Evaluating Bacterial Diversity and Pest Control Efficacy of Steam Disinfestation Treatments in the Salinas Valley Spinach and Lettuce Fields

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Introduction

This project investigates the efficacy of steam disinfestation for sustainable pest and pathogen control in Salinas Valley spinach and lettuce fields. Using a custom-built steam applicator, the study validates its practicality by assessing weed control, pathogen management, and the treatment's impact on the soil microbial community. The trials were conducted from July to September 2023 on lettuce and spinach fields at the USDA-ARS/UCCE Hartnell research farm

Research Design

Trial 1: Spinach Field Microbiome Analysis

- Steam treatment consisted of four beds, 60 ft long by 3.33 ft wide plots.
- The two control beds were 45 ft long by 3.33ft wide plots
- 10 soil samples were gathered pre and post treatment

Trial 2: Lettuce Field Steam Disinfestation Efficacy for Pest Management

- Tested 4 different band configurations (width by depth)
- Each treatment was replicated 4 times in a single 40-in wide by 120 ft long bed
- Treatments:
  - Non-treated
  - Kerb
  - 4"Wx3"D
  - 4"Wx5"D
  - 5"Wx3"D
  - 5"Wx5"D

Methodology

Trial 1

Soil Sampling:
- 2 samples were collected per steamed bed at 20ft and 40ft Pre and Post treatments (n=20)
- 1 sample per control bed was collected at 22 ft

DNA Extraction and Sequencing
- DNA extraction with Qiagen DNeasy PowerSoil Pro Kit
- 16S amplicon sequencing processed in the Illumina MiSeq System QIIME2

Statistical Analysis
- Clam method, Principal Components Analysis (PCA) and Non-metric multidimensional scaling (NMDS) in Rstudio

Trial 2

Weed Assessments
- Weed density counts by species were numbered per 1 ft². Total weed counts were normalized based on plots

Soil temperatures
- Monitored by HOBO data loggers (Onset computers)

Statistical Analysis
- Factorial analysis of variance on ARM, with LSD's (p-value<0.05) for mean separation
- Pairwise comparisons and t-test in RStudio

Soil Sampling:
- Treatments were sampled and combined at 40ft and 80ft along the beds (n=20 Pre/Post)
- Microbiome analysis in progress

Pythium Protocol:

- **Day 1** • Prepare Pythium medium
- **Day 2** • Weight 1g (x3 tubes) for each sample with autoclaved water.
- **Day 2 part 2** • Place the soil plate into five plates
- **Day 3** • 1st Reading
- **Day 4** • 2nd Reading

Image 1. Plate with Pythium spp. colonies
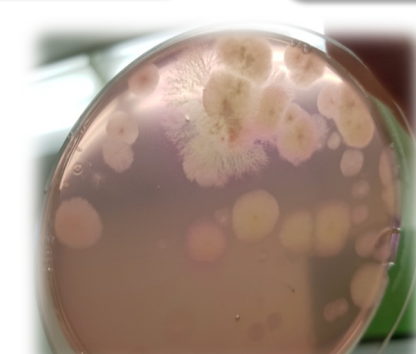
Steam Process

Image 2. Steam applicator in Hartnell research farm

			Timeline		
Trial/ Crop	Steam	Planting	Weed Assessment	Pre-Soil Sample	Post Soil Sample
1. Spinach	7.27.23	7.30.23	na	7.26.23	7.28.23
2. Lettuce	8.29.23	8.31.23	9.19.23	8.28.23	8.30.23
	8.30.23				8.31.23
					10.4.23

Table 1. Critical dates for the two trials in Salinas, CA
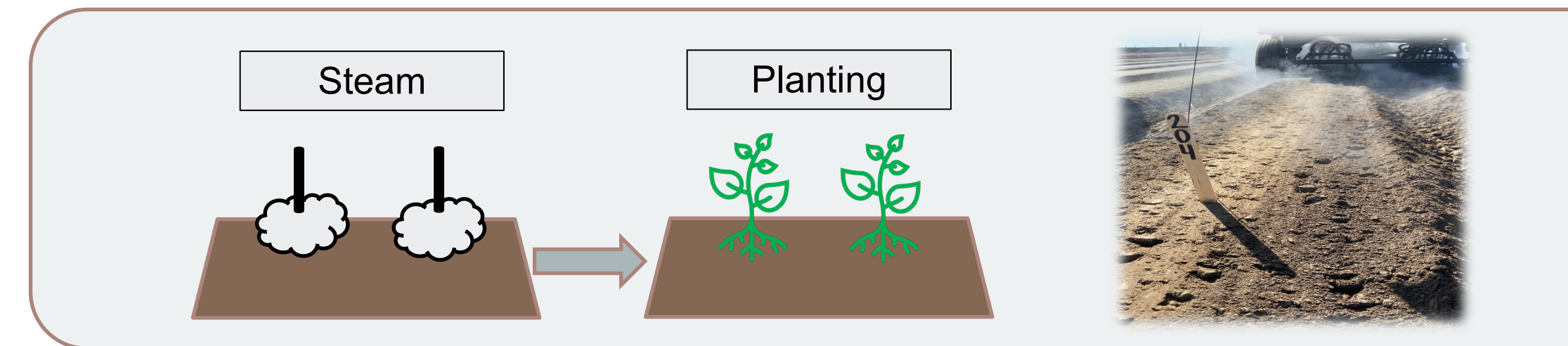
Image 3. Steam Applicator Process Diagram

