Microbial Community Analysis by Examination of Carbon Source Utilization Patterns by Gifford Pinchot National Forest Tree Canopy Soils

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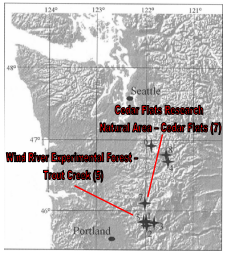


Figure 1. Location of sample collection in the Washington state (Van Pelt & Nadkarni)

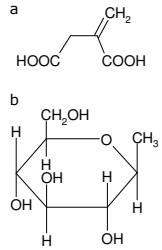


Figure 4. Structures of (a) itaconic acid and (b) β-methyl-D-glucoside

Introduction:

Although trees cover one-third of Earth's land surfaces, relatively little is known about the natural communities within forest canopies. Chemical analyses reveal distinct differences between arboreal and terrestrial soils; arboreal soil is higher in organic content, more acidic, and lower in concentrations of extractable ions (Nadkarni *et al*.). Although microbes undoubtedly play critical roles in forest-canopy nutrient cycling and arboreal soil formation, little work has assessed these microbial communities or the significance of distinct members.

In this study, microbiologists and ecologists collaborated to evaluate and compare the biochemical activities of the microbial communities in two Douglas-fir/western hemlock forests in southwestern Washington. Canopy and ground soil samples were collected from sites within Gifford Pinchot National Forest in southwestern WA using rope climbing techniques (Figure 1). Trees within the Cedar Flats Research Natural Area were sampled during Summer 2005 and the soils were found to be high in volcanic ash content due to their close proximity to Mt. St. Helens. Subsequently, trees from the Wind River Experimental Forest at Trout Creek were sampled during the Summer 2006 and contained a reduced ash content.

In the past decade, researchers have begun to characterize microbial communities using Biolog microtiter plate technology to monitor carbon source utilization patterns of natural communities (Garland and Mills). Using Biolog EcoPlates, followed by multivariate statistical analysis, we analyzed microbial community carbon source utilization patterns from temperate coniferous forest tree canopy soils compared to ground soils.

Hypothesis: Bacterial community composition differs significantly between forest-canopy arboreal soils and corresponding terrestrial soils.

Main Objectives:

- Identify biochemical differences between soil microbial communities of the forest floor and the canopy.
- Examine differences in soil microbial communities between the two different tree species sampled: Douglas-fir & western hemlock.
- Examