biology and life sciences forum

Organic and Regenerative Farms are a Gold Mine for Potentially Novel Species of Soil Bacteria

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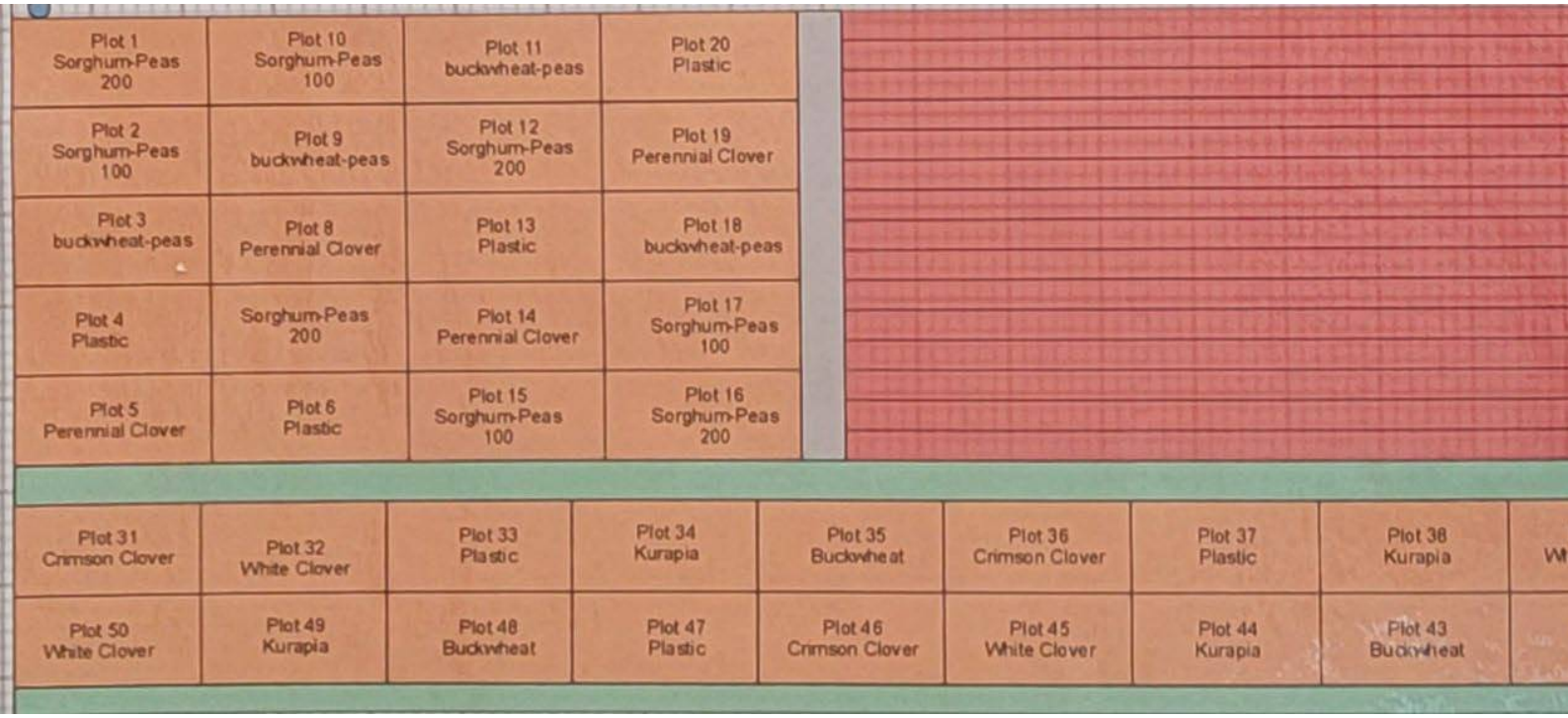


Artichoke plots were sampled for soil 3 months after the cover crops were seeded.

Methods

- Between Fall 2021- Spring 2023 soil metabarcoding samples and soil bacterial isolates were collected from Los Angeles Pierce College farm and local farms, and sequenced on Illumina MiSeq and Sanger platforms.

Materials and Methods

- Soil physical and chemical properties from the sites were also measured including pH, EC, TDS, organic matter by dry combustion, texture by touch, and nitrogen, phosphorus and potassium by spectrophotometry.

Materials and methods

- Soil physical and chemical properties data were compared with reference measurements from the USDA NRCS soil surveys.

- Comparisons between fields were carried out in the R stats package.

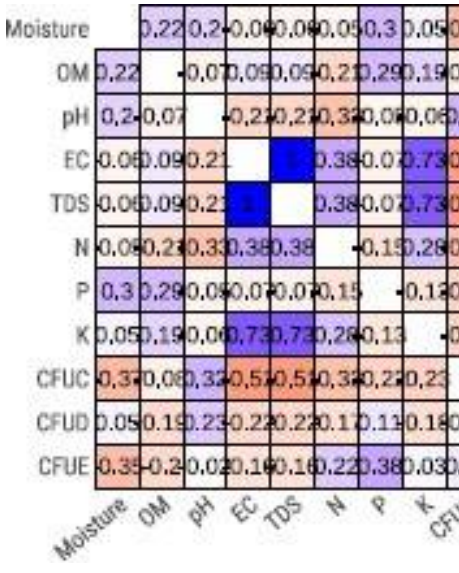
Barcoding and Metabarcoding Methods

- The cultivated bacteria were isolated on Nutrient Agar, Luria Broth Agar, or ISP-6 medium.
- Barcoding isolate DNA was extracted using 10% chelex solution heated for 10 minutes at 100 C.
- Quality control was achieved through electrophoresis using the E-gel system.

- DNA extraction from soil samples used the Qiagen DNEasy Power Soil Kit.
- Quality control was achieved with spectrophotometry using the ThermoFisher MultiSkan SkyHigh Microplate Spectrophotometer.
- Barcoding data analysis was performed on the DNA Subway Blue Line and EZBioCloud; metabarcoding data analysis was carried out using the DNA Subway Purple Line.

Results- Soil Science

Preliminary data showed that the conditions between the fields differed significantly in values for organic matter, N, K, and pH ($p<0.05$); this offered a diverse panel of substrates for discovery.



It is apparent that Potassium concentrations are making the highest contribution to EC and TDS, based on the Pearson correlation matrix.

It also appears that the CFU concentrations for the 10^-5 dilution were mildly associated with higher N and P levels.

Organic Matter (OM)

According to the results of the nonparametric Kruskal-Wallis rank sum test, Field was associated with OM (p=0.02).

```
##
##  Pairwise comparisons using Wilcoxon rank sum exact test
##
## data:  OM and Field
##
##            Arboretum Marquis _C Marquis_A Marquis_B N_side Rodale S_Side
## Marquis _C 0.37      -          -         -         -      -      -
## Marquis_A  0.74      0.29       -         -         -      -      -
## Marquis_B  0.23      0.23       0.74      -         -      -      -
## N_side     0.23      0.18       0.35      0.78      -      -      -
## Rodale     0.58      0.29       0.85      1.00      0.98   -      -
## S_Side     0.18      0.18       0.29      0.28      0.58   0.85   -
## Vineyard   0.72      0.29       1.00      0.58      0.30   1.00   0.35
##
## P value adjustment method: BH
```

Nitrogen (N)

According to the results of the nonparametric Kruskal-Wallis rank sum test, Field was associated with N (p=0.02).

```
##
##  Pairwise comparisons using Wilcoxon rank sum exact test
##
## data:  N and Field
##
##            Arboretum Marquis _C Marquis_A Marquis_B N_side Rodale S_Side
## Marquis _C 0.70      -          -         -         -      -      -
## Marquis_A  0.70      0.12       -         -         -      -      -
## Marquis_B  0.90      0.70       0.70      -         -      -      -
## N_side     0.75      0.70       0.20      1.00      -      -      -
## Rodale     0.70      1.00       0.21      1.00      0.70   -      -
## S_Side     0.68      0.70       0.12      0.70      0.80   0.70   -
## Vineyard   0.20      0.20       0.12      0.70      0.12   0.28   0.19
##
## P value adjustment method: BH
```

Potassium (K)

According to the results of the nonparametric Kruskal-Wallis rank sum test, Field was associated with K (p=3.24*10^-5).

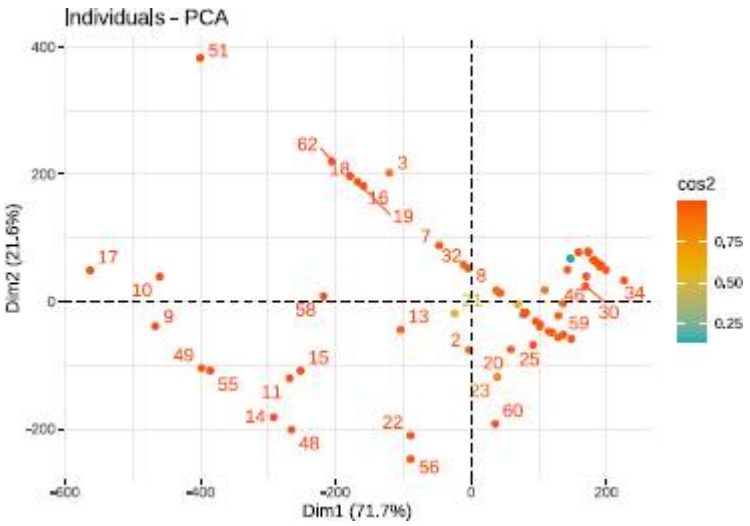
```
##
##  Pairwise comparisons using Wilcoxon rank sum exact test
##
## data:  K and Field
##
##           Arboretum Marquis _C Marquis_A Marquis_B N_side Rodale S_Side
## Marquis _C 0.2333    -         -         -         -      -      -
## Marquis_A  0.0975    0.1063    -         -         -      -      -
## Marquis_B  0.5091    0.5091    0.8969    -         -      -      -
## N_side     0.1063    0.0346    0.0099    0.2333    -      -      -
## Rodale     0.1600    0.2333    1.0000    1.0000    0.0711 -      -
## S_Side     0.7333    0.5091    0.1600    0.6087    0.1063 0.2333 -
## Vineyard   0.2333    0.2333    0.3742    1.0000    0.1063 0.5091 0.3294
##
```

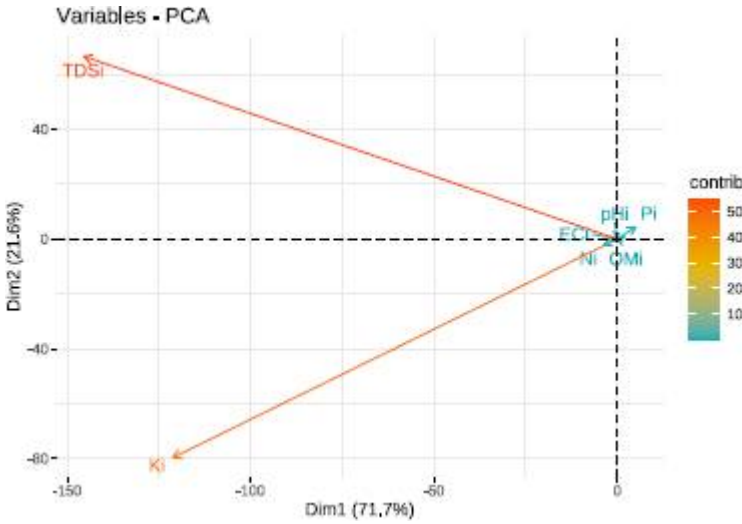
pH

According to the results of the nonparametric Kruskal-Wallis rank sum test, Field was associated with pH (p=0.002).

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  pH and Field
##
##           Arboretum Marquis _C Marquis_A Marquis_B N_side Rodale S_Side
## Marquis _C 1.000     -         -         -         -      -      -
## Marquis_A  0.093     1.000     -         -         -      -      -
## Marquis_B  0.691     1.000     0.642     -         -      -      -
## N_side     0.103     0.673     0.651     0.370     -      -      -
## Rodale     0.103     0.123     0.063     0.123     0.063  -      -
## S_Side     0.138     0.493     0.258     0.210     0.493  0.119  -
## Vineyard   0.123     1.000     0.093     0.143     0.063  0.123  0.103
##
## P value adjustment method: BH
```

Principal Components Analysis





Results- Barcoding

- In spring 2021 and fall 2022, 137 bacterial isolates from Pierce College, Rodale Institute for Organic Agriculture in Camarillo, and Tulare, CA were screened; this

investigation revealed up to 30 potentially novel species based on the partial 16S sequences.

- The soil samples from fall 2022 were taken from pasture, fallow, cover

  crop, and hemp plantings.

- The purpose of the cover crop trial was to test replacement of plastic mulch with living mulch and cover crops in strawberry and artichoke production.

Sample Name	Number	Gel Number	Lane	Pass QC?	Passed sequencing?	Concensus sequence	% Similarity	% Completeness
21-C1-ER	56	1	7	Yes	Yes	ANGACGCTGGCGGCNNG	86.63	51.5
21-C1B-ER	61	6	2	Yes	Yes	TGTCGGCAGCGTCAGATG	93.68	36.8
21-C2-ER	58	1	9	Yes	Yes	CCTACGGGGGGCTGCAGT	91.16	31.4
21-C3-ER	57	1	8	Yes	Yes	CCTACGGGGGGCTGCAGT	89.39	31.7
21-C3A-ER	59	1	10	Yes	Yes	TCGGCAGCGTCAGATGTG	94.21	36.3
21-E1-ER	55	1	6	Yes	Yes	AATACGNTGGCACCTTGA	83.17	31.3
21-E1A-ER	60	1	11	Yes	Yes	CANTTCGTCGGCAGCGTC	93.53	38.8
38-D1-CW	49	13	10	Yes	Yes	TTTTGTATAAGGATAAACC	91.41	26.9
38-E1-CW	66	6	7	Yes	Yes	CAGTTTGTCGGCAGCGTCA	92.51	38.7
38-E3-CW	65	6	6	Yes	Yes	TTCGTCGGCAGCGTCAGA	93.06	39.6
4-D1-ms	75	8	6	Yes	Yes	TCGTNGGCAGCGTCAGAT	95.03	35
41-E1-GR	21	10	2	Yes	Yes	GTTGATNATGGCTCAGAA	95.86	57.3
43-E2-DY	19	4	10	Yes	Yes	GAGCCCGNGTCGCATTAG	95.94	37.6
47-D1-EV	46	13	7	Yes	Yes	ACGCTGGCGGCNNGCCTA	94.12	51.6
47-D1-SR	63	6	4	Yes	Yes	GTCGGCAGCGTCAGATGT	93.61	36.3
47-D2-SR	64	6	5	Yes	Yes	GTCGGCAGCGTCAGATGT	92.68	38
47-D3-AM2	88	11	9	Yes	Yes	AGAAGGCACCTTGACGGT	73.69	39.2
47-E1-SR	62	6	3	Yes	Yes	CCTACGGGGGGCTGCAGT	89.95	31.3
ARB-C2-YK	72	8	3	Yes	Yes	CCTACGGGGGGCTGCAGT	92.17	31.9
ARB-D1-MA	68	6	9	Yes	Yes	GTNGGCAGCGTCAGATGT	94.51	36.1
ARB-D1-YK	70	6	11	Yes	Yes	TTGTNGGCAGCGTCAGAT	94.57	36.9
ARB-D2-MA	67	6	8	Yes	Yes	GTCGGCAGCGTCAGATGT	92.95	36.2
ARB-D2-YK	71	8	2	Yes	Yes	GTCGGCAGCGTCAGATGT	93.9	38.8
ARB-E2-DB	14	4	5	Yes	Yes	CTTTTTGCGGCGGACGGGT	85.93	44.3
Arb-e3-ma	69	6	10	Yes	Yes	TGTCGGCAGCGTCAGATG	94.57	36
D1-47-SR	24	10	5	Yes	Yes	CAGTAGAGTTTGATCATGG	90.28	51.5
D2-21-AL	29	10	10	Yes	Yes	CTGGCGGCNTGCTNNNAC	92.76	49.3
D6-21-AL	27	10	8	Yes	Yes	TGACGCTGGCGGCNNGCT	87.32	45.3
P24-C1-AE	10	2	11	Yes	Yes	CTTGCTCCCGGGATTAGTG	94.06%	46.10%
P24-E1-AE	9	2	10	Yes	Yes	TGACGCTGGCGGCGTGCT	95.05%	50.60%

Barcoding- Example Potentially novel isolates from our study

JinMa-D5-SS Bacillus pumilus ATCC 7061 % ID 92.94 Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus

JinMa-E6-SS Streptomyces montanus NEAU-C151 % ID 94.51 Bacteria;Actinobacteria;Actinomycetia;Streptomycetales;Streptomycetaceae;Streptomyces

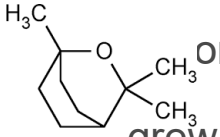
JinMa-D6-MAR Lysobacter antibioticus ATCC 29479 % ID 79.81 Bacteria;Proteobacteria;Gammaproteobacteria;Lysobacterales;Lysobacteraceae;Lysobacter

Results- Essential Oil effect on microbes

- Some of the more notable genera discovered from the isolates were *Lysobacter, Streptomyces,* and *Bacillus*, which include antibiotic producers.
- Some of the bacterial isolates were challenged with crude ethanolic extracts of tea tree and lemon balm. Percent transmittance was measured at 600 nm.
- The growth was similar between replicated treatments for

putative *Bacillus mojavensis, Curvibacter lanceolatus, Streptomyces bobili.*

- Both tea tree and lemon balm were effective at controlling *Bacillus pumilus* compared with the control, whereas putative *Pseudomonas segetis* grew more when treated with either