Results

Similarity and Variation of Bacterial Population

Figure 1 and 2 : Figure 1 (Left): Non-metric multidimensional scaling (NMDS) plot on microbial communities' similarity before and after treatments in spinach fields (n=20). Figure 2 (right) PCA plot on microbiome composition's variability before and after treatment in spinach fields to investigate beta diversity of microbes (n=20)

Bacterial Species Grouping Analysis
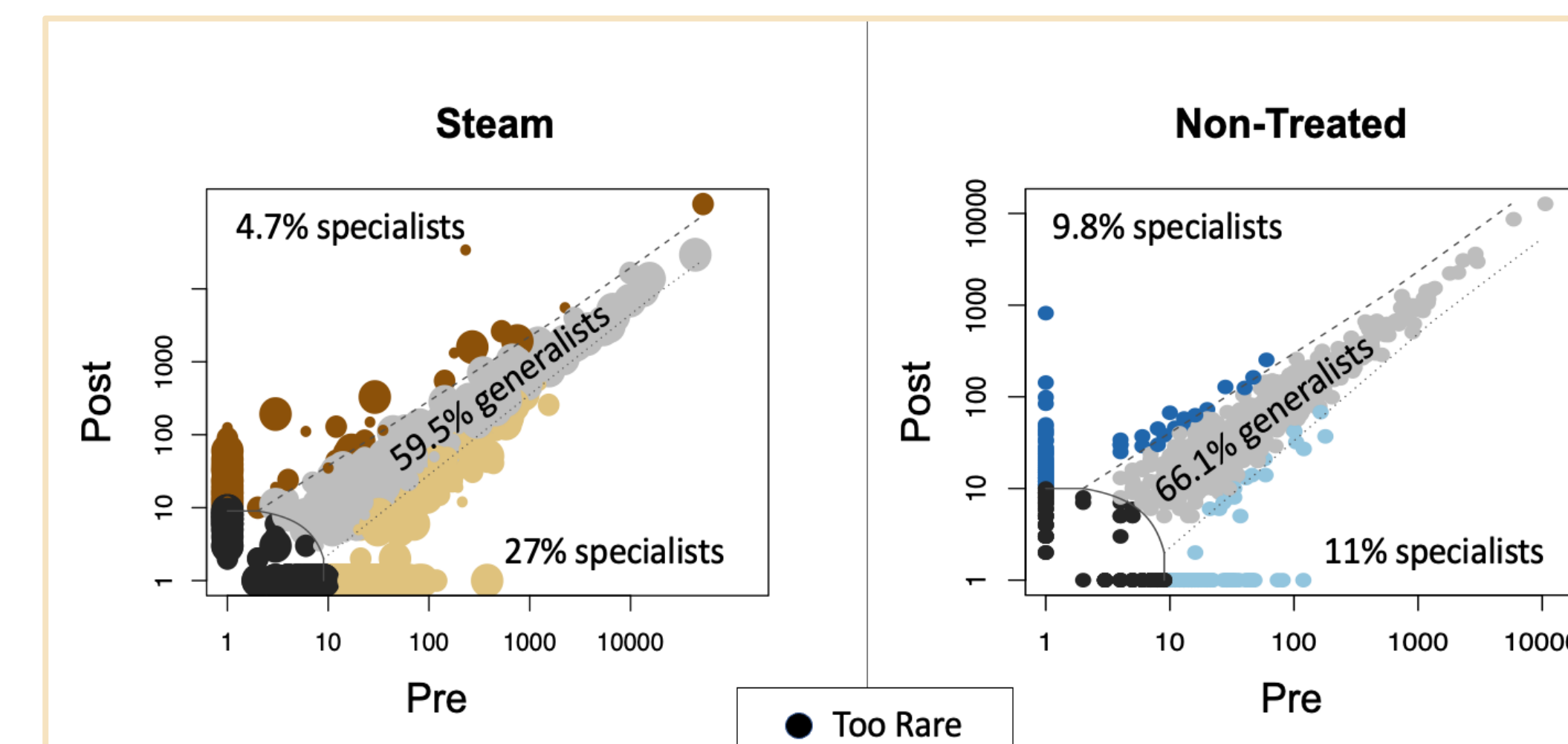
Figure 3 and 4 : Figure 3 (Left): Multinomial species classification method (CLAM) for the niche occupancy test for steam pre and post treatment (n=16). Figure 4 (right) CLAM for the niche occupancy for the non-treated pre and post sampling (n=4)

