

EFFECT OF MOLASSES IN ANAEROBIC SOIL DISINFESTATION: FOCUS ON THE SOIL MICROBIOME

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Growers in Florida have begun to apply molasses to increase the soil microbial biodiversity. Previous reports have indicated that a greater microbial diversity could cause disease suppressive soils (van Bruggen and Semenov, 2000). Furthermore, it has been shown that under moderate temperature, 15-20 °C, an increase of carbon could be more effective in for creating a disease suppressive soil (Butler et al., 2014). The current standard for applying ASD in Florida consists of incorporating composted broiler litter (CBL), 22 Mg ha⁻¹, and molasses, 13.9 m³ ha⁻¹ as the soil amendments, covering the soil with a polyethylene film, and saturating the soil through irrigation. The objective of this study was to compare various rates of molasses, 0-4 times the standard rate (treatment 2): treatment 1 = 0, 2 = 13.9, 3 = 27.7, 4 = 41.6, and 5 = 55.5 m³ ha⁻¹. This study compared the effect of the molasses on the soil microbiome and factors that are associated with the microbiome including, soil pH, moisture, and cumulative redox potential (Eh). In conjunction with the microbial data, tomato plant vigor and weed count were also measured.

The experiment was performed during the fall/winter season of 2015 in greenhouse conditions at University of Florida, IFAS SWFREC (Immokalee, FL). Soil contained in 10 L plastic pots was amended with CBL and the various rates of molasses. Soil was then saturated with 5 cm of water and covered with Vaporsafe Totally Impermeable Film. Three-week post ASD treatment, the film was removed and a tomato seedling, cv Ridge Runner (Syngenta), per pot was transplanted. The experiment was conducted in a randomized complete block design, with four replications. Five pots made up an experimental unit.

Soil cores for each replication of each treatment were homogenized. Samples were taken at 1, 3, 7, 14, and 21 days post ASD treatment (dpt), and were frozen at -20 °C. DNA was extracted using a modified protocol of the MO BIO PowerSoil® DNA Isolation Kit. Length heterogeneity-PCR (LH-PCR) was used to determine bacterial population. LH-PCR was performed as a normal PCR, using the V2 and V3 primers, except the V2 primer was tagged with fluorescent tag (6-fluorescein amidite) on the 5' region. Detected amplicons were interpreted by Genemapper v5.0 (Applied Biosystems, Foster City, CA, USA) and analyzed by PRIMER v6 and PERMANOVA+ software packages.

Shifts in the microbiome correlated to molasses rate, changes in soil pH, and reduction potential (Figure 1). During the treatment, pH dropped for all of the treatments except for treatment 1, and treatments 3-5 remained acidic at 21 dpt. All of the treatments with molasses went anaerobic. From day 1 to day 3 the bacterial communities, as detected by LH-PCR, shifted except for treatment 1 (Figure 2). Bacterial diversity decreased for the treatments 3-5 at 7 dpt, and these communities were static. The relative abundance of population 435 was 438 were greater in treatments 3-5 than treatments 1 and 2. These populations correspond to members of the *Bacteroidetes* and *Proteobacteria* phyla respectfully.

Plant phytotoxicity and mortality were observed in treatments 4 and 5. Regarding leaf, stem, and total dry plant biomass, treatments 1 and 2 were similar, while these variables were inversely proportional to the molasses rate. In conclusion, CBL alone did not change the soil pH and had little effect on the soil microbiome over the 21-day treatment. Furthermore, molasses applied greater than $13.9 \text{ m}^3 \text{ ha}^{-1}$ can select for bacterial communities that lower the pH and can cause phytotoxic environments.

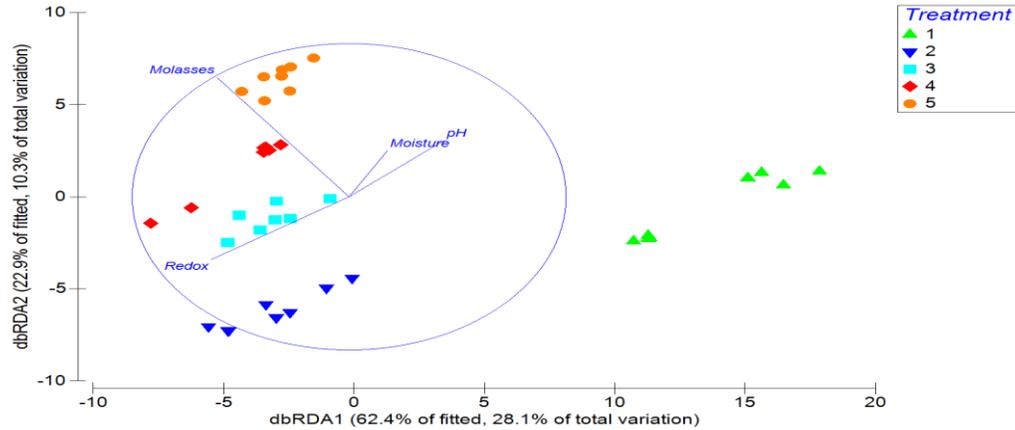


Figure 1. Comparison of microbial populations during anaerobic soil disinfestation to environmental factor: molasses concentration (Molasses), soil moisture (Moisture), soil pH (pH), and cumulative redox potential (Redox).

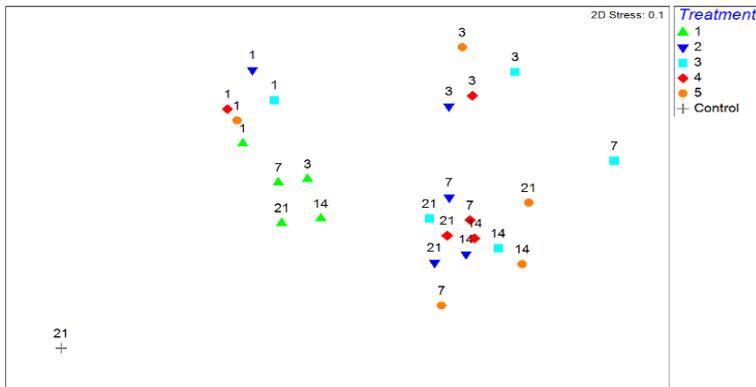


Figure 2. Non-metric multidimensional scaling of the average abundances of soil bacterial populations sampled 5 times, 1, 3, 7, 14, and 21 days post anaerobic soil disinfestation (ASD), as indicated in the figure.

References

- Butler D. M., Shrestha U., Dee M. E., Eichler Inwood S. E., McCarty D. G., Ownley B. H., Kokalis-Burelle, N., and Roskopf E. N. (2014) Low carbon amendment rates during anaerobic soil disinfestation (ASD) at moderate soil temperatures do not decrease viability of *Sclerotinia sclerotiorum* sclerotia or fusarium root rot of common bean. In: VIII international symposium on chemical and non-chemical soil and substrate disinfestation, vol 1044, Torino, Italy, pp 203–208.
- Van Bruggen, A. H. C., and Semenov, A. M. 2000. In search of biological indicators for soil health and disease suppression. *Appl. Soil Ecol.* 15:13-24.