchecked, these diseases and many other soilborne pathogens can result in complete crop loss and potentially infect subsequent crops (Martin and Loper 1999). For the past 40 years, methyl bromide (MeBr) has been the dominant soil fumigant used to control soilborne pests for a wide range of high value crops from strawberries (Shennan *et al.* 2009) and tomatoes (Locascio *et al.* 1997), to fruit and nut trees (Ramos 1998). However, the 1993 *Montreal Protocol* required a complete phase-out of MeBr by 2005 in developed countries (http://www.epa.gov/Ozone/mbr), though certain crops and nurseries have been exempt. Since these exemptions will end soon, there is an increasing demand for alternatives (Browne *et al.* 2013; Hanson *et al.* 2013). In addition, current MeBr

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alternatives, such as 1,3-dichloropropene and chloropicrin, are facing increased regulatory pressure as well. For example, there are 47 counties in California, USA, alone with binding township caps that limit application of chloropicrin (Carpenter *et al.* 2001). Barrier films and expanding buffer zones where fumigation is limited are also adding to the cost and difficulty of soil fumigation (Gao *et al.* 2011; Fennimore *et al.* 2013).

Given the limitations and regulations of chemical fumigation, there is a need to develop a biologically-based, integrated management strategy for soilborne diseases that facilitates profitable and sustainable production without the use of chemical fumigants. One such method showing great promise is anaerobic soil disinfestation (ASD), also termed biological soil disinfestation (Blok *et al.* 2000; Butler *et al.* 2012a). In this method, anaerobic conditions are generated after soil is amended with a carbon source (C-source), irrigated to field capacity, and covered with an impermeable plastic tarp (Blok *et al.* 2000; Momma *et al.* 2006). Independently developed in Japan (Momma *et al.* 2006) and the Netherlands (Blok *et al.* 2000), this method is currently being used on a commercial basis in California for strawberries (Muramoto *et al.* 2014), Japan for tomatoes, melons, and cut flowers (Momma *et al.* 2013), and the Netherlands for strawberries, asparagus, and tree nurseries (Shennan *et al.* 2014).

While the basic technique of ASD treatment is straight forward, the efficacy against a given target pathogen varies based on three key parameters: C-source used, tarp type, and soil temperature. As the use of ASD increases, it is critical that growers are provided with options and methods that are most effective for their target pathogens, crop, environmental conditions, and available carbon sources. Here, we provide an overview of the different options available when applying ASD as a pre-plant strategy to control soilborne pests and pathogens. In addition, we summarize the current understanding of the mechanisms that mediate ASD disease suppression.

2. Modes of action

The mode of action of ASD is not completely understood. In preliminary trials, others and we have documented ASD induced changes in the soilborne microbial community composition, production of volatile organic compounds, and the generation of anaerobic conditions that have all been shown to contribute to the suppression of phytopathogenic agents. Each of these factors will be discussed here.

ASD dramatically induced changes in the soil microbial community (Messiha *et al.* 2007; Mowlick *et al.* 2013, 2014), which is hypothesized to antagonize resident phytopathogenic microbial agents (Messiha *et al.* 2007). A next-generation sequence analysis was performed for bacterial 16S rRNA genes amplified from total DNA extracted from soil exposed to ASD conditions for 7 weeks. In these trials using 20.2 t ha^{-1} rice bran, we observed a significantly different bacterial community composition compared to adjacent non-treated soils (Fig. 1). Some of the most dramatic changes we observed in species composition included a significant increase in the abundance of *Clostridiales*, *Acidobacteria*, and *Burkholderia* (Fig. 2). Increases in *Clostridia*, a strict anaerobe, also were found in greenhouse ASD trials using wheat bran, *Brassica juncea*, or *Avena satvia* plants as a C-source (Mowlick *et al.* 2013, 2014).

Another contributing factor to ASD-mediated pathogen suppression is the production of volatile organic compounds (VOCs) such as isothiocyanates, alcohols, organic acids, organic sulfides, and esters (Hewavitharana *et al.* 2014), all of which were reported to contribute to the suppression of *Pythium ultimum*, *Fusarium oxysporum* and *Rhizoctonia solani* AG-5 in greenhouse trials (Hewavitharana *et al.* 2014). The production of acetic, propionic, and butyric acid in ASD treatments using a commercial organic amendment also were correlated with suppression of potato cyst nematode *Globodera pallida* (Runia *et al.* 2014). While similar compounds were generated in ASD trials using different C-sources, the proportions of these compounds differed as

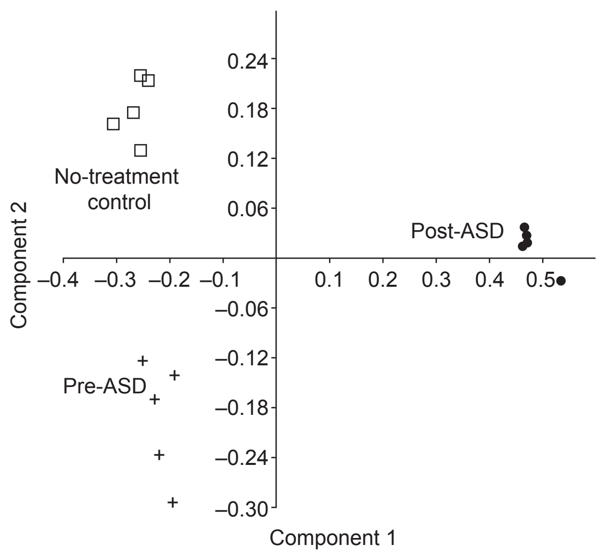

Fig. 1 PCA (variance-covariance) analysis of bacterial community composition data based on unweighted distance measurements (Unifrac) between samples of soils pre-ASD (+), post-ASD (•), and a no-treatment control (□). Post-ASD and no-treatment control samples were collected immediately following 7 weeks of ASD. No-treatment control plots were adjacent to ASD plots. ASD began in August 2013 at the University of California Kearney Agricultural Research and Extension Center in Parlier, CA. Soil was classified as Hanford sandy loam. Each treatment had *n*=5.

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