Pathogen Control

Figure 5. Affect of steam band width and depth treatments on Pythium Spp. before and after treatment (two-sided p-value <.*05, **.01,***.001)

Weed Control

Figure 6. Total weed counts among four steamed treatments, Kerb, and control in trial 2 (dunnett method versus control, LSD (p=0.05))

Figure 7. Average Temperatures of HOBOS across four steam treatments over a 1-hour span
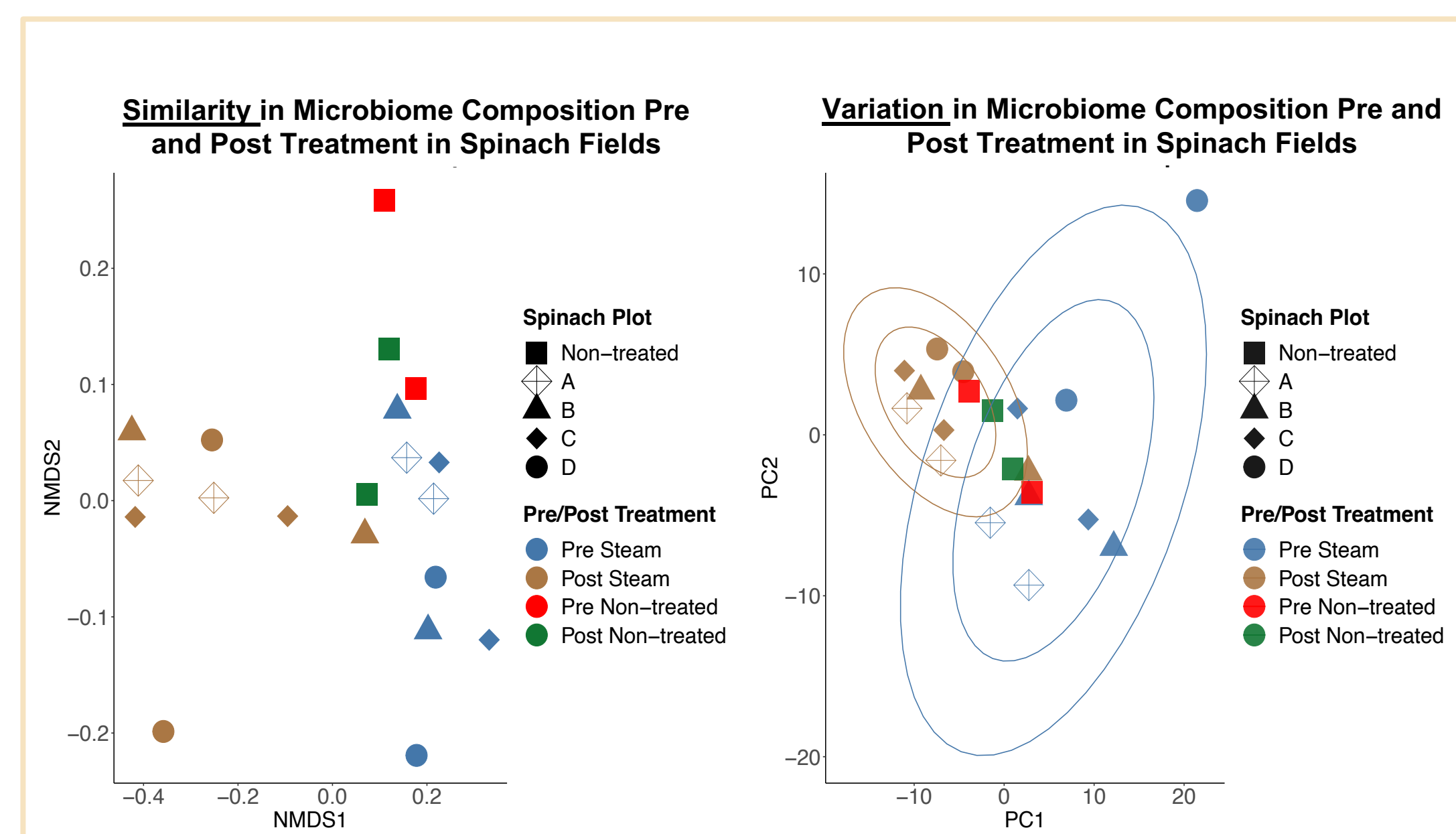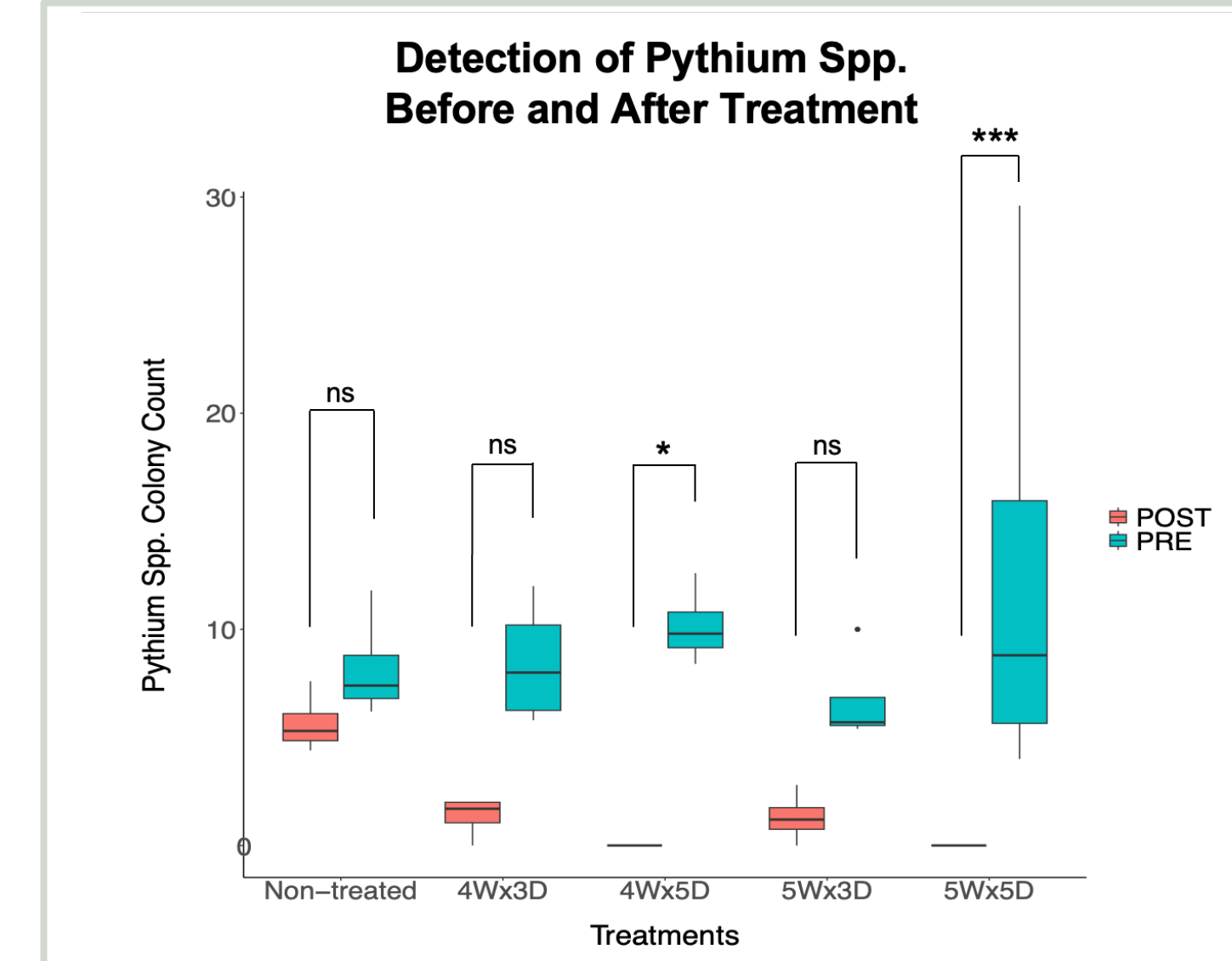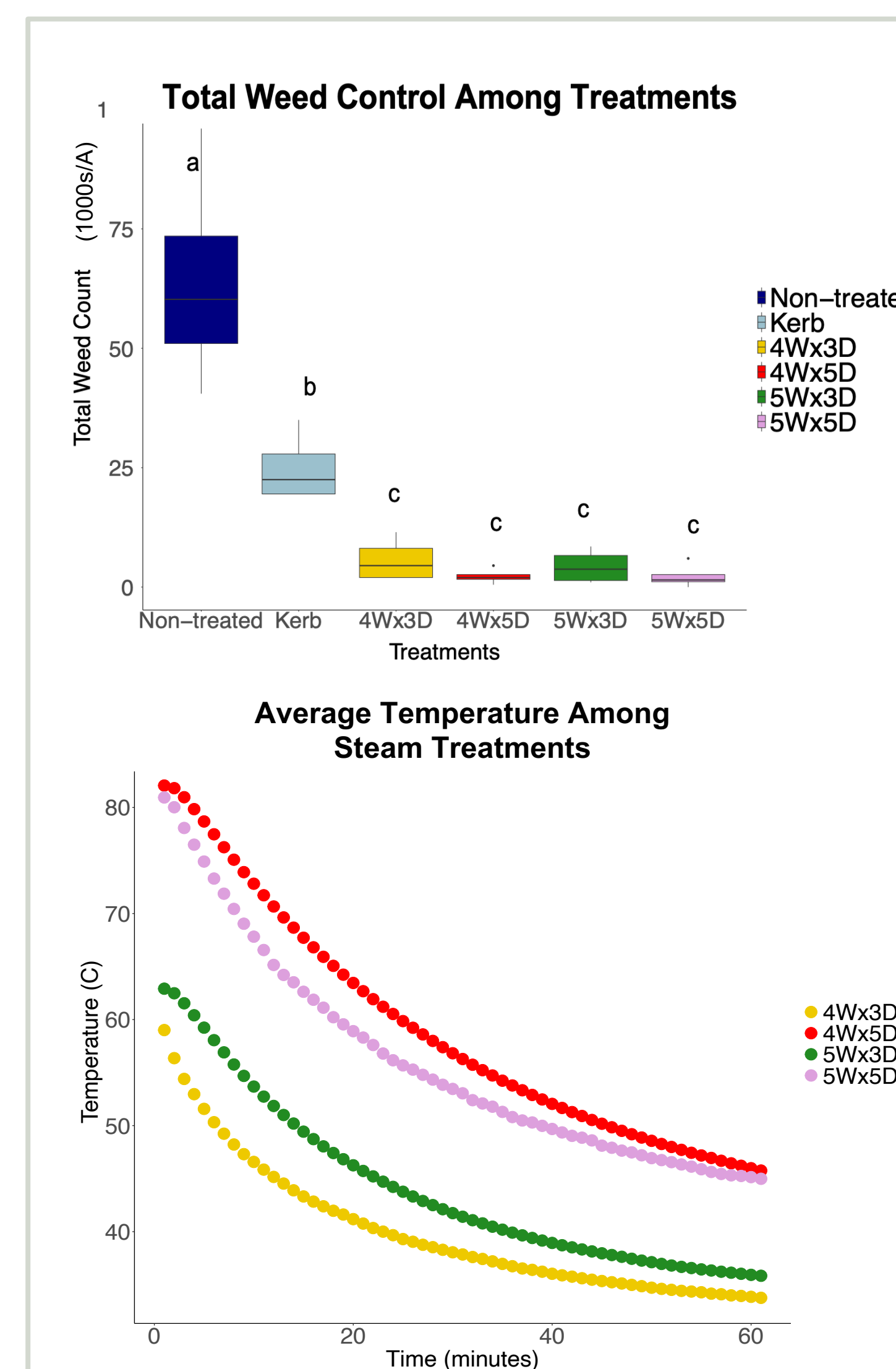
Discussion

Trial 1

- Dissimilarities between pre- and post-treatment samples indicating a population change (figure 1)
- The variance decreased after steam treatment indicating a uniform transformation (figure 2)
- Bacteria specialist were reduced after treatment compared to non-treated possibly due to sampling error (figure 3 and 4)

Trial 2

- The total weed count in all steam treatments was statistically significantly higher than in the non-treated (figure 6)
- The 4Wx5D steam treatment had the fewest weeds and the highest temperature (figure 6 and figure 7)
- Pathogen control significantly improved with a 5'' depth (figure 5)

Future Work

- Continue steam disinfestation efficacy for the summer of 2024
- Soil samples from Trial 2 were sequenced and will be assessed for bacterial diversity
- Follow-up analysis on the lettuce and spinach trials will focus on identifying specific taxonomic losses in bacterial populations

Image 4. Trial 2 lettuce field in Hartnell research farm

Acknowledgements