Cultivating Growth: Exploring the Symbiotic Interplay Between Domestic and Foreign Hop Cultivars and Nitrogen-Fixing Bacteria

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Introduction

- Plants rely on effective microbial relationships for success, particularly with nitrogen-fixing bacteria essential for converting atmospheric nitrogen into usable forms.
- Nitrogen, obtained as nitrate by plants from microbial communities, is vital for amino acids, proteins, and enzymes crucial for life functions.
- The interaction between plants and bacteria alters soil elemental composition, impacting growth patterns based on the plant's needs and soil elements available.
- Biodiversity of nitrogen-fixing bacteria varies across regions due to soil nitrogen content and climatic conditions, affecting plant growth rates.
- Plant traits determine nitrogen uptake, shaping microbial communities and impacting bacterial abundance in soils.
- Nitrogen availability affects bacterial community structure, with high nitrogen uptake plants impacting the abundance of nitrate reducing microorganisms.
- The diversity and abundance of nitrogen-fixing bacteria reflect plant traits and optimal climatic conditions for growth and survival.
- Specific nitrogen-fixing bacteria abundance correlates with the compatibility and strength of relationships between Hop cultivars, impacting growth rates and soil compositions.

Hypothesis

In the course of our investigation, we developed questions concerning the dynamics of plant growth and the relationships between hop cultivars and nitrogen-fixing bacteria. Initially, we hypothesized that domestic cultivars would exhibit a greater uptake of extractable nitrogen from the available soil compared to foreign cultivars.
This presumption stemmed from the anticipation that domestic cultivars would establish more efficient symbiotic relationships with nitrogen-fixing bacteria in the common soil. This led us to our second inquiry, postulating that domestic cultivars would demonstrate a higher abundance of nitrogen-fixing bacteria compared to their foreign counterparts. Ultimately, considering the cumulative impact of these variables, we anticipated that domestic cultivars would manifest higher growth rates in contrast to foreign cultivars.

H0- N levels are lower in US cultivars compared to non US cultivars

Ha- N levels are higher in US cultivars compared to non US cultivars

H0- US cultivars have higher growth rates compared to non US cultivars

Ha- US cultivars have lower growth rates compared to non US cultivars

H0- There is more N fixing bacteria in US cultivars compared to non US cultivars

Ha- There is less N fixing bacteria in US cultivars compared to non US cultivars

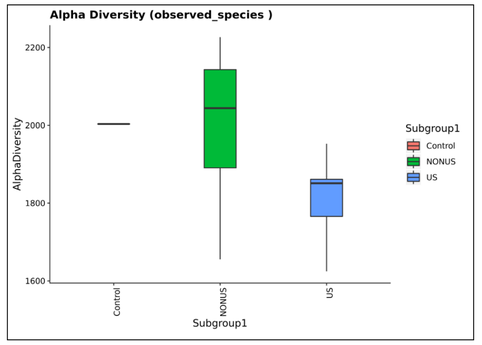
Materials and Methods

Nitrogen extraction-
-Weighed out 10 g of sieved soil and add 40 ml of 2M KCl. Shook for 1 hour on a shaker table
-Filtered and collected the liquid (supernatant), storing it in labeled 30 ml plastic bottles in the freezer.
-Analyzed extracts for NH4 and NO3 concentration using a spectrophotometer at 667 nm (NH4) and 540 nm (NO3), following Mulvaney (1996) for NH4 and DeForest (2007) / Miranda et al. (2001) for NO3.

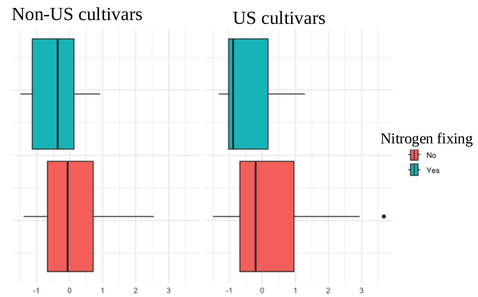
Soil DNA extraction-
-Utilized Zymobiomics bash beading lysis tubes and Zymo-Spin III-F-Filter to gain a filtrate
-Employed ZymoBiomics DNA Binding Buffer and Zymo-Spin IC Column in order to obtain a matrix that DNA can then be eluted from.
-Eluted DNA utilizing ZymoBIOMICS HRC Prep Solution and transferred to a clean 1.5 ml microcentrifuge tube.

Sequencing of Soil Samples based on 16S Region-
-DNA extraction utilizing ZymoBiomics DNA miniprep kit
-Targeted library preparation made by employing Quick-16S and custom ITS2 primers for targeted sequencing.
-ZymoBiomics standards as positive controls
-Sequencing was done by running libraries on Illumina Nextseq and Bioinformatically analyzed using the DADA2 pipeline .
-Quantification was done through qPCR with standard curves for absolute gene copies as well as estimated DNA quantity per sample.

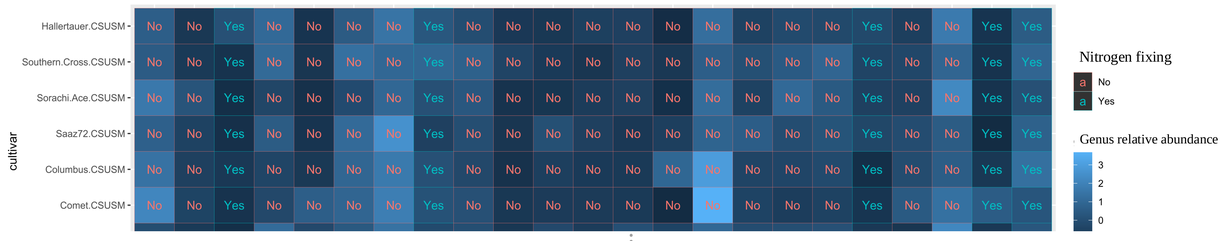
Results



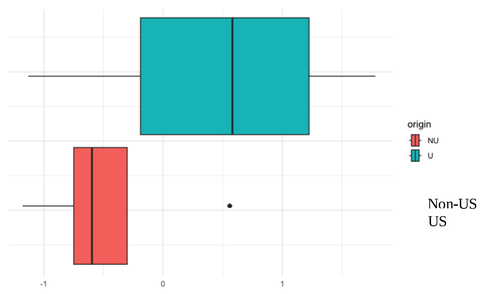
Alpha diversity stratified by cultivar origin



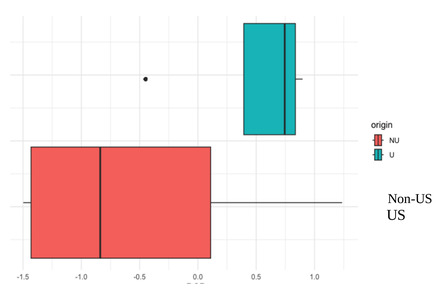
Normalized genus relative abundance differences in nitrogen fixing bacteria versus non-nitrogen fixing bacteria stratified by the cultivar origin



Heatmap of genus relative abundance per cultivar and nitrogen fixing ability



Normalized dissolvable Nitrogen



Normalized mean relative growth

Levels of normalized dissolvable nitrogen (left) and normalized mean relative growth rates (right) estimated between US and non-US cultivars in a common-garden setting at CSUSM.

Conclusions

- Statistically significant relationship between bacterial alpha diversity, dissolvable nitrogen in soil, and growth rate of hop cultivars with respect to their dominant geographic origin.
- The precise nature of the relationships between nitrogen fixing bacteria and domestic or foreign hop cultivars remains unclear
- The presence of hops of certain geographic origin will have separate effects on the soil elemental composition and its bacterial community.
- Dissolvable Nitrogen levels were observed to be lower in foreign hop cultivars as a direct result of a higher alpha diversity of nitrogen fixing bacteria present in the soil when compared to Domestic cultivars.
- US cultivars were able to form more effective relationships with selective nitrogen fixing bacterial species enabling those bacteria to outcompete other nitrogen cyclers resulting in the lower abundance of Nitrogen fixing bacteria observed in the soils of US cultivars compared to foreign cultivars.
- This explains why Dissolvable Nitrogen levels are higher in domestic cultivars as there are few identified Nitrogen fixing species of bacteria that have formed effective symbiotic relationships by efficiently fixing Nitrogen to a bioavailable form in the soil. The nitrogen fixing bacteria are expected to form better symbiotic relationships with the domestic cultivars due to their coevolution, allowing for the bacteria to produce more bioavailable nitrogen to be released into the soil compared to that of the bacteria associated with the foreign hop cultivars. Thus, leading to the surplus of dissolvable nitrogen levels observed in domestic samples explaining higher growth rates.

References

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