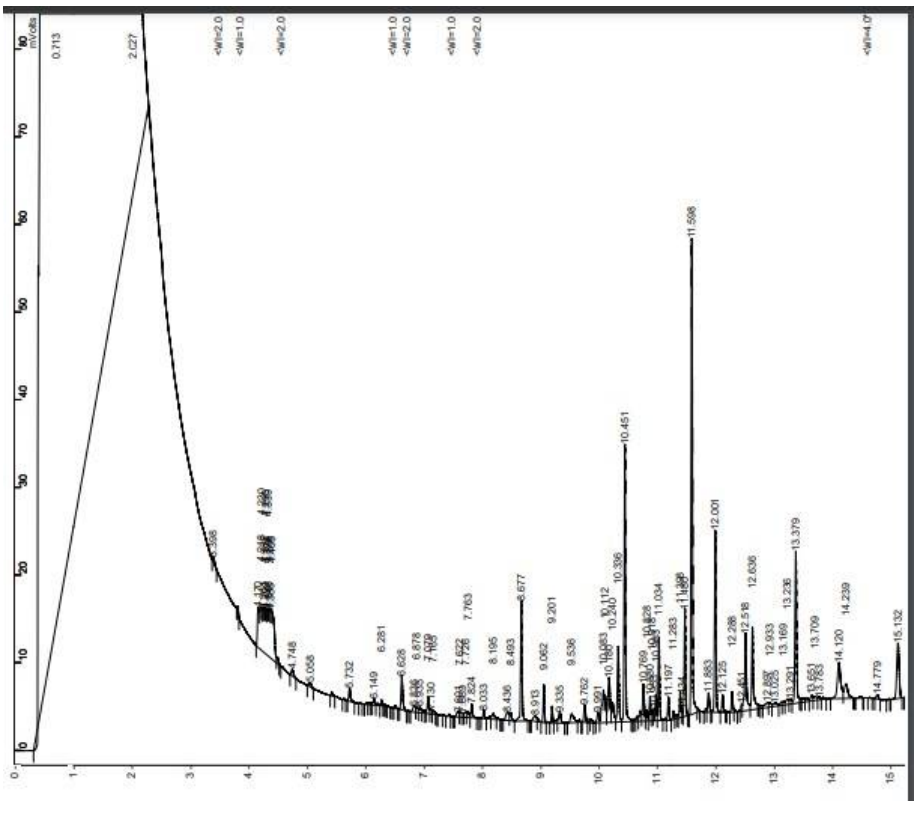


on balm or tea tree extracts, and *Paracoccus marcusii* grew more when treated with tea tree oil versus the control.

Eucalyptol Caryophyllene

GC Preliminary Results: Lemon Balm

The GC Standard used was Eucalyptol (1,8-Cineole) and Caryophyllene Oxide (Cannabis Terpene Standard #2, Restek) with 2500 ug/mL



Chromatogram of

Lemon Balm crude extract (5.2 uL/mL IPA)

Lemon balm is expected to have caryophyllene oxide but does not

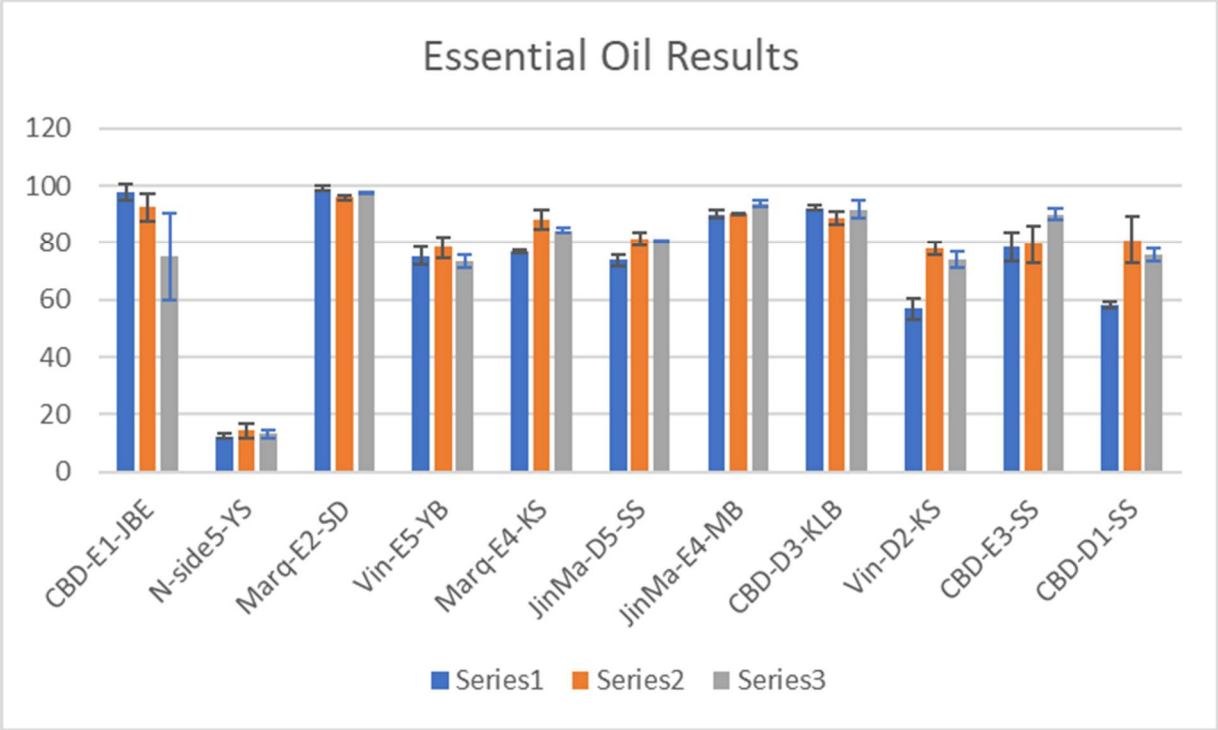
contain eucalyptol.

The main peak is believed to be Caryophyllene

Further studies will report percent composition of the main terpene components.

Essential Oil Testing Results

	Paracoccus marcusii	Bacillus mojavensis	Curvibacter lanceolatus	Curvibacter lanceolatus	Unknown	Bacillus pumilus	Streptomyces bobili	Curvibacter lanceolatus	Pseudomo
	CBD-E1-JBE	N-side5-YS	Marq-E2-SD	VIn-E5-YB	Marq-E4-KS	JinMa-D5-SS	JinMa-E4-MB	CBD-D3-KLB	VIn-D2-KS
C	97.93333333	12.43333333	99.06666667	75.26666667	76.93333333	73.93333333	90.16666667	92.4	
Std. Error C	2.878850079	0.676592771	0.920748488	3.090487196	0.698410895	2.073912031	1.476858528	0.723417814	
T	92.53333333	14.43333333	96.03333333	78.43333333	88.16666667	81.5	90.2	88.76666667	
Std. Error T	4.838847429	2.611725696	0.876229295	3.725736318	3.235394532	2.163330765	0.37859389	2.503553031	
L	75.23333333	13.1	97.83333333	73.5	84.46666667	80.6	93.9	91.8	
Std. Error L	15.14621331	1.42243922	0.260841656	2.357965225	0.788106028	0.458257569	0.929157324	3.113411848	



Discussion and Conclusion

- Additional work is needed to characterize the plant terpenes. Soil properties should be remeasured since the cover crop trial is now in year 2.
- More investigation into the taxonomic

composition and

predicted functions of the potentially novel soil bacteria is necessary.

- Further research will focus on identification of the main terpene

components of the plant extracts by gas chromatography.