Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Impacts of anaerobic soil disinfestation and chemical fumigation on soil microbial communities in field tomato production system

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ARTICLE INFO

Keywords:
Phospholipid fatty acid analysis
Molasses
Composted poultry litter
Herbicide
Rhizosphere
Bulk soil

ABSTRACT

Anaerobic soil disinfestation (ASD), a potential alternative to pre-plant chemical fumigation for controlling soilborne pathogens, has been demonstrated in several agricultural production systems. The effect of ASD on the soil microbial community is considered one of the major factors responsible for pathogen suppression. However, rather limited information is available regarding the response of the soil microbial community to ASD throughout the cropping season, particularly in sandy soils. A field experiment was conducted to optimize the ASD technique for tomato production in Florida, utilizing two rates of molasses and composted poultry litter (CPL), and a pre-emergent herbicide application. The pre-plant soil treatments included ASD with 6.9 $m^3 ha^{-1}$ of molasses and 11 $Mg ha^{-1}$ of CPL (ASD0.5), ASD with 13.9 $m^3 ha^{-1}$ of molasses and 22 $Mg ha^{-1}$ of CPL (ASD1.0), and chemical soil fumigation control (CSF). The herbicide treatments included with and without halosulfuron application. Soil microbial community composition was monitored using phospholipid fatty acid (PLFA) analysis during the fall 2015 tomato production season. Halosulfuron application did not result in changes in the soil microbial community during the season. CSF led to significantly lower levels of bulk soil total microbial biomass, Gram negative bacteria, Gram positive bacteria and actinomycetes, compared to the ASD treatments. However, the rhizosphere effect of plants under CSF alleviated the microbial suppression and stimulated the growth of Gram negative bacteria and protozoa to reach similar levels to that of rhizosphere soils under the ASD treatments. Compared to 0 day after transplanting (DAT), Gram positive bacteria in bulk soils under the three pre-plant soil treatments significantly decreased while the fungi:bacteria ratio in bulk soils under CSF significantly increased at 36 DAT, and then remained stable throughout the season. Despite the similar microbial composition in bulk and rhizosphere soils between the two ASD treatments, the dynamic changes of some biomarker groups in bulk soils during 0–99 DAT showed distinct patterns particularly for total microbial biomass, Gram negative bacteria, actinomycetes, and fungi. Compared to 0 DAT, bulk soil microbial community composition shifted after 36 DAT under all soil treatments and remained stable until the end of the season. The changes in soil microbial community composition over time were related to changes in soil nutrient availability.

1. Introduction

Many studies have shown that anaerobic soil disinfestation (ASD) is a potential alternative to pre-plant chemical-dependent fumigation for controlling soilborne pathogens and plant-parasitic nematodes across a range of environments and crop production systems (Momma et al., 2013; Shennan et al., 2014). The ASD method, also known as biological soil disinfestation (Momma, 2015) or reductive soil disinfestation (Huang et al., 2016), was originally developed in Japan (Shinmura et al., 1999; Momma et al., 2006) and the Netherlands (Blok et al., 2000). It involves incorporation of a labile organic carbon source such as molasses, rice bran, wheat bran, cover crop residue, or ethanol into the soil (Momma, 2015), tarping the soil, and saturation of top soil with water to facilitate anaerobic decomposition of the organic carbon (Blok et al., 2000; Butler et al., 2012a, 2014). The treatment can be conducted for as short as 2 weeks to as long as 21 weeks (Strauss and Kluepfel,

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https://doi.org/10.1016/j.apsoil.2017.12.018
Received 26 April 2017; Received in revised form 13 December 2017; Accepted 17 December 2017

