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Characterization of bacterial communities in solarized soil amended with lignocellulosic organic matter

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ABSTRACT

Solarization can provide thermal inactivation of weed seeds and phytopathogens through passive solar heating of moist soil covered with clear plastic tarp. Microbial respiration in soils, especially those with increasing levels of organic matter, can augment solarization to produce soil temperatures higher than those achieved by solar heating alone. Currently, it is unclear how solarization affects microbial community structure in soils amended with organic matter to promote microbial activity. In this study, a field trial was conducted in the San Joaquin Valley of California to solarize an irrigated, agricultural field soil which was either amended with mature green waste compost destabilized with wheat bran, or not amended. Following 22 days of treatment during July–August 2011, soil from three depth increments (0–5.8, 5.8–11.6, and 11.6–17.4 cm) was subjected to 16S ribosomal RNA gene sequencing to characterize microbial communities. The sequencing data obtained revealed similar microbial species richness and evenness in both solarized amended and non-amended soil. However, the taxonomic composition of communities differed by treatment. Furthermore, community structure within each treatment changed with soil depth, indicating potential enrichment of thermophilic bacteria in layers that experienced greatest heating, as well as changes related to alterations in the soil atmosphere. Certain bacteria detected in solarized, compost-amended soil may be relevant to agriculture and plant biomass deconstruction processes.

1. Introduction

Solarization is a method of soil disinfestation which may be used as an alternative to synthetic chemical application. It is primarily accomplished by inducing thermal inactivation of soil pathogens and weed propagules. The technique involves covering moist soil with clear plastic tarp during the warmest months of the year (Stapleton, 2000). The resulting passive solar heating of the mulched soil can lead to soil temperatures much higher than ambient, and beyond the tolerance of many mesophilic organisms, including certain undesirable microbes and weeds (Candido et al., 2011; Pullman et al., 1981). However, the pesticidal efficacy of solarization may be limited by factors including climate, time of year, treatment duration, soil depth, susceptibility of target pest organisms, and others (Stapleton, 2000). Numerous studies have shown that combining various organic soil amendments with solarization may result in increased pesticidal activity. Prior work has shown that amending soil with mature green waste compost destabilized with wheat bran prior to solarization can increase temperatures during solarization as a result of heat generation stemming from microbial consumption of organic matter (Simmons et al., 2013). Activated spores and other survival structures of thermophilic/thermotolerant organisms present within the mature compost likely complement those of the altered

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microbial community within heated soil to drive rapid degradation of incorporated organic matter (Simmons et al., 2013). While coupling biological activity from organic amendments with solarization to increase soil heating can lead to more effective thermal inactivation of undesirable organisms, it is currently unclear how this strategy affects soil microbial communities.

Previous studies have examined microbial community shifts in soil as a result of compost addition and solarization (Culman et al., 2006; Gelsomino and Cacco, 2006; Schönfeld et al., 2003). However, microbial community changes that occur in response to the targeted destabilization of soil through addition of destabilized compost have not been studied in the context of solarization. Furthermore, no studies have utilized the sensitivity of next-generation 16S rDNA gene sequencing technology to capture the taxonomic diversity of microbial communities in amended and solarized soil. Decomposition of lignocellulosic plant matter in high-temperature environments at low moisture levels is also relevant to the discovery of industrially relevant, thermotolerant/thermophilic microorganisms and enzymes potentially useful for biofuel production (Reddy et al., 2011). Examination of the microbial communities that develop during decomposition of organic matter under the dynamic temperature regimens characteristic of soil solarization may yield information important to the understanding of organic matter decomposition in soil and in extreme environments.

The objective of this study was to investigate the effects of soil amendment with destabilized compost on temporal and spatial changes in soil microbial community structure during solarization. The specific emphasis was on solarized soil due to the limited research on the effects of organic amendment on microbial community structure during solarization. It also complimented prior research on the effects of organic amendment on soil heating during solarization (Simmons et al., 2013). The hypothesis was that soil community structure during solarization would be affected by the incorporation of compost. In this report, microbial community compositional changes occurring in compost-amended and non-amended, solarized field soil, as analyzed by high-throughput 16S rRNA gene sequencing are presented. Ordination and ecological analyses were performed to measure differences in community structure between amended and non-amended, solarized soils as a function of soil depth.

