Comparative study of the microbiome of the native plant *Ceanothus velutinus* (snowbrush) from different locations and greenhouse studies

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Abstract

Climatic change is one of the biggest threats to the ecosystem and biodiversity by enhancing environmental stresses. Environmental stresses such as biotic and abiotic stresses affect plant health and reduce crop production. The rhizosphere microbiome of a plant plays a significant role in a plant's defense against various biotic and abiotic stresses. In this study, we are investigating the microbiome diversity of bulk soil, rhizosphere, and endosphere of *Ceanothus velutinus*, snowbrush. *Ceanothus* is an evergreen native plant that is usually found in dry areas and thrives well in harsh conditions. The snowbrush samples were collected from different locations 1920m, 1950m, and 2289m of the Tony Grove area of the Intermountain West region of the United States. The snowbrush plants propagated from cuttings under the greenhouse conditions were treated with the native soil. The rhizosphere and roots samples were collected from treatment and control plants after 3 months of inoculation for microbiome studies. The DNA was isolated from all the samples of native plants and greenhouse plants and sequenced for 16s rRNA for bacteria and ITS for fungi. The NGS data has been analyzed by the QIIME tool to investigate microbial diversity. The results revealed the dominance of *Proteobacteria* followed by *Actinobacteria* and *Acidobacteria* in all the bulk soil; *Actinobacteria*, *Proteobacteria* and *Gemmatimonadetes* in rhizosphere; *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* in endosphere native soil samples; *Proteobacteria*, *Actinobacteria* and *Verrucomicrobia* in the control and treatment samples from the greenhouse. There were a few phyla that were absent in control but present in the treated plants and the native soil like *Nitrospirae*. The taxonomic classification of the native soil samples revealed the presence of various Plant Growth Promoting Rhizobacteria (PGPR) which were also found in the treated plants in the greenhouse but absent in control. They include *Rhodococcus* that helps in phosphate solubilization. *Dyadobacter* that fixes atmospheric nitrogen and *Sphingobium* that helps in siderophore production. The ITS sequencing analysis of the native soil samples revealed the presence of an Arbuscular Mycorrhizal Fungi (AMF) that helps in glycoprotein production in plants. These microorganisms will further be isolated, characterized for their functions in promoting plant growth and development.

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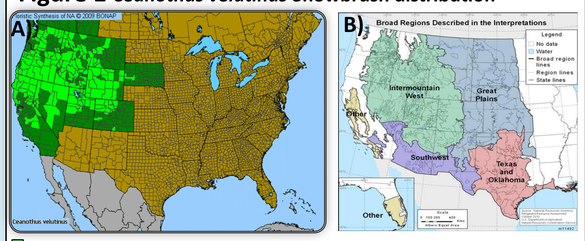
Objectives

1. Identification and characterization of microbial communities in endosphere, rhizosphere and bulk soil of the native plant *Ceanothus velutinus*
2. Comparative study of the microbial community present in the rhizosphere of the native soil treated and non-treated snowbrush plants

Summary

1. Sample collection of bulk soil, rhizosphere and endosphere of Native plant *Ceanothus velutinus* (snow brush) from the elevations 1920m, 1950m and 2289m of the Tony Grove, Logan canyon, Utah in Intermountain West region.
2. The 16s rRNA sequencing analysis revealed a dominance of the phyla *Actinobacteria* and *Proteobacteria* in all the bulk, rhizosphere and endosphere samples.
3. The alpha diversity showed a higher diversity in the bulk soil samples followed by rhizosphere and the lowest diversity was observed in the endosphere samples.
4. The beta diversity analysis revealed that the abundance of the bacterial diversity of the bulk soil and the rhizosphere samples were similar to each other and the endospheric diversity differed from that of the bulk soil and rhizosphere. The results also showed a higher diversity in the elevation 1920m in the endosphere.
5. The taxonomic classification showed the presence of several Plant Growth Promoting Rhizobacteria.
6. The fungal diversity in the elevation 2289m was observed to be more in all of the bulk soil, rhizosphere and endosphere samples. Endosphere contain more fungal communities.
7. The microbial analysis of the rhizosphere and endosphere of the cutting plants showed a dominance of *Proteobacteria* followed by *Actinobacteria* in all the plants .
8. The taxonomic classification revealed the presence of several PGPRs that were absent in control but were present in the treated plants indicating the effect of native soil inoculation as they were also identified in the native soil.

Figure 1 *Ceanothus velutinus* Snowbrush distribution



Present in state/Native (Native and Present in state, but not Present in a county)

Present in county/Native (Native and Present in state, and Present in county)

Unreported (Absent for area)

Figure 2 Identification and characterization of bacterial communities in endosphere, rhizosphere and bulk soil of the native plant *Ceanothus velutinus*

A)

	Bulk soil			Endosphere			Rhizosphere		
	1920m	1950m	2289m	1920m	1950m	2289m	1920m	1950m	2289m
Actinobacteriota	34.2	37.1	27.3	54.1	39.4	39	29.7	29.7	28
Proteobacteria	30	31.1	36.2	18	42.1	31.2	40.2	36.9	39.1
Cyanobacteria	0.1	0	0.1	0	9.5	13.2	1.8	2.2	1.9
Myxococcota	7.2	2.2	3.4	3	4.1	8.4	4.4	2.2	2.4
Acidobacteriota	6	7.8	7.4	0.3	0.4	1.1	5.7	6.4	4.6
Gemmatimonadota	1.3	3.7	3.6	20.1	0	0.1	0.6	2.2	2.1
Bacteroidota	6.8	2.8	4.2	2	1	2.2	6.1	2.4	4.5
Verrucomicrobiota	4.2	2.7	4.8	0.4	0.3	0.3	4.3	4.7	6.8
Firmicutes	2.6	5.5	5.7	0.5	0.8	1.2	2.7	4.2	4.8
Chloroflexi	1.9	2.2	2.7	0.5	0.4	0.8	1.4	4.2	1.7
Planctomycetota	1.3	1.1	1.3	0.1	0.1	0	1.8	1.9	1.4
Patescibacteria	0.2	0	0.1	0.8	1.6	1.8	0.2	0	0.1
Crenarchaeota	0.7	1.8	0.6	0	0	0	0.3	1.6	1.2
Bdellovibrionota	0.9	0.3	0.3	0.1	0	0.4	0.2	0.1	0.2
Nitrospirota	0.1	0.5	0.7	0	0	0	0	0.3	0.4
Methylomirabilota	0	0.4	0.4	0	0	0	0	0.4	0.1
Desulfobacterota	1.3	0.1	0.2	0	0	0	0.3	0	0.1
RCP2-54	0	0	0.1	0	0	0	0	0.3	0.1
Latescibacterota	0.5	0	0.3	0	0	0	0.3	0	0.1
Dependentiae	0.1	0.1	0	0.1	0	0.1	0.1	0	0.1
Armatimonadota	0	0	0.1	0	0	0	0.1	0	0.1
Fibrobacterota	0.1	0	0.1	0	0	0.2	0	0	0
Elusimicrobiota	0.1	0.1	0.2	0	0	0	0	0	0.1
Deinococcota	0	0	0	0	0.1	0	0	0	0
NB1-j	0	0	0	0	0	0	0	0.1	0

% Read Abundance: 50, 40, 30, 20, 10

Figure 2A) Cumulative result of bacterial abundance in the endosphere, rhizosphere and bulk soil at three elevations at the phyla level of the 16s rRNA sequencing analyzed by QIIME. The results revealed the dominance of *Actinobacteria* and *Proteobacteria* in all the samples.

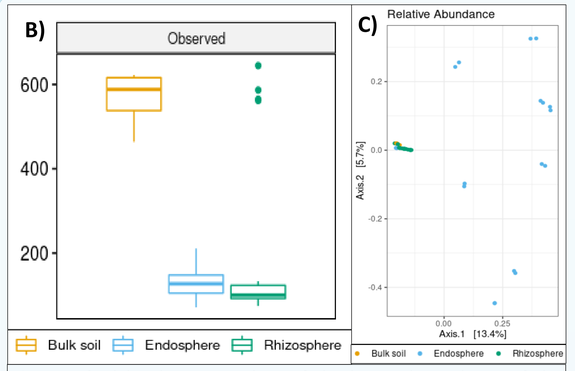


Figure 2B) Alpha diversity analysis of the bacterial population of the endosphere, rhizosphere and bulk soil. Bulk soil showed a higher diversity than that of the rhizosphere and endosphere. **2C)** Beta diversity analysis of the bacterial population of the endosphere, rhizosphere and bulk soil. The rhizosphere and bulk soil diversity abundance was closer to each other whereas the endospheric population was scattered.

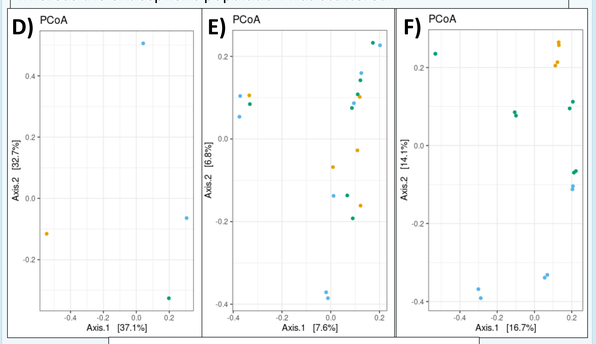


Figure 2D) Beta diversity analysis of the bulk soil at three different elevations. **2E)** Beta diversity of the rhizospheric bacterial population in native soil at three elevations. The abundance at was scattered indicating the population at the different elevations are not significantly different. **2F)** Beta diversity plot of the endosphere population at three elevations. The diversity at the elevation 1920m was observed to be higher than the other two elevations. It was followed by the population abundance in 2289m and 1950m respectively.

Figure 3 Identification and characterization of fungal communities in endosphere, rhizosphere and bulk soil of the native plant *Ceanothus velutinus*

A)

	Bulk soil			Endosphere			Rhizosphere		
	1920m	1950m	2289m	1920m	1950m	2289m	1920m	1950m	2289m
Basidiomycota	100	97.5	98.4	100	99.9	100	36.4	39.6	39.7
Ascomycota	0	2.4	1.6	0	0	0	48.6	38.3	47
Mortierellomycota	0	0	0	0	0	0	5.5	8.6	5.6
k__Fungi_OTU147	0	0	0	0	0	0	0.9	5.6	0.8
Mucoromycota	0	0	0	0	0	0	0.8	1	0.3
k__Fungi_OTU149	0	0	0	0	0	0	0	0.7	1.1
k__Fungi_OTU193	0	0	0	0	0	0	0.2	1.3	0.3
k__Fungi_OTU229	0	0	0	0	0	0	0	0	0.9
k__Fungi_OTU374	0	0	0	0	0	0	0	0.8	0
Rozellomycota	0	0	0	0	0	0	1	0	0.1
k__Fungi_OTU511	0	0	0	0	0	0	0	0.5	0
k__Fungi_OTU400	0	0	0	0	0	0	0.9	0	0
k__Fungi_OTU513	0	0	0	0	0	0	0.8	0	0
Glomeromycota	0	0	0	0	0	0	0.1	0.3	0
k__Fungi_OTU779	0	0	0	0	0	0	0.5	0	0
Olpidiomycota	0	0	0	0	0	0	0	0	0.3
Chytridiomycota	0	0	0	0	0	0	0.3	0.1	0.1
k__Fungi_OTU753	0	0	0	0	0	0	0	0.3	0
k__Fungi_OTU725	0	0	0	0	0	0	0	0	0.3
k__Fungi_OTU906	0	0	0	0	0	0	0.4	0	0
k__Fungi_OTU912	0	0	0	0	0	0	0.1	0.2	0
k__Fungi_OTU614	0	0	0	0	0	0	0	0	0.2
Zoopagomycota	0	0	0	0	0	0	0.1	0.1	0.1
k__Fungi_OTU776	0	0	0	0	0	0	0	0.1	0.2
k__Fungi_OTU994	0	0	0	0	0	0	0	0	0.2

