

The Microbiome of *Acer saccharum*: environmental drivers of microbial communities in different plant structures



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Introduction

Bacteria inhabit all surfaces and tissues of living organisms. The bacterial communities that inhabit plant structures have many beneficial effects on the host's functions such as protecting against pathogens, synthesizing growth hormones and providing nutrients (Davison 1988).

The microbial communities found on the deciduous tree species *Acer saccharum* were investigated from samples taken along the altitudinal gradient of Mont Megantic, Quebec where a distinct sugar maple tree line occurs at approximately 2500 feet (Brown & Vellend, 2014).

Objective:

To use high-throughput DNA sequencing to compare the bacterial communities of different structures and tissues of seedlings such as the surface of the leaves and roots, as well as their interior tissues. Also to compare bacterial communities from seedlings found at either the altitudinal limit of the *A. saccharum* range or from just below the limit.

Distinct bacterial communities were found between seedlings from the range limit (Edge) and from below the limit (Within) ($p=0.006$). Significant differences in the communities were found to be present in every structure and tissue.

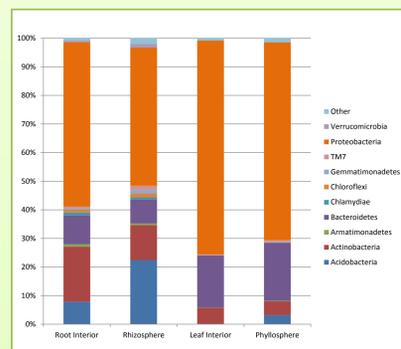


Figure 2: Relative abundance of bacterial phyla in the different plant structures

Distinct communities were also found between the four plant structures using both the Bray – Curtis method (fig 1) and the Unifrac method. These differences were found to be very large even at the phylum level. The microbiome of the plant structures were mostly composed of Proteobacteria, Acidobacteria, Actinobacteria and Bacteroidetes with highly varying levels between the structures (fig 2).

The bacterial communities found on the rhizosphere of the plants had higher similarity to the communities found in the interior of the roots compared to the other structures. Also the interior of the leaves had higher similarity to the phyllosphere communities than the root structures. However there was a high degree of variability in the phyllosphere samples (fig 1).

Using LEfSe (Segata *et al.* 2011) to search for biomarkers, indicator species were found to be higher in the Within samples in every structure except the interior of the leaves. The highest amount of indicator species from the Within samples was found in the root tissues (fig 3).

Results

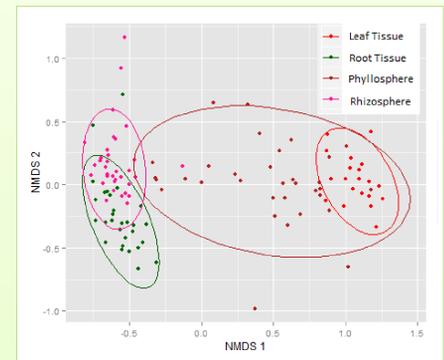


Figure 1: Ordination NMDS using Bray-Curtis distance of the taxonomic variation between communities from different plant structures.

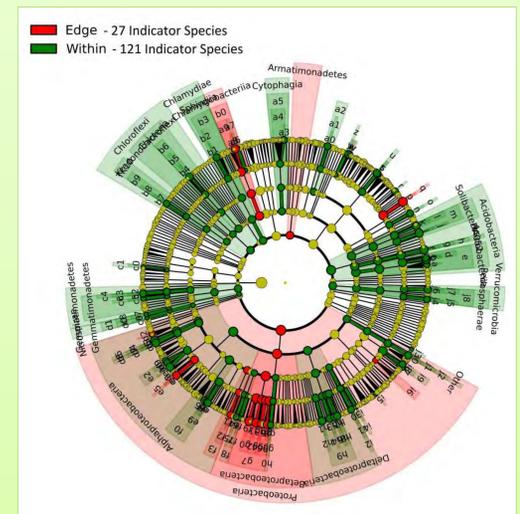


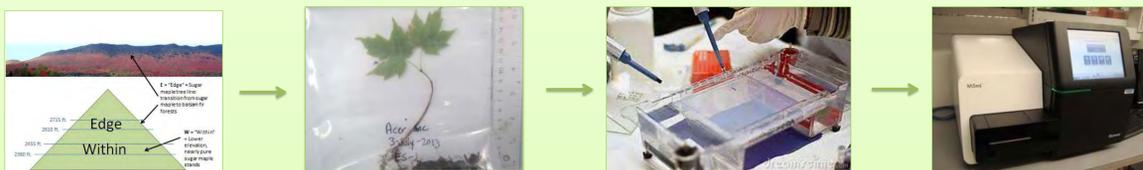
Figure 3: Cladogram of LEfSe results showing indicator species for the root interior samples only. Red indicates a high presence in the Edge samples while green indicates Within samples

Methods

Forty *Acer saccharum* seedlings were collected from the Eastern slope of Mont Megantic. Twenty plants were sampled from along the edge of the altitudinal limit (2600 to 2715 feet) while another twenty were taken from slightly below this tree line and within *A. saccharum*'s range (2360 to 2450 feet). The samples were labeled as either **Edge** or **Within** respectively.

DNA samples were collected from four different plant surfaces or tissues:

- Rhizosphere** - the surface of the roots and the soil within 1 mm from the roots
- Phyllosphere** - the surface of the leaves
- Leaf Tissue** – the interior of the leaves
- Root Tissue** – the interior of the roots



DNA was extracted from the samples using the MoBio PowerSoil DNA Isolation kit. The samples were amplified with PCR using primers to target the V5-V6 region of the bacterial 16S rRNA gene [799F and 1115R]. The sequencing was performed on an Illumina MiSeq at the University of Montreal.

Data returned from the sequencing centre was processed using QIIME software. Sequences were binned into OTUs at a 97% similarity cut-off rate. The OTUs were assigned taxonomy using the RDP classifier. Singletons below a count of 20 were removed and each sample was rarefied to 4500 OTUs. This resulted in a total of 116 usable samples from 37 seedlings with a total OUT count of 3785.

From the sequencing data we quantified the community structure of the four different plant structures. Using a nonmetric multidimensional scaling ordination of the Bray – Curtis distances we calculated dissimilarity between the samples from the tree line and below as well as between the different plant structures. Using LEfSe we investigated the presence of indicator species in the four structures from the different elevations.

Conclusions

Acer saccharum seedlings have distinct bacterial communities inhabiting their leaves, roots and within different tissues.

The communities between the exterior of the leaves and roots and their interior tissues have greater similarity than between different structures of the plant.

There are significant differences in the bacterial communities at the plants altitude range limit in every structure.

Acknowledgments

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References

- Brown, C.D., and Vellend, M., 2014. Non-climatic constraints on upper elevational plant range expansion under climate change. *Proc Royal Soc B*. **281**: 1471-2954
- Davison, J., 1988. Plant Beneficial Bacteria. *Nature Biotechnol.* **6**: 282 - 286
- Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. *Genome Biol* **12**:R60