Microbial community of the rhizosphere of *Ceanothus velutinus* improves the plant's growth and development under greenhouse conditions

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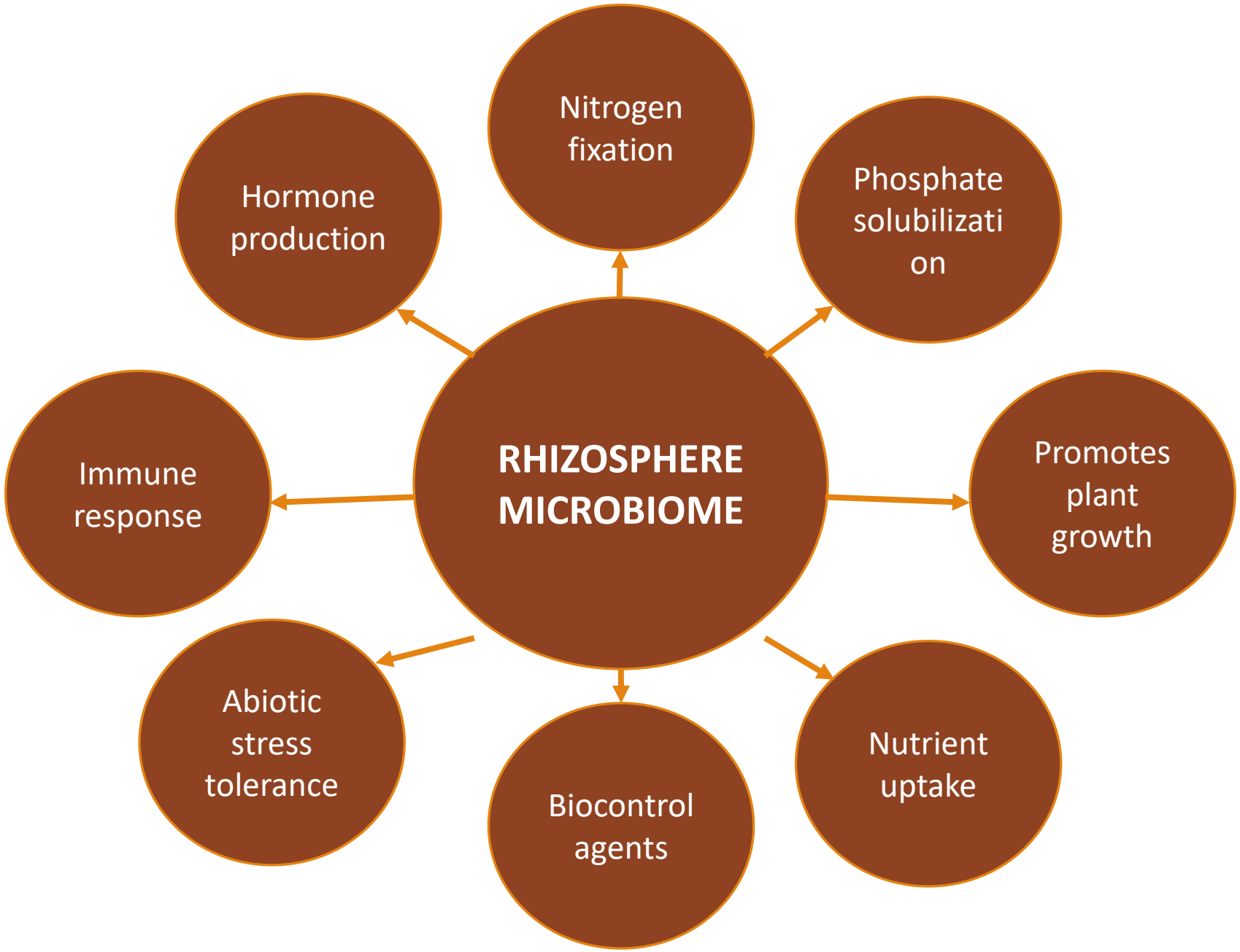
Introduction to the problem

1. Plant's **rhizosphere microbiome** plays a significant role in growth and development of the plant.
2. **Endosphere** constitutes of all the endophytes in a plant
3. Endophytes are microorganisms (usually bacteria or fungi) living inside the plant
4. **Rhizosphere** soil can be defined as the soil that is attached to the roots of a plant and the microorganisms present are the rhizospheric microbiome
5. **Bulk soil** is soil outside the rhizosphere which surrounds the plant roots
6. **Root nodules** are special organs formed by plant-microbe symbiosis which fixes atmospheric nitrogen in the soil for the plant to uptake

Bulk soil rhizosphere soil and endosphere

https://fineartamerica.com/featured/modules-of-rhizobium-leguminosarum-dr-jeremy-burgesscience-photo-library.html


RHIZOSPHERE MICROBIOME
Nitrogen fixation
Phosphate solubilization
Promotes plant growth
Nutrient uptake
Biocontrol agents
Abiotic stress tolerance
Immune response
Hormone production

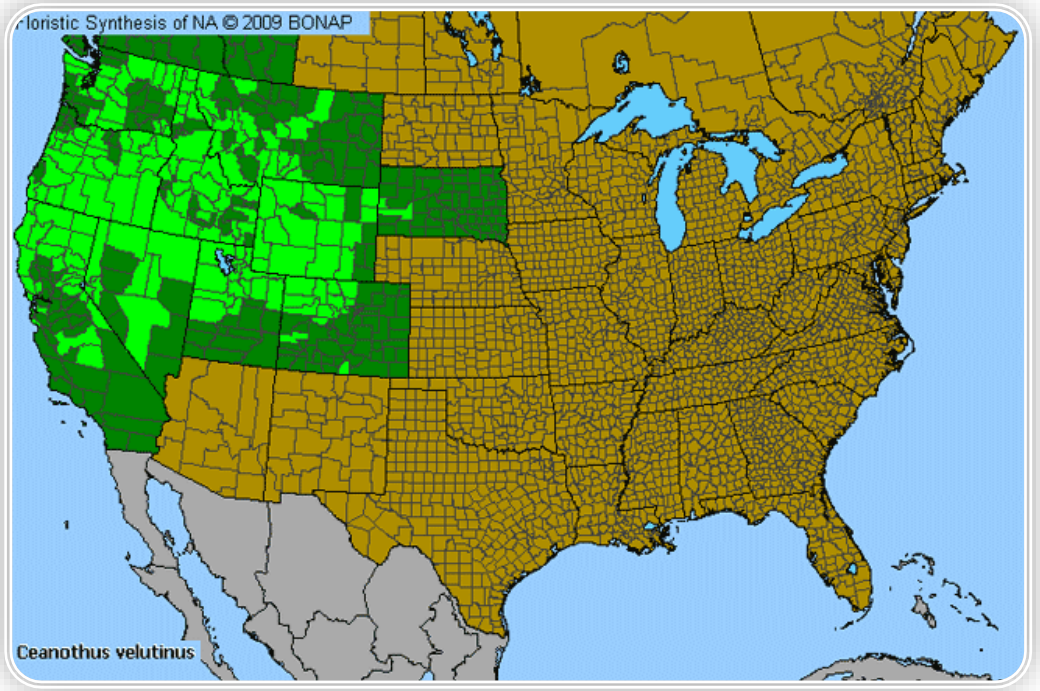
Native plants

- Plants that have thrived in a region and have evolved in the same place for years
- Have the ability to sustain and survive in difficult environments
- The USU **Centre for Water-Efficient Landscaping** (CWEL) recommended a list of native plants for use in low water-use landscaping
- Mountain Mahogany, Silver Buffaloberry, Sagebrush etc are well known native plants for their drought tolerance and low water use landscaping.

https://cwel.usu.edu/
https://waterwiseplants.utah.gov/default.asp?Cart=

Ceanothus velutinus (snowbrush)

- Evergreen native plant found in the Intermountain West region
- Can thrive well in dry and harsh conditions
- Hard to locate nodules in native conditions as their roots are deep and wide-spread
- No record of nodulation observed in snowbrush under the greenhouse conditions
- Difficult to propagate under landscape conditions
- Recent metagenomic study conducted in the microbiome of the native soil revealed the presence of several Plant Growth Promoting Rhizobacteria



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)

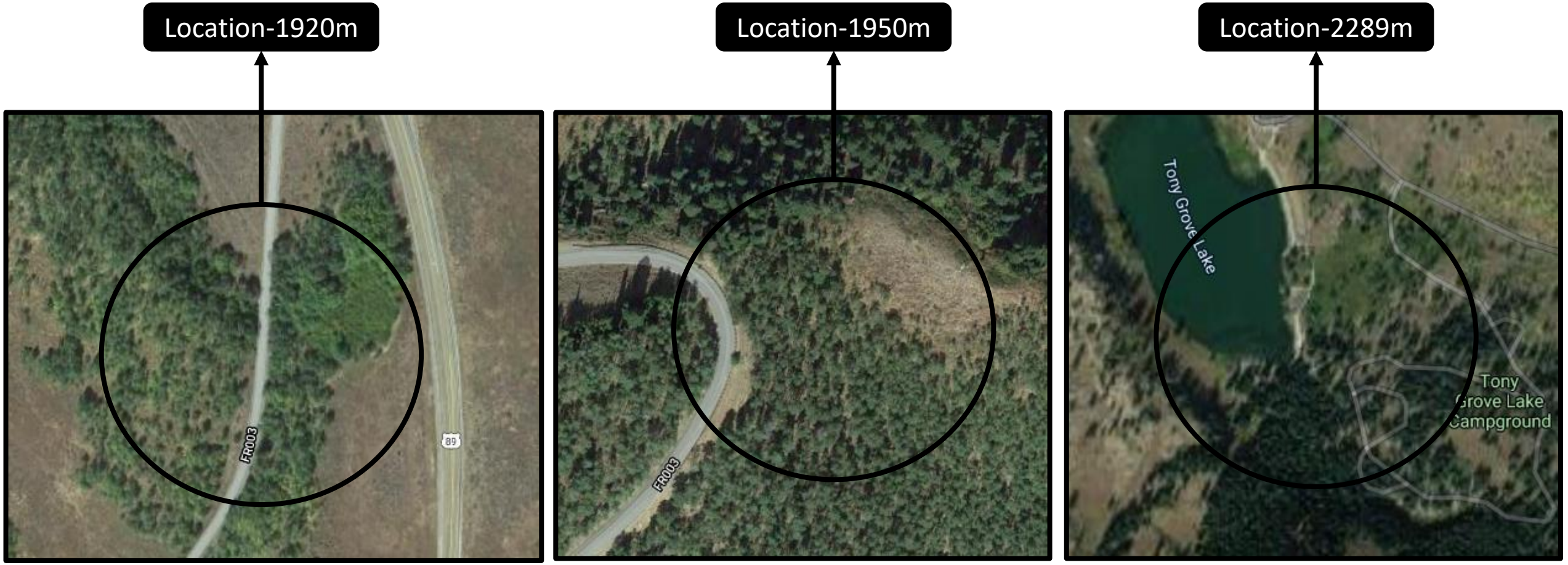
http://www.pollenlibrary.com/map.aspx?map=Ceanothus-velutinus.png

https://digitalcommons.usu.edu/extension_curall/797/

Objectives

1. Effect of native soil on the rooting of *Ceanothus velutinus* cuttings under the greenhouse conditions
2. Effect of native soil on the growth and development of *Ceanothus velutinus* plants grown via cuttings under the greenhouse conditions
3. Effect of native soil on the growth and nodulation of *Ceanothus velutinus* plants grown via seedlings under the greenhouse conditions

Sample collection



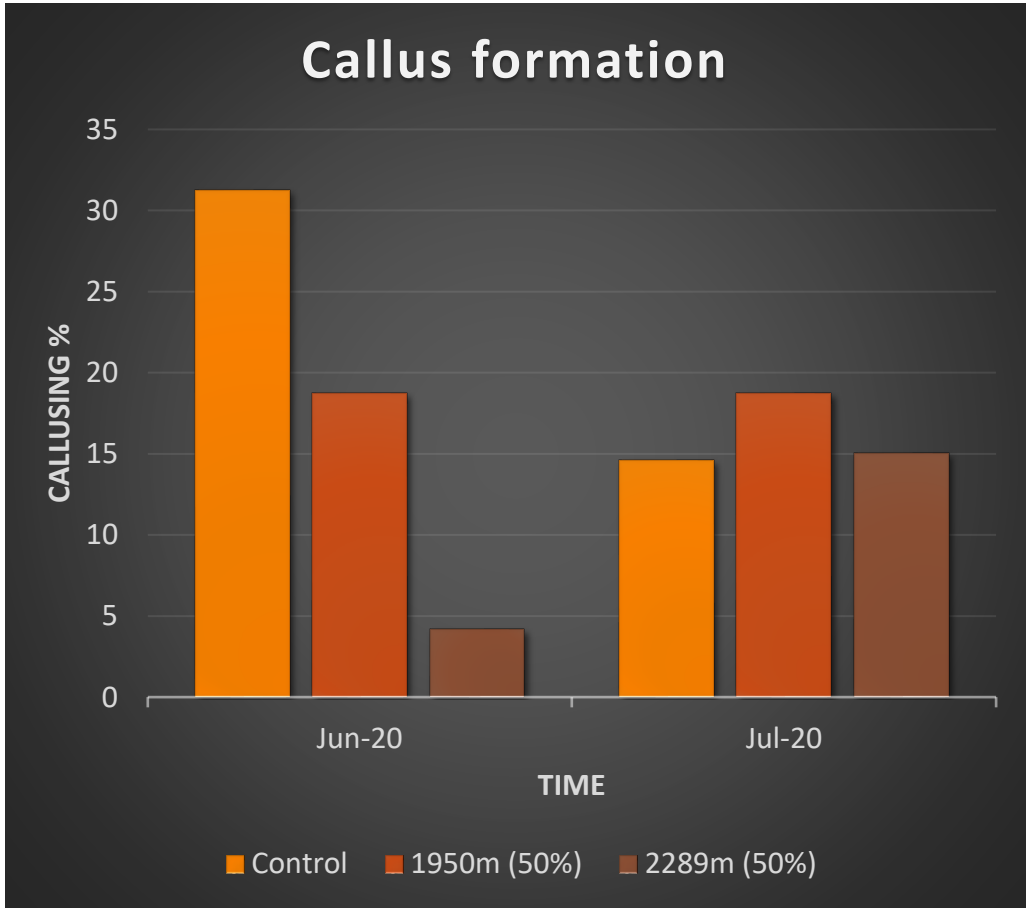
Effect of native soil on the rooting of *Ceanothus velutinus* cuttings under the greenhouse conditions

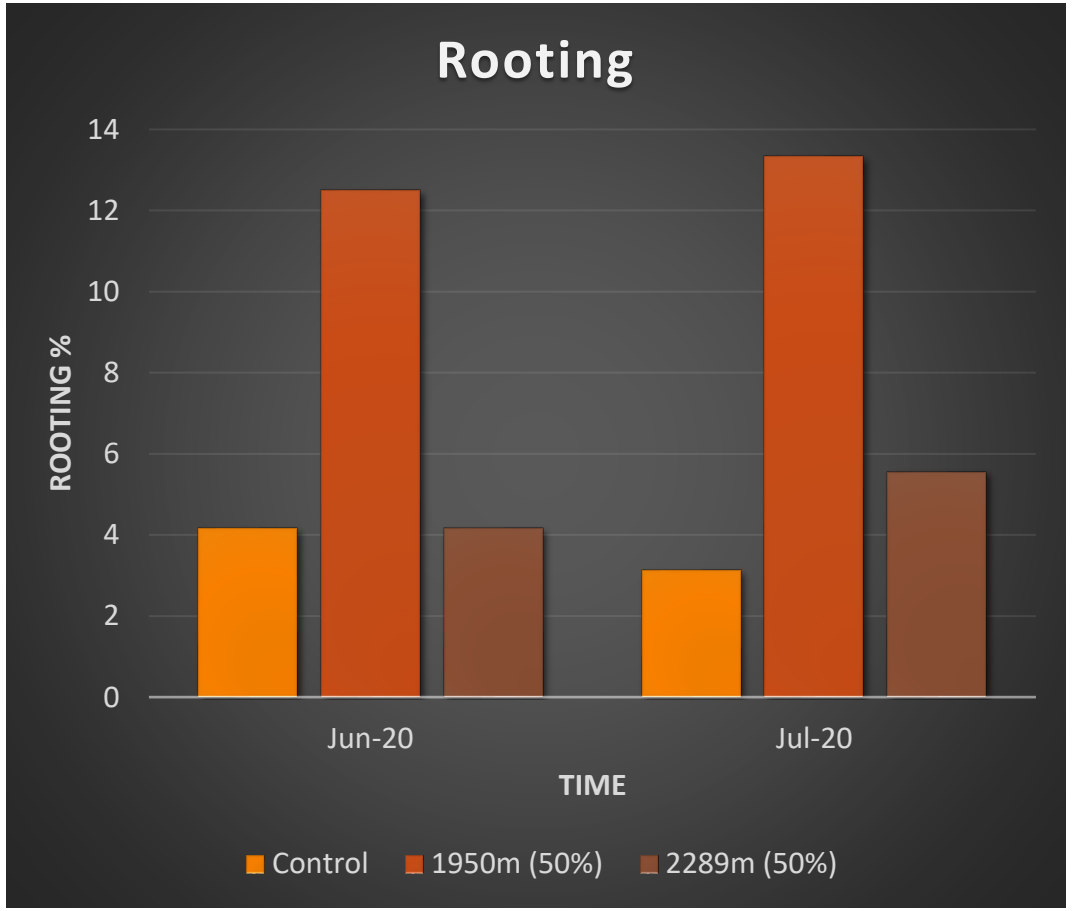
Cutting propagation of snowbrush cuttings with and without native soil inoculation

Rooting

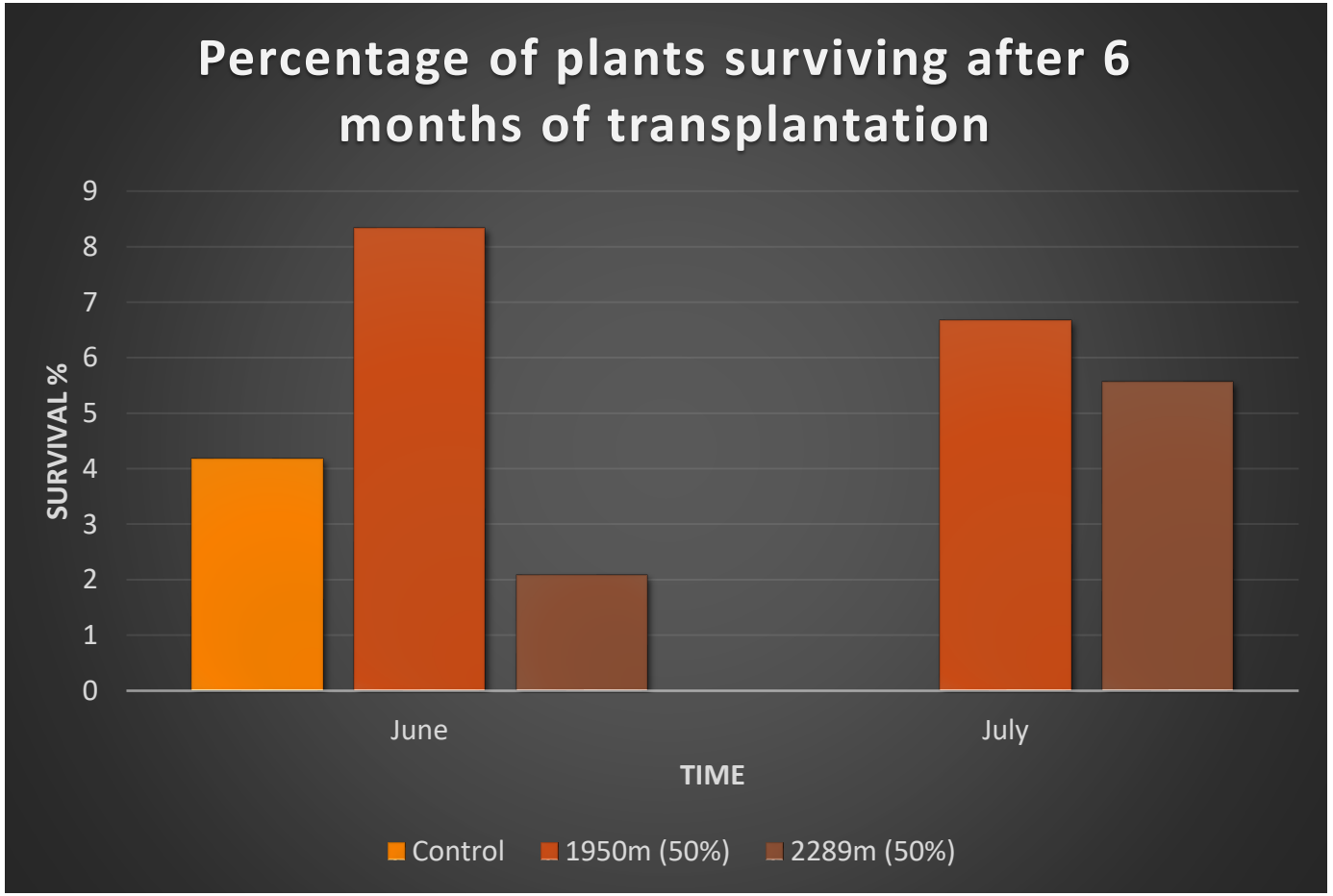
Callusing

Percentage of callus formation and rooting in snowbrush via cutting propagation- control vs treatment





