

**PROGRESS REPORT SUBMITTED TO
THE SOUTHERN REGION SMALL FRUIT CONSORTIUM**

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**TITLE: IMPROVING SOIL HEALTH FOR STRAWBERRY PRODUCTION IN
NORTH CAROLINA**

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ABSTRACT

Soil-borne disease complex is a persistent constraint in strawberry production systems in North Carolina and the Southeast. Plant pathogenic fungi and poor soil conditions cause this disease complex. Anaerobic soil disinfestation (ASD) may be a viable and environmentally safe strategy to manage this complex. Our main objective of this project was to advance biologically based solutions for strawberry production systems that contribute to soil health, microbial communities, disease suppression, and marketable yield. We conducted this experiment at Horticultural Crops Research Station, Castle Hayne, NC in the 2021-2022 season. We evaluated and compared the ASD using a full rate of molasses and mustard meal, each, and a combination of half rate of molasses and mustard meal each compared to fumigation with PicClor-60, and an untreated control. Four weeks after soil treatments were initiated, cv. ‘Chandler’ was transplanted, and soil health and plant biomass data were collected to assess treatment effects. Total yield was assessed bi-weekly and cumulative yields were calculated in lbs./A. Soil and root samples were collected at peak harvest to determine the abundance of bacterial and fungal communities through an amplicon sequencing approach. All ASD treatments, and fumigant PicClor-60, significantly increased total yield compared to untreated control. The relative abundance of bacteria phyla *Gemmatimonadota*, *Planctomycetota*, *Patescibacteria*, *Acidobacteriota*, *Chloroflexi*, *Bacteriodota*, *Cyanobacteria*, and *Actinobacteriota* generally increased whereas *Firmicutes* and *Proteobacteria* decreased. Similarly, the phylum *Ascomycota* was dominant in rhizosphere soil samples whereas phyla *Mycoromycota*, *Blastocladiomycota*, *Basidiobolomycota*, and *Ascomycota* were dominant in root samples. These findings provide insights into the impacts of ASD on soil health, strawberry yield, and microbial communities, and can be used to optimize ASD as a biologically based solution for sustainable regenerative agriculture.

INTRODUCTION

Black root rot (BRR) is a major disease complex of cultivated strawberries, caused by pathogenic fungi (*Pythium irregulare*, and *Rhizoctonia fragariae*) in North Carolina and surrounding states

(Abad et al. 1999; LaMondia 2003). BRR causes the death of feeder roots and the degradation of structural roots resulting in an overall decrease in productivity (Maas 1998) and can cause up to 40% yield losses (<https://content.ces.ncsu.edu/black-root-rot-of-strawberry>). To manage this disease complex, methyl bromide (MeBr), a soil fumigant, has been used in strawberry production. However, due to health and environmental concerns, MeBr was phased out for commercial use. Non-chemical cultural practices such as soil amendments, composts or crop residues, and cover crops other than host resistance, have been utilized and significantly enhanced C: N ratios and favorable soil chemical properties to improve soil health indicators, increase yield, and reduce soil-borne pathogens in vegetables and strawberry (Bernard et al. 2012; Cohen and Mazzola 2006; Fang et al. 2012; Larkin et al. 2011; Leandro et al. 2007a, 2007b; Litterick et al. 2004; Watanabe et al. 2011). Evaluating the performances of organic amendments and comparing them with soil fumigant (PicClor 60), and untreated control (UTC) treatments may help develop biologically sustainable disease control options in strawberry production systems.

RESEARCH OBJECTIVES:

The specific objectives of this study were to:

- 1). evaluate the efficacy of anaerobic soil disinfestation (ASD) + organic amendments in comparison with soil fumigation on marketable fruit yield
- 2). determine the effects of ASD + organic amendments on soil health, soil microbial communities, and disease suppression

MATERIALS AND METHODS:

The field trial was conducted at Castle Hayne Research Station, Castle, Hayne, NC. The soil of this research station consisted of a history of black root rot complex. We arranged the experiment in a randomized complete block design with four replications. Each replication had three beds side-by-side and each plot was 30' long. Based on previous experiments, we selected and further evaluated five treatments in the 2021-2022 season. These treatments were (i) Group 1 = PiClor-60 @ 350 lbs./treated A (control); (ii) Group 2 = no fumigation (untreated control); (iii) Group 3 = molasses (full rate) @ 5000 lbs./A; (iv) Group 4 = mustard meal (full rate) @ 2000 lbs./A, and, (v) Group 5 = molasses (half rate) 2500 lbs./A + mustard meal (half rate) @ 1000 lbs./A. These plots had the same treatments in the previous two years.

We prepared beds from late August to early September, incorporated organic amendments, and covered them with plastic mulch. To induce high moisture and anaerobic conditions in the topsoil, we applied drip irrigation via the buried lines. We used impermeable film and installed redox electrodes hooked up to data loggers. We transplanted the strawberry cultivar 'Chandler in October and soil samples were collected using standard protocols at the time of land preparation (baseline), pre-planting, and peak harvest. Brookside Lab, OH, analyzed soil health analysis. We calculated the soil health index using the 1-day CO₂-C divided by the organic C: N ratio plus a weighted organic carbon and organic N addition. The 1-day CO₂-C was determined as an indicator of microbial respiration utilizing an IR Gas Analyzer CO₂-C and was expressed as ppm/24 hrs. after the soil has been dried and then rewetted using protocols developed by Cornell Soil Health Testing protocols (<https://soilhealth.cals.cornell.edu>) and analyzed by Brookside laboratories, Inc, Ohio.

To determine the effects of organic amendments on microbial communities, we collected soil (rhizosphere) and roots (endosphere) within the harvested area of each plot at peak harvest. DNA was extracted and 16S (for bacterial communities) and ITS (for fungal communities) regions were sequenced at GSL, NCSU, and Novogene Company, Sacramento, CA. Sequence data were analyzed to find the relative abundance of bacterial and fungal phylum in each treatment or group. Strawberries were harvested on a semiweekly basis from each treatment for eight weeks. Marketable yield was analyzed and compared means and treatment effects (treated vs control plots) using SigmaPlot v.14 (Systa Software Inc.).

RESULTS AND DISCUSSION

Soil health parameters analysis. Treatment had a high impact on soil health indices at planting but decreased over time or at peak harvest. The application of the full rate of molasses sharply increased soil health value (which is ~ seven value) followed by the full rate of the mustard meal (Fig. 1). ASD with half rate of molasses and a half rate of mustard meal did not create as strong of a soil health value response but it was stable until at peak harvest. The fumigated plots and UTC decreased soil health values at planting and they slowly recovered by Peak Harvest.

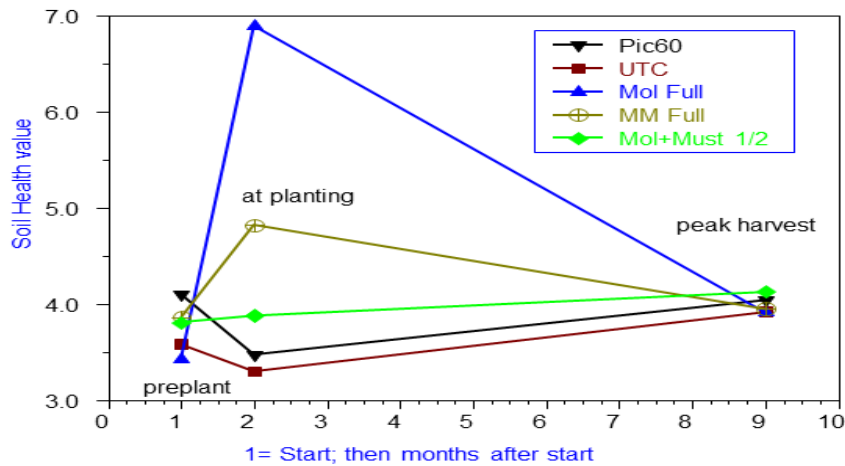


Fig. 1. Soil health values as calculated from soil samples taken just before field preparation, planting time (Oct 2021), and peak harvest (June 2022) the following spring.

Treatments also affected the soil pH values. Changes in soil pH following ASD varied with treatments (Fig. 2). The overall mean pH values were higher in the treated plots and all plots seemed to normalize by the spring.

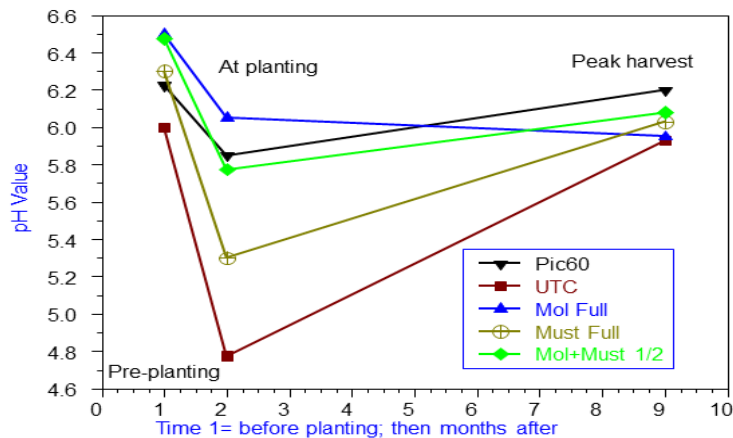


Fig. 2. pH values were measured from soil samples taken just before land preparation, planting time (Oct 2021), and peak harvest (June 2022) the following spring.