% Read Abundance: 100, 75, 50, 25

Figure 2A) Cumulative result of the fungal population in the endosphere, rhizosphere and bulk soil at three elevations of the ITS sequencing analyzed by QIIME. The results revealed the dominance of Basidiomycota and Ascomytota in all the samples. The rhizosphere samples showed more fungal diversity.

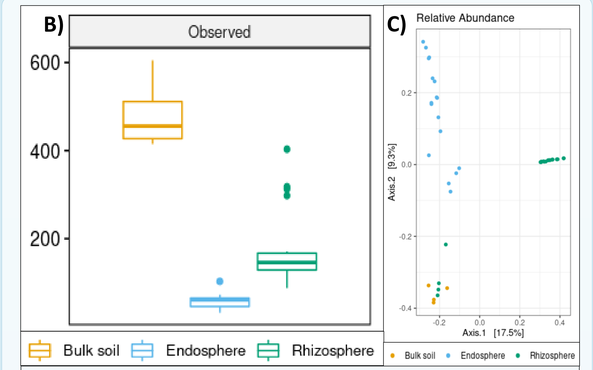


Figure 3B) Alpha diversity analysis of the fungal population of the endosphere, rhizosphere and bulk soil. Bulk soil showed a higher diversity than that of the rhizosphere and endosphere. **3C)** Beta diversity analysis of the fungal population of the endosphere, rhizosphere and bulk soil. The rhizosphere and bulk soil diversity abundance was closer to each other whereas the endospheric population was scattered.

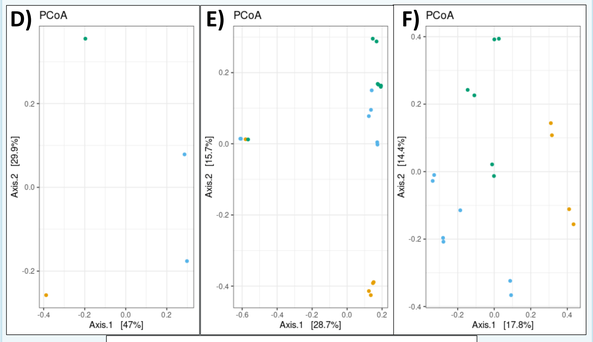


Figure 3D) Beta diversity analysis of the bulk soil at three different elevations. The fungal diversity observed in the elevation 2289m was higher than that of 1920m and 2289m **3E)** Beta diversity of the rhizospheric bacterial population in native soil at three elevations. The diversity in the elevations 1950m and 2289m was observed to be higher than that of 1920m. **3F)** Beta diversity plot of the endosphere population at three elevations. The diversity at 2289m was observed to be higher than the other two elevations. The fungal diversity in the elevation 2289m was observed to be more in all of the samples.

Figure 4 Comparative study of the microbial community in the rhizosphere of the snowbrush cuttings treated with and without native soil inoculum.

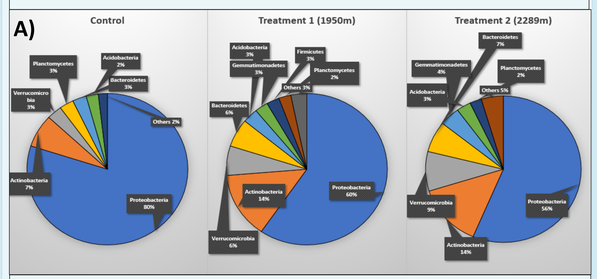


Figure 4: A) The resultant bacterial population of the rhizosphere of the treated plants (inoculated with native soil) and control/non-treated plants (no inoculum) of the 16s rRNA sequencing analyzed by QIIME. Bacterial abundance in control and treated plants at the phyla level

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