2015). The effectiveness of ASD as an alternative to chemical soil fumigation has been demonstrated worldwide and used on a commercial basis for strawberry and tomato production (Momma et al., 2013; Shennan et al., 2014). However, the underlying mechanisms of ASD are not completely understood (Momma, 2015; Strauss and Kluepfel, 2015). Organic amendments have been widely used for biological control of soilborne pathogens in plant production (Bailey and Lazarovits, 2003), but the suppressive effect has been inconsistent (McSorley, 2011). The mechanism for effective control of a wide range of soilborne pathogens and nematodes in a variety of crop production systems using ASD is thought to be similar to the paddy-upland rotation system (Momma et al., 2013). Several factors may contribute to ASD-mediated pathogen suppression via a combination of effects involving production of volatile organic compounds and organic acids, generation of lethal anaerobic conditions, release of metal ions into soil water, and changes in the soil microbial community and diversity (Momma, 2015; Strauss and Kluepfel, 2015; van Agtmaal et al., 2015).

The soil microbial community plays a vital role throughout the ASD treatment. After irrigation to reach soil saturation, it is believed that incorporation of a readily-available carbon source stimulates rapid growth of aerobic microorganisms, which consume the remaining soil oxygen and induce anaerobic soil conditions (Reddy et al., 1986; Messiha et al., 2007). This then could lead to the soil bacterial community to shift to facultative and obligate anaerobes in a short period of time. The amount of time that anaerobic conditions are maintained during ASD is related to soil water content, soil texture and structure, quality and quantity of labile carbon source, and soil temperature (Butler et al., 2012a; Shennan et al., 2014). During ASD treatment, members of the *Firmicutes* phylum, including *Bacillus*, *Clostridia,* and *Paenibacillus,* become the dominant bacterial population (Mowlick et al., 2013, 2014; Strauss and Kluepfel, 2015; Huang et al., 2016). Organic carbon source can significantly influence the dominant bacterial species. For example, using rice bran as a carbon source significantly increased the abundance of *Acidobacteria* and *Burkholderia* (Strauss and Kluepfel, 2015), while *Flavisolibacter*, *Rhodanobacter*, and unclassified-*Ruminococcaceae* became the dominant bacterial genera when using alfalfa as the carbon source (Huang et al., 2016). For vegetable production, polyethylene tarp/mulch can either be removed or punched with holes for crop planting after ASD application, which introduces oxygen into the soil. The restoration of the soil to an aerobic state then restructures the microbial community diversity and biogeochemistry (Husson, 2013). Momma (2015) found that the bacterial community structure shifted 2–15 days after ASD initiation using 1% (v/v) ethanol as the carbon source. van Agtmaal et al. (2015) reported a clear legacy effect of the former anaerobic stress on bacterial community composition three months after the ASD treatment.

Fresh-market tomato is a tremendous vegetable industry for the state of Florida in the United States. In 2015, 13,355 hectares of tomatoes were planted in Florida with a production value of $453 million, which contributed to 35% of the U.S. tomato production and ranked first nationwide (USDA-NASS, 2016). To develop cost-effective and commercially viable approaches to ASD as an alternative to chemical-dependent fumigation for tomato production in Florida, a field experiment was conducted to optimize ASD treatments utilizing different amounts of molasses and composted poultry litter (CPL) and in addition to an herbicide application, with chemical soil fumigation used as the control. Changes in the soil microbial community after the ASD treatment are considered to be responsible for site-specific soil suppression of plant pathogens (Weller et al., 2002; Mazzola, 2004), which may also play an important role in maintaining the suppressive effect of ASD on soilborne pathogens during the cropping season (Momma, 2015). However, rather limited information is available regarding the impacts of ASD on the compositional changes of soil microbial communities throughout the cropping season under field conditions, and effects on the soil microbial community structure of the tomato rhizosphere. Therefore, the objective of this study was to explore the responses of soil microbial communities to differing levels of molasses and CPL amendments and in combination with pre-emergent herbicide application during the whole crop season in a tomato production system. Phospholipid fatty acid (PLFA) analysis was used to measure changes regarding soil microbial biomass and community composition.

2. Materials and methods

2.1. Field experiment

The field experiment was carried out at the University of Florida Plant Science Research and Education Unit in Citra, Florida from August to December 2015. The soil type was classified as Gainesville loamy sand (Hyperthermic, coated Typic Quartzipsamments), and the field had high weed (predominantly nutsedge) and root-knot nematode pressure based on observations from previous production cycles at this location. During field preparation, the entire plot area was thoroughly rototilled at a depth of 15 cm. A split plot design with four replications was used in the field experiment. The pre-plant soil treatment was the whole plot factor, while the herbicide treatment was the subplot factor. The soil treatments included ASD with 6.9 $m^3 ha^{-1}$ of molasses (Agricultural Carbon Source, TerraFeed, LLC, Plant City, FL, USA) and 11 Mg ha^{-1} of CPL (ASD0.5), ASD with 13.9 $m^3 ha^{-1}$ of molasses and 22 Mg ha^{-1} of CPL (ASD1.0), and chemical soil fumigation control with Pic-Clor 60 (Soil Chemical Corporation, Hollister, CA, USA) at a rate of 224 kg ha^{-1} (CSF). No non-treated soil control was included in this study because the focus was on optimizing the ASD treatment in comparison with the soil fumigation practice used by tomato growers (Guo et al., 2017). The herbicide treatments included halosulfuron (Sandea®, Gowan Company, Yuma, AZ, USA, containing 75% active ingredient) application at a rate of 70 g ha^{-1} (with halosulfuron), and the without halosulfuron application control.

Soil treatments in the whole plots were arranged in a randomized complete block design with four replications (blocks). In each of the four blocks, three raised beds (24.4 m long, 0.9 m wide, 0.30 m high, and 1.8 m between centers) were formed with each assigned randomly to ASD0.5, ASD1.0, or CSF. The pre-plant compound fertilizer (10 N-10 P_2O_5-10 K_2O) was applied to each bed to provide 56, 56, and 56 kg ha^{-1} of N, P_2O_5, and K_2O, respectively. For setting up the ASD0.5 and ASD1.0 treatments, CPL and molasses with a 1:1 (v:v) water dilution were applied to the bed top and then tilled to a depth of 15 cm with a rotary cultivator to work the amendments into the soil. The 24.4 m long bed in each whole plot was divided in half, and herbicide halosulfuron (Sandea) treatment and no-herbicide control were randomly assigned to each of the half bed plots. Accordingly, each treatment combination occupied a bed length of 12.2 m. After herbicide application, beds with CSF treatments were fumigated by shank injection. All beds were reformed and covered with a 0.025-mm white (on black) VaporSafe® TIF (Raven Industries Inc., Sioux Falls, SD, USA) polyethylene mulch containing an ethylene vinyl alcohol (EVOH) barrier layer. Two drip irrigation lines were installed approximately 2.5 cm below the soil surface under the mulch in each bed. Only beds with ASD treatments were irrigated once using 69 kPa water pressure for four hours to saturate air-filled soil pore space in the top 10 cm of the bed (5-cm irrigation) to enhance the development of anaerobic conditions (Butler et al., 2012a). All beds were monitored for three weeks prior to tomato transplanting.

After the three-week soil treatment, tomato cultivar 'Tribute' (Sakata Seed America, Morgan Hill, USA) at the fourth-true-leaf stage were transplanted on September 3, 2015, with in-row spacing of 0.45 m and 26 plants in each subplot. Plants were watered twice a day using a timer-controlled drip irrigation system, with irrigation time initially set at 30 min and adjusted as plants grew. Fertilizer 6 N-0 P_2O_5-8 K_2O plus micro blend (2% Ca, 0.4% Mg, 0.02% Zn, and 0.02% B) (Mayo Fertilizer Inc., Mayo, FL, USA) was injected weekly through the drip tape starting 7 days after transplanting (DAT) to provide the remaining