Marketable yield. Plants were relatively uniform in the middle beds of each plot. Total yield was assessed semi-weekly; cumulative yields were calculated in lbs./A (Fig. 3). Molasses + Mustard combined at half rates each produced the highest yield (~21,000 lbs). Molasses applied at full rate (Mol Full), Mustard meal (Must Full) full rate, and PicClor60 generated similar total yields and were not significantly different (Fig. 1). The UTC plots had the lowest yields.

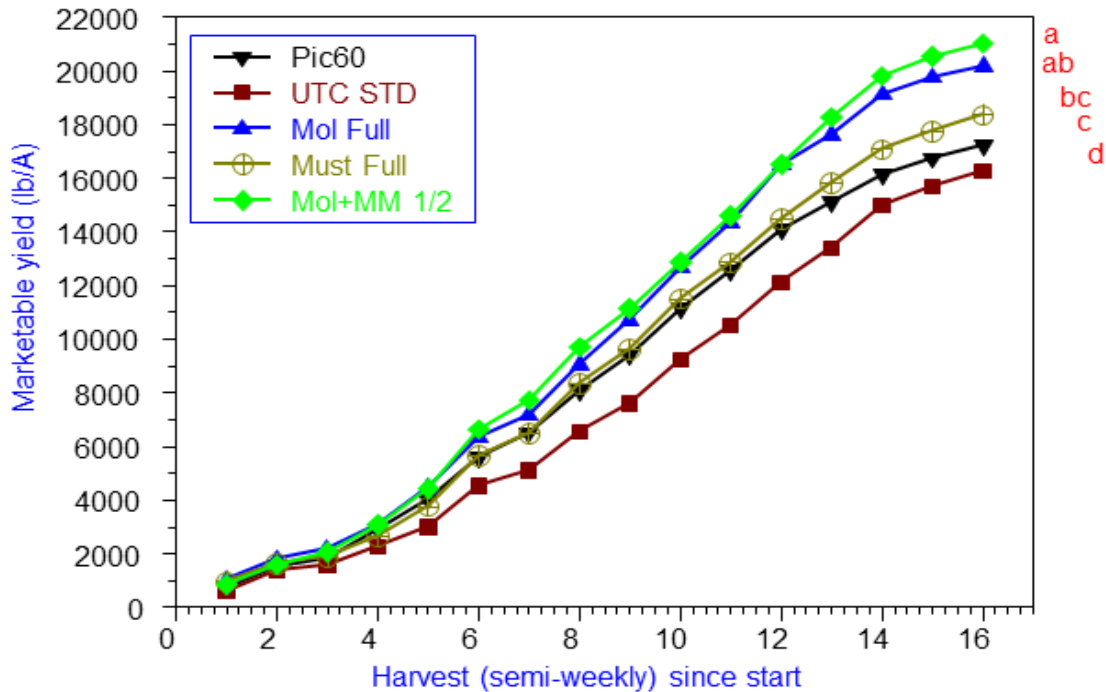


Fig. 3. Cumulative yield (April to June) over eight weekly harvests as impacted by pre-plant soil treatments. Progress curves followed by the same letter are not significantly different from each other based on repeated measures analysis and the Fisher Protected LSD (P=0.05). Acronyms were described in the text.

Microbial communities. The amplicon sequencing of the rhizosphere and root samples from all treatments was performed according to the 16S rRNA protocols for bacterial communities, and ITS1/ITS2 protocols for fungal communities. The bacterial community composition in the rhizosphere and root samples varied at the phylum level. Among the rhizosphere samples, the bacterial community composition was notably different across ASD-treated plots. Overall, the relative abundance of *Firmicutes* and *Proteobacteria* decreased whereas that of *Gemmatimonadota*, *Planctomycetota*, *Patescibacteria*, *Acidobacteriota*, *Chloroflexi*, *Bacteriodota*, *Cyanobacteria*, and *Actinobacteriota* increased with organic amendments (Fig. 4A). In root samples, *Patescibacteria*, *Gemmatimonadota*, *Planctomycetota*, and *Acidobacteriota* were the dominant phyla. In general, the relative abundance of *Proteobacteria* and *Cyanobacteria* in roots decreased in all treatments (Fig. 4B).

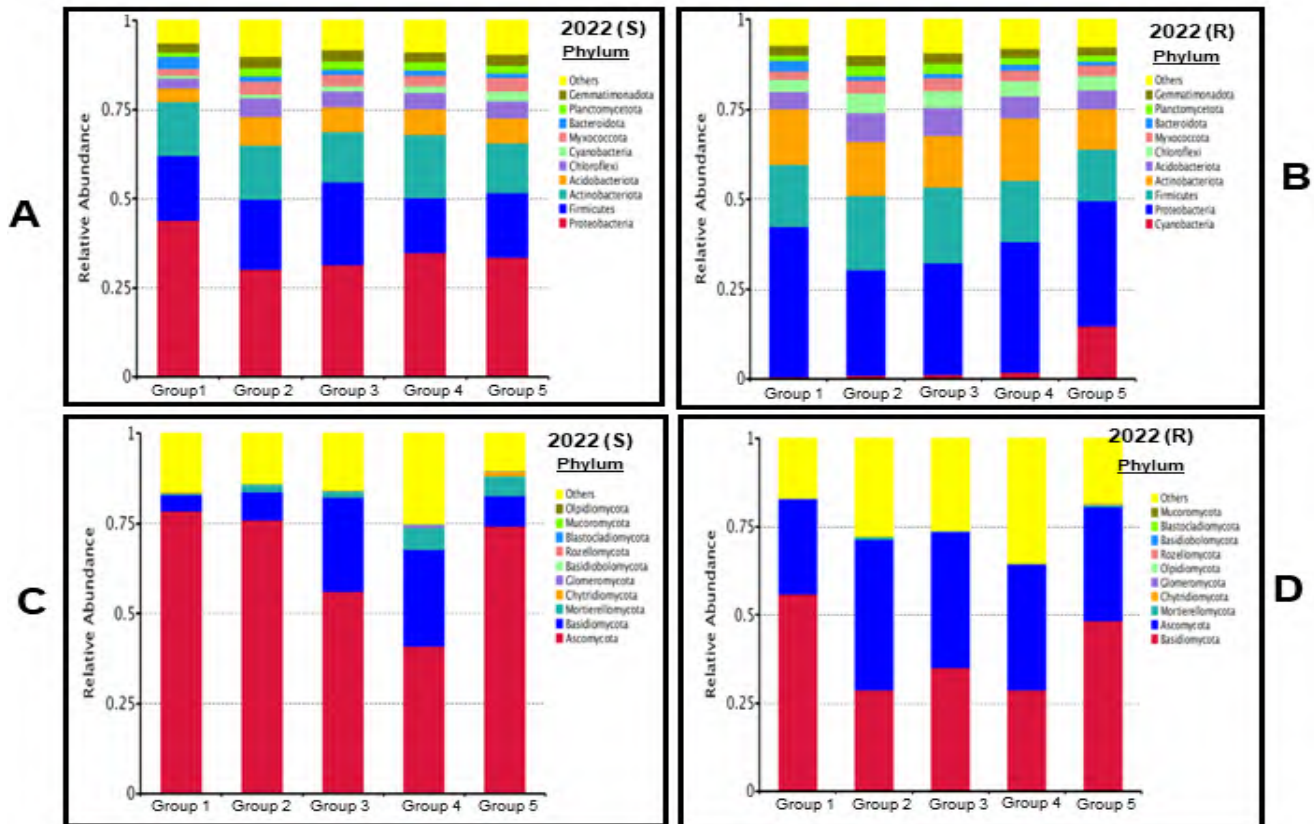


Fig. 4. Effects of ASD-mediated organic amendments on the relative abundance of microbial communities in the experimental field. The bacterial community compositions of the rhizosphere (S) and endosphere (R) at the Phylum level. Fungal composition of the rhizosphere (S) and endosphere (R) at the Phylum level. Group 1 = Pic-Clor 60; Group 2 = Untreated control; Group 3 = ASD + Molasses (5,000 lbs/A); Group 4 = ASD + Mustard meal (2,000 lbs/A), and Group 5 = ASD + ½ Molasses + ½ Mustard meal.

The fungal community composition at the phylum in the rhizosphere soil and root samples are shown in Fig. 4C and 4D, respectively. *Ascomycota* was the dominant phylum in all rhizosphere soil samples. The relative abundance of *Olpidiomycotia* was higher in ASD with half-rate molasses and half-rate mustard meal compared to other treatments. In root samples, the relative abundance was highest among the phyla of *Mucoromycota*, *Blastocladiomycota*, *Basidiobolomycota*, and *Ascomycota*, among others.

IMPACTS: The research project generated a robust data set that impacts soil health. Biologically based soil treatments were as effective as or better than the standard fumigant in advancing plant growth and increasing marketable yields. Comparatively, the ASD treatments produced higher plant biomass, marketable yield, and affected microbial communities. This study represents a step toward a more mechanistic understanding of how ASD influences plant growth and yield, and how microbial community composition mediates strawberry plant health and soil-borne disease suppression. Future work will focus on investigating additional sources of locally available carbon sources and the biological functions of the beneficial microbiome complemented with optimizing selected beneficial strains for commercialization in strawberry production systems.

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