2. Materials and methods

2.1. Soil and microcosm preparation

Full details describing soil collection and amendment are presented elsewhere (Simmons et al., 2013). Briefly, these studies were performed on experimental soil contained within microcosms buried at a field site at the University of California Kearney Agricultural Research and Extension Center, (KARE) in Parlier, CA (36.6° N; 119.5° W; elevation 96.9 m), in order to finely control conditions relating to soil amendment, temperature measurement and soil collection by depth. Soil for microcosms was collected from the field site prior to preparation. The soil was classified as Hanford fine sandy loam and the contents of organic matter, sand, silt and clay were 0.0151 g (dry g)$^{-1}$, 0.41 g (dry g)$^{-1}$, 0.37 g (dry g)$^{-1}$ and 0.22 g (dry g)$^{-1}$, respectively (Marshall et al., 2013). Mature green waste compost (Zamora Compost, Yolo County, CA) and autoclaved wheat bran (Giusto's Vita Grain, South San Francisco, CA) were used as soil amendments. Autoclaved wheat bran was added to increase the organic matter level and microbial activity in the soil + compost mixture. To prepare amended soil, compost and wheat bran were added to the soil mass to achieve 8% and 2% (dry weight basis), respectively. Non-amended field soil served as a control. Soil mixtures were wetted with water to a moisture content of 400% (dry weight basis), sealed in plastic bags, and equilibrated overnight in ambient conditions. Microcosms were prepared by loading 3.8 l (17.8 cm diameter, 17.4 cm height when filled) black plastic Grow Bags (neHydro, 114 Southampton, MA) with soil mixture. Thermisters (Onset Computer, Bourne, MA) were positioned at depths of 5.1 and 12.7 cm in select microcosms to measure temperature during solarization.

2.2. Solarization

Solarization was performed between July 8, 2011 and July 30, 2011. Full details regarding field preparation are presented elsewhere (Simmons et al., 2013). The field site consisted of 5 separate plots arranged linearly. One microcosm containing amended soil and one control microcosm containing non-amended soil were buried within each plot, such that there were five replicates for each treatment across all plots. The top of each buried microcosm was flush with the soil line. Microcosms were spaced 0.6 m from one another within plots and were at least 0.9 m from plot borders to avoid edge effects (all measurements were from the center of the microcosm). Arrangement of microcosms within plots was random. Plots were covered with 0.7 mil (17.8 μm) transparent plastic tarp (Huskey Painter Plastic from Home Depot). The tarp was gently flattened to remove air bubbles from beneath it. Tarp edges were buried in trenches along the border of each plot. Solarization lasted 22 days with temperature readings taken every 10 min. Following solarization, microcosms were exhumed and stored at 1 °C overnight. Microcosms were transported from the field site to the laboratory under ambient conditions (approximately 3 h). Microcosms were then sectioned horizontally every 5.8 cm to isolate various depths. Microcosm sections were stored at −20 °C.

2.3. DNA extraction and 16S rDNA sequencing

Microbial DNA was extracted from solarized, non-amended and amended soil samples using a Powersoil DNA Isolation Kit (MO-BIO, Carlsbad, CA). DNA was also extracted from non-solarized field soil, green waste compost, and compost plus wheat bran-amended soil for comparison to solarized samples. Soil and compost samples were well-mixed prior to DNA extraction. Duplicate extractions were performed for each sample and the purified DNA was pooled. Sequences corresponding to the 16S rRNA gene were amplified from DNA extracts using PCR. Each 50 μl PCR was comprised of 1× 5 Prime HotMasterMix (Fisher, Pittsburgh, PA), 0.2 μM forward primer, 0.2 μM reverse primer, and approximately 0.4 μg/ml template. Forward primer was 515f primer with a 5′ Illumina adapter amended via pad and linker sequences (5′AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGGTAA). Reverse primer was 806r primer with Illumina adapter compliment, barcode, pad and linker sequences amended to the 5′ end (5′CAAGCAGAAGACGGCATACGAGAT(12 bp barcode)CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTCC(1–3 bp pad)GGACTACHVGGGTWTCTAAT). Reactions underwent an initial incubation at 94 °C for 3 min followed by 35 cycles of 94 °C for 45 s, 50 °C for 1 min, and 72 °C for 1.5 min. Reactions concluded with a final incubation at 72 °C for 10 min and a hold at 94 °C. Reaction products were purified with Agencourt AMPure XP (Beckman Coulter, Brea, CA) in accordance with the manufacturer's instructions using 1.2× volume of bead solution. The size of purified products was measured on a DNA 7500 LabChip (Agilent, Santa Clara, CA) with an Agilent 2100 Bioanalyzer using the manufacturer's protocol. Amplicons were sequenced on an Illumina MiSeq system as described by the manufacturer's protocol. A PhiX Control v3 library (Illumina, San Diego, CA) was spiked