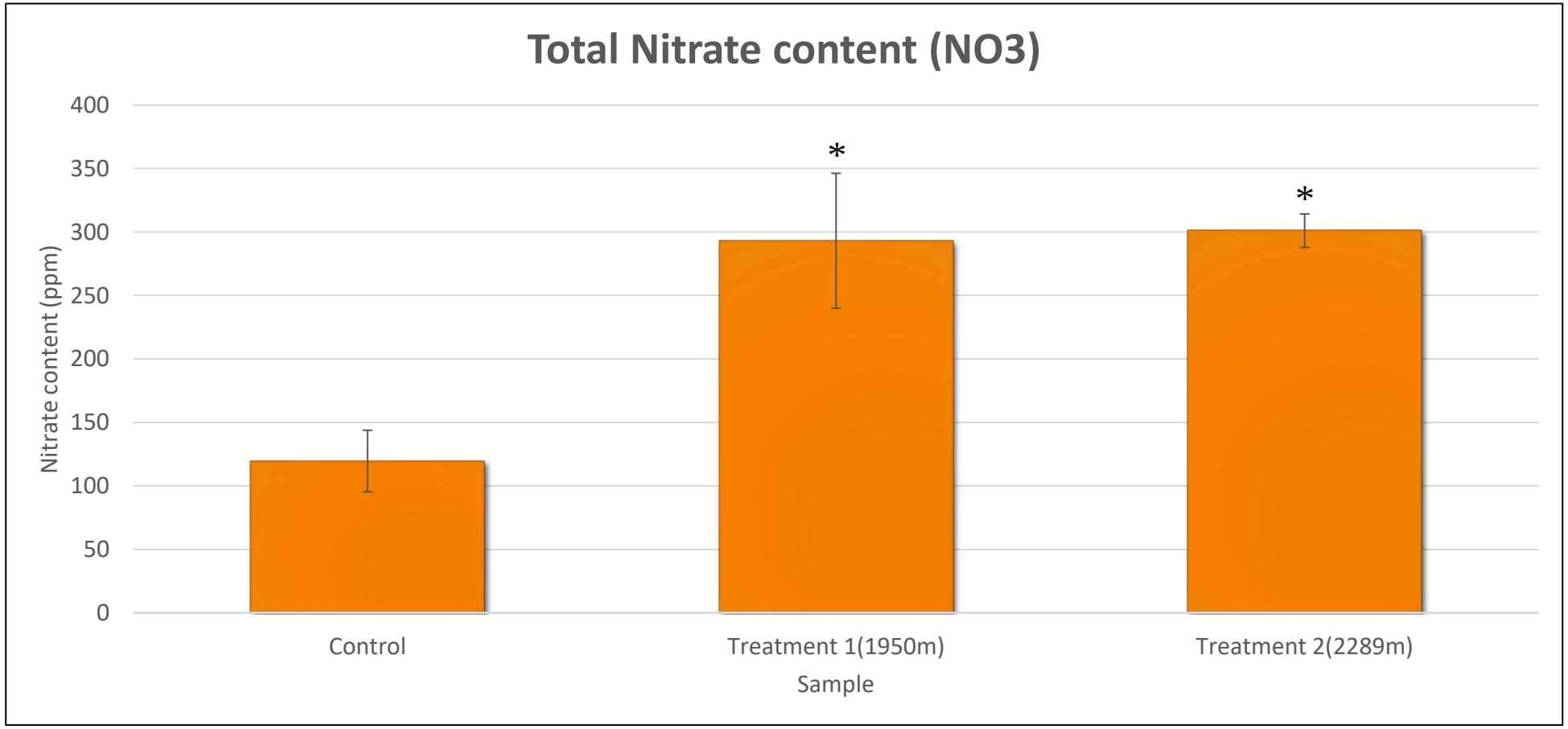
Higher survival percentage of snowbrush cuttings treated with native soil was observed after 6 months of transplantation into potting soil



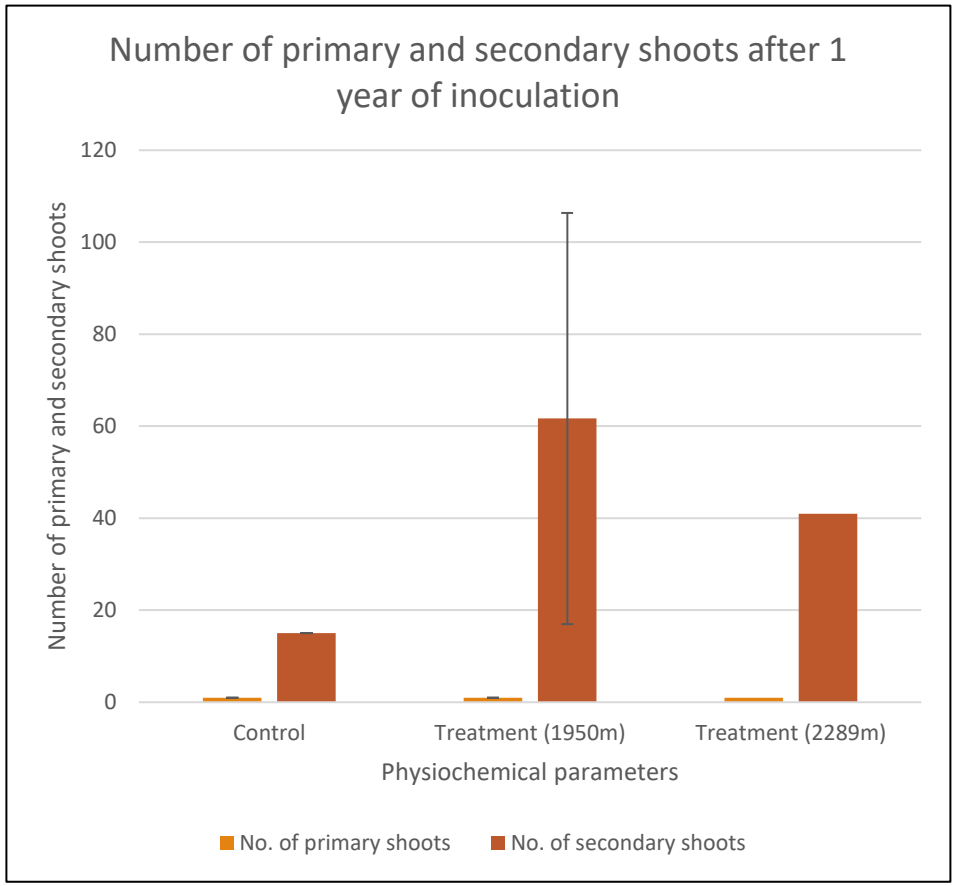
Effect of native soil on the growth and development of *Ceanothus velutinus* plants grown via **cuttings** under the greenhouse conditions

Change in Snowbrush plants from cuttings observed show increase in growth after 12 weeks of inoculation

Increased amounts of available nitrogen in treated snowbrush plants when compared to control

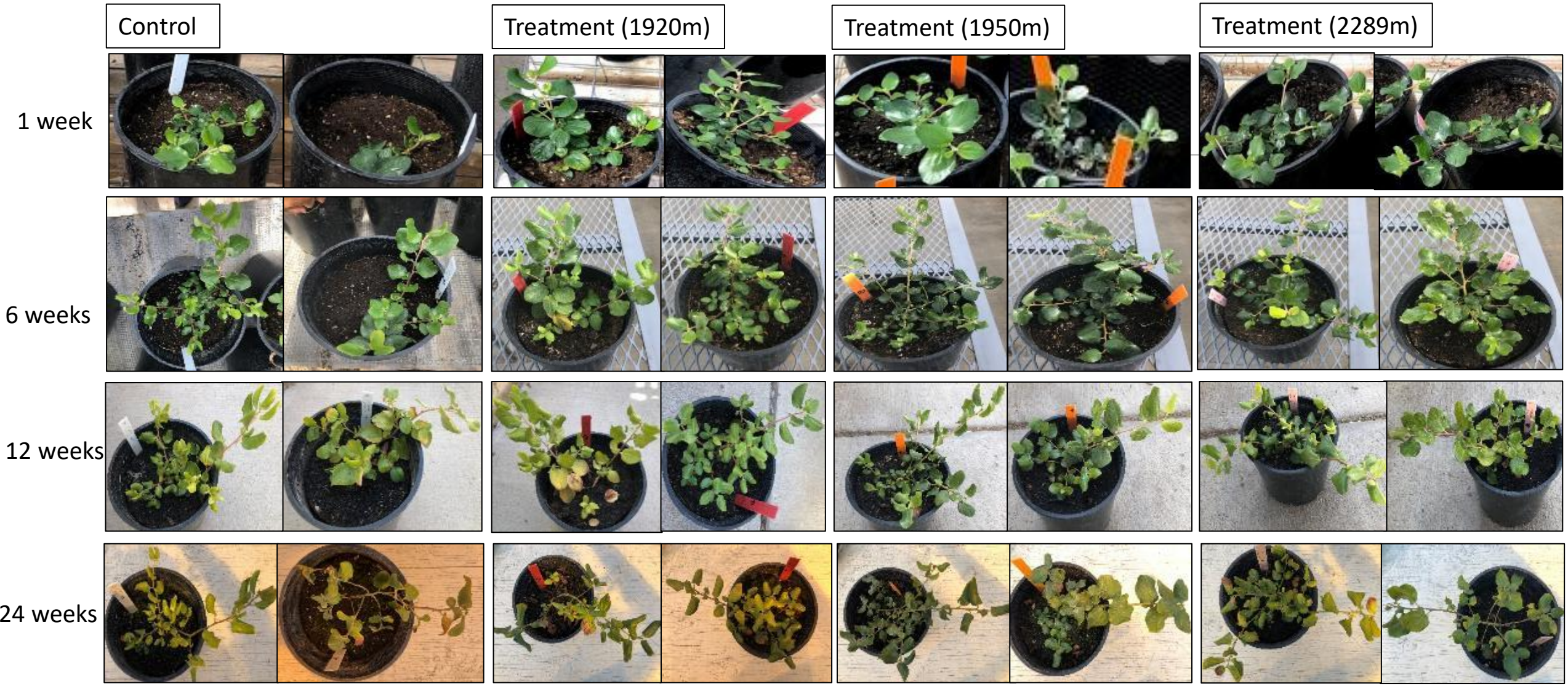


Increased number of primary shoots and plant width in snowbrush treated plants as compared to control after 1 year of inoculation



Effect of native soil on the growth and nodulation of *Ceanothus velutinus* plants grown via **seedlings** under the greenhouse conditions

Snowbrush seedlings show increase in growth after 12 weeks of inoculation



Significant change in the macro and micronutrient contents observed in the native soil at three different elevations

Native soil									
Sample	**Carbon**	**Phosphorus**	**Potassium**	**Zinc**	**Iron**	**Copper**	**Manganese**	**pH**	**EC**
1920m	2.21±0.77a	31.62±2a	469.5±115.5a	1.79±0.3a	70.35±11.45a	1.355±0.35a	19.9±1.2a	6.575±0.26a	0.19±0.01a
1950m	24.80±1.75b	6.00±0.60a	600.5±57.23a	2.17±0.03ab	83.875±4.7a	0.8375±0.12a	26.7±1.44b	6.2425±0.26a	0.24±0.01a
2289m	12.78±1.82c	34.80±0.32a	485.5±7.5a	2.56±0.2b	74.05±5.65a	1.005±0.14a	18.5±0.8a	6.3867±0.15a	0.125±0.005b

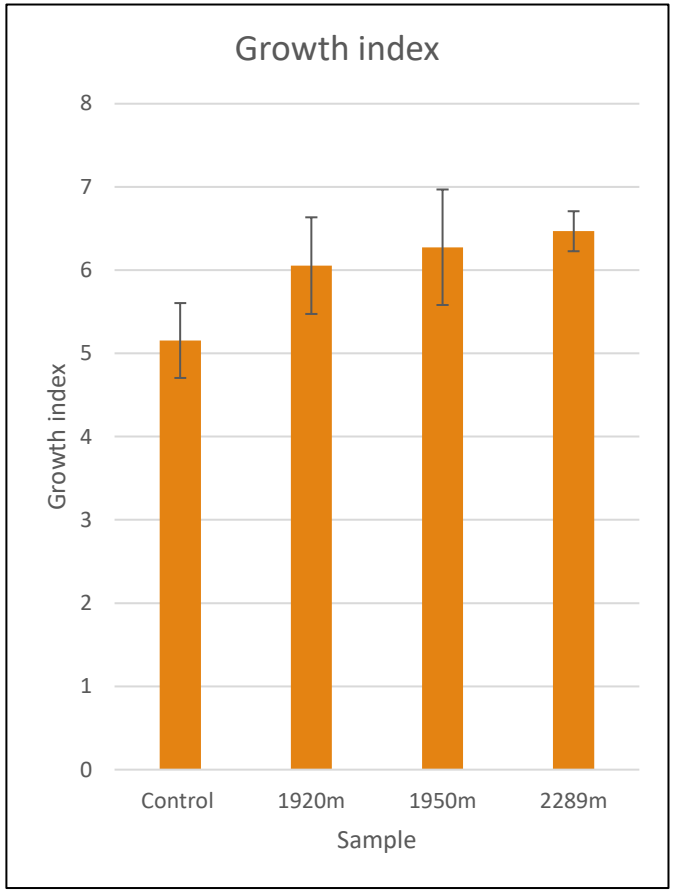
- More total carbon and manganese content in the soil from the elevation 1950m as compared to 1920m and 2289m
- More zinc content in the soil from the elevation 2289m as compared to 1920m and 1950m

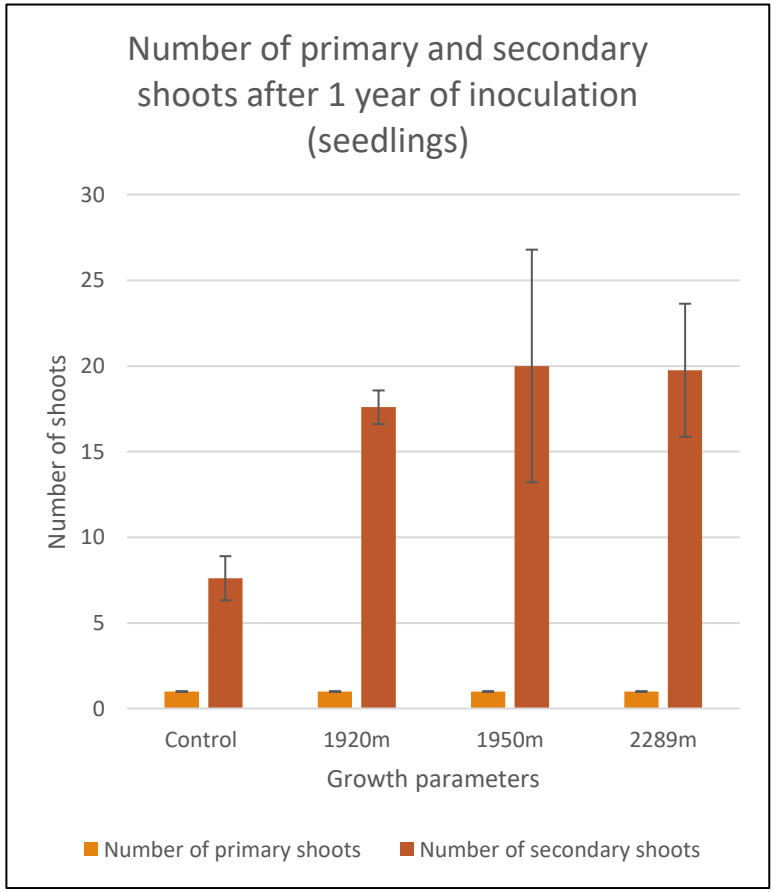
Difference in the macro and micronutrient content in control vs treated soil

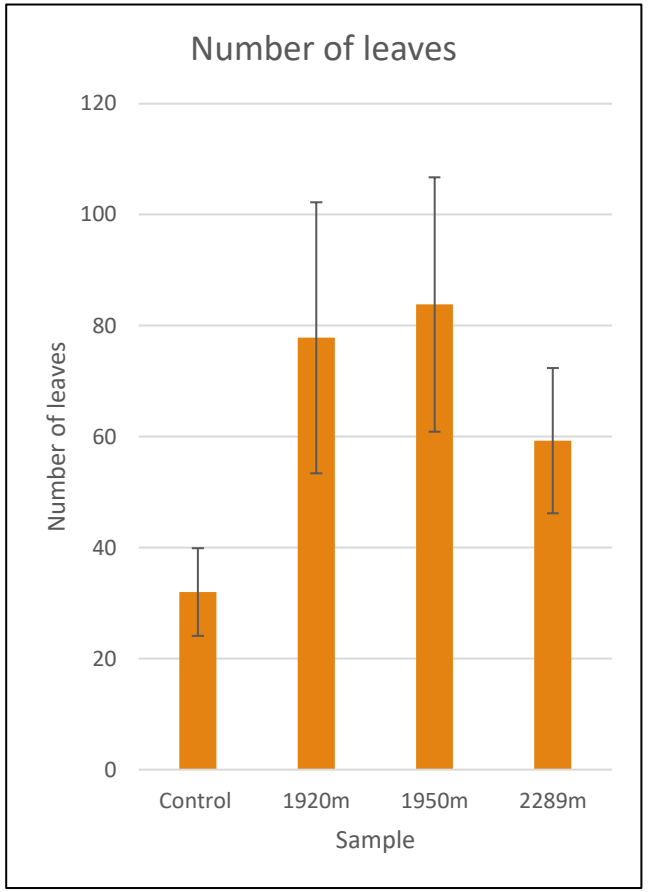
Seedlings										
Sample	**Carbon**	**Nitrate**	**Phosphorus**	**Potassium**	**Zinc**	**Iron**	**Copper**	**Manganese**	**pH**	**EC**
Control	64.17±3.92a	301.2±16.76a	50.61±2.84a	968±84a	15.2±1.4a	140±10a	26±0.7a	49±4.2a	8.376±0.06a	366±13.32a
1920m	6.98±1.54b	647.2±33.40b	12.33±0.52b	481.33±23.02b	2.79±0.27b	58.23±6.41b	6.43±1.16b	18.77±0.77b	8.654±0.08a	303.2±25.72a
1950m	28.86±1.27c	797.6±44.20b	15.83±0.72b	608.33±19.70b	5.82±1.32b	96.7±14.85b	7.08±1.41b	29.4±4.98b	8.498±0.11a	348.2±34.36a
2289m	22.27±2.16c	618±20.88b	12.24±0.54b	519.67±27.17b	5.23±0.36b	75.7±3.51b	7.12±0.18b	33.5±2.70ab	8.76±0.08	340.25±37.95a

- More amounts of total carbon, phosphorus, potassium, zinc, iron, copper and manganese in control soil when compared to the treated soil indicating a potential higher nutrient absorption by the native soil treated plants when compared to control (no inoculation).

Increased growth index, number of secondary shoots and number of leaves in the plants treated with native soil when compared to control (no inoculation) after 1 year of treatment







Increased growth parameters in treated snowbrush plants when compared to control

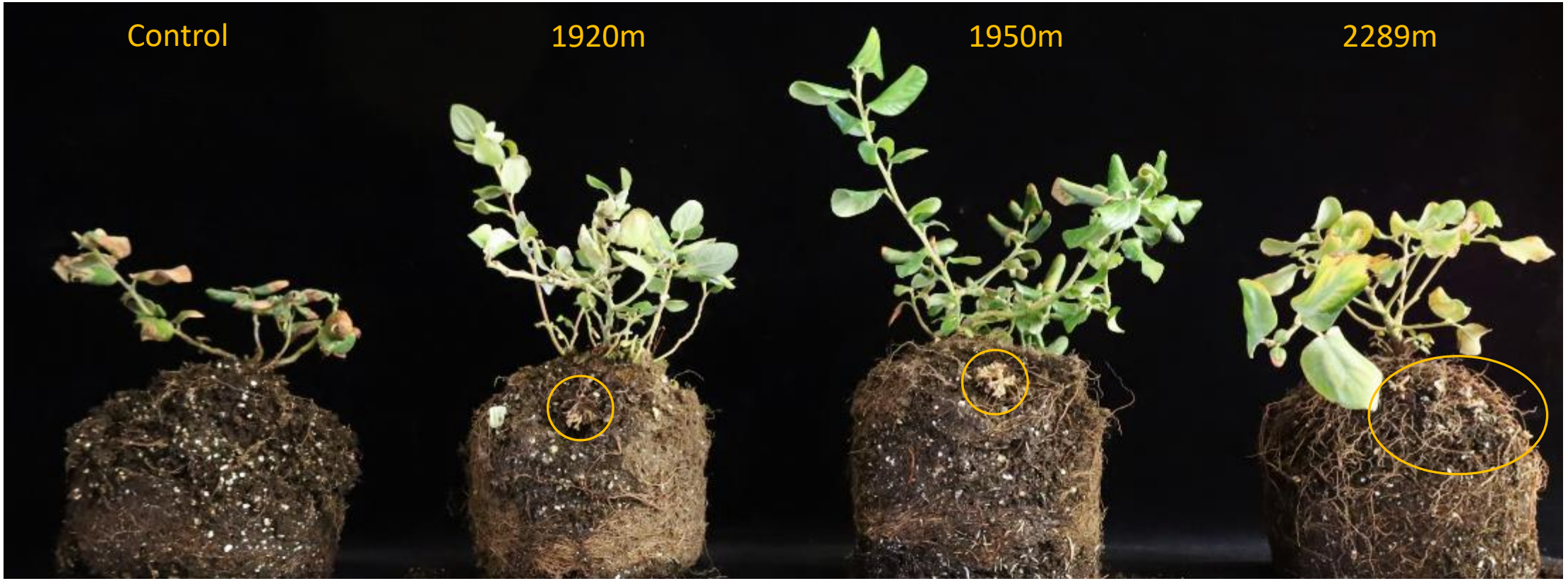
Sample	Growth index	Number of leaves	Chlorophyll content	Chlorophyll fluorescence	Number of primary shoots	Number of secondary shoots
Control	5.153±0.45a	32±7.91a	37.44±4.27a	0.715±0.02a	1±0a	7.6±1.29a
Treatment 1920m	6.053±0.58a	77.8±24.42a	43.56±3.54a	0.7272±0.02a	1±0a	17.6±0.98a
Treatment 1950m	6.273±0.69a	83.8±22.93a	43.46±2.60a	0.763±0.01a	1±0a	20±6.79a
Treatment 2289m	6.467±0.24a	59.25±13.09a	36.9±3.80a	0.7045±0.02a	1±0a	19.75±3.89a

- Increased amounts growth index, number of leaves and number of secondary shoots in snowbrush plants treated with native soil when compared to control though change is not significant as the sample size was low.

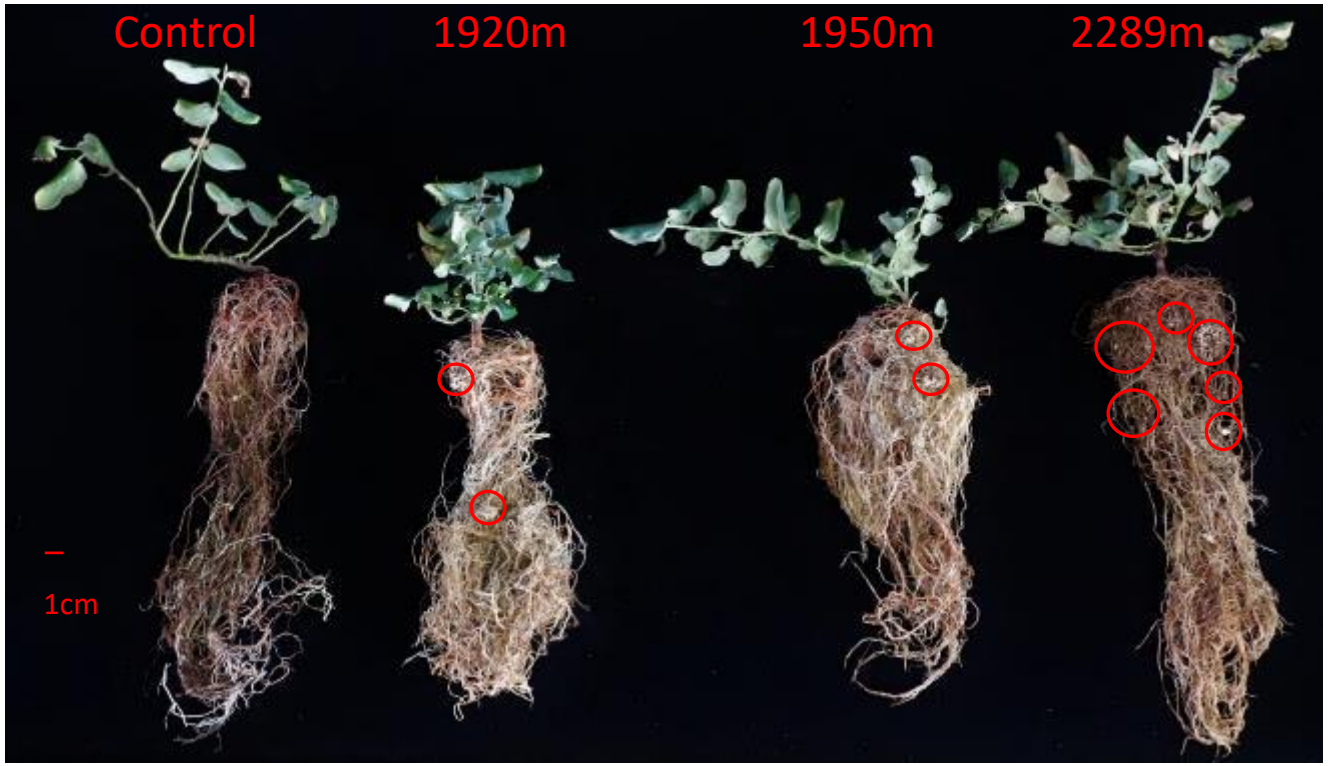
Nodulation in snowbrush plants has been reported, but it is difficult to get nodules from wild native plants

- This plant is a bush and is deep rooted
- An attempt was made to induce nodulation by inoculating snowbrush plants from seedlings with native soil in greenhouse conditions

Nodulation observed in the snowbrush plants via seedlings treated with native soil, but not observed in control (no inoculation)

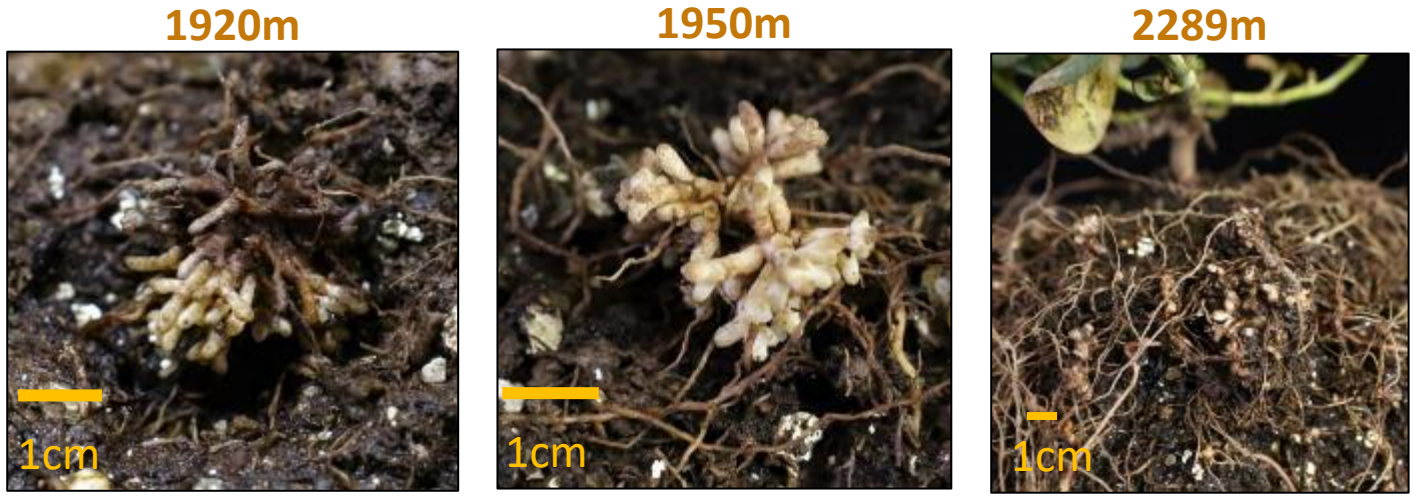


Nodulation percentage in snowbrush plants after 1 year of native soil inoculation (control and treatment)



Sample	Nodulation%
Control	0%
Treatment 1920m	100%
Treatment 1950m	83.33%
Treatment 2289m	100%

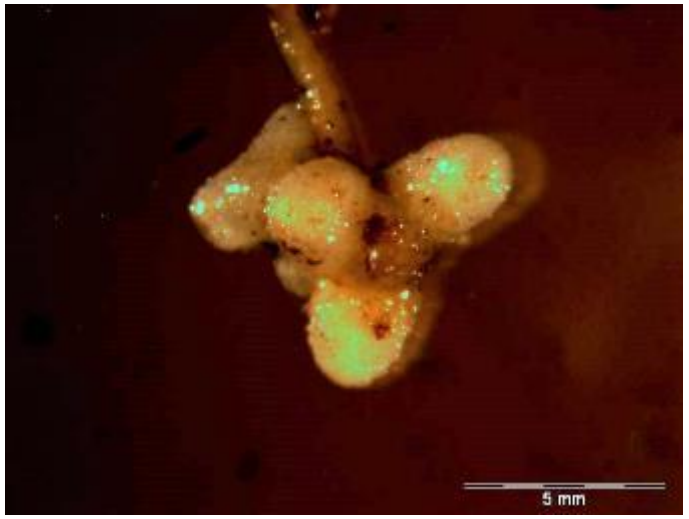
Average nodule diameter and the largest nodule found in the snowbrush plants treated with native soil after 1 year of inoculation



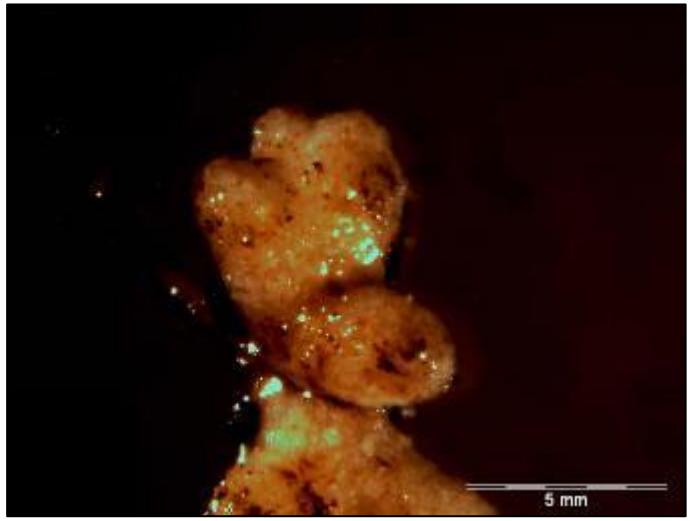
Sample	Average diameter of nodules (cm)	Diameter of the largest nodule (cm)	Average number of nodules per plant
Control	0	0	0
Treatment 1920m	1.14±0.17	2.7	3
Treatment 1950m	1.37±0.16	2.4	2
Treatment 2289m	1.1±0.13	2.4	31

Microscopic view of the nodules formed in the snowbrush plants treated with native soil

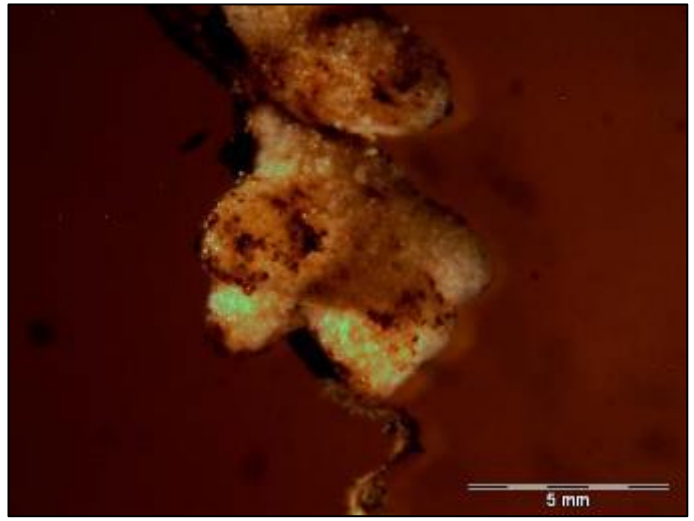
1920m



1950m



2289m



Conclusions

- Increased rooting percentage as well as denser roots was observed in the cuttings treated with native soil when compared to control (no inoculation)
- Improved growth; increased nitrate content, plant width and number of secondary shoots observed in the native soil treated snowbrush plants from cuttings when compared to control
- Increase in the number of secondary shoots, leaved and plant width along with nitrate content was observed in the snowbrush plants from seedlings treated with native soil when compared to control
- The presence of macro and micronutrient contents was found less in the soil of treated snowbrush plants via seedlings after 1 year of inoculation then control plants, indicate the enhanced nutrient absorption by plants by the presence of microbes from the native soil.
- Nodulation observed in almost all of the snowbrush plants treated with native soil but not in control indicating the effect of native soil on the nodule formation of snowbrush plants

Future plan

- Isolation and identification of endophyte microorganisms from the nodules
- Isolation of the PGPR from the treated plant's rhizosphere and native plant's rhizosphere
- Effect of these microorganisms in the propagation of snowbrush seedlings
- Effect of endophytes and rhizosphere microorganisms on the growth and development of model plants like *Arabidopsis thaliana* and *Medicago truncatula* and crop plants like maize and alfalfa.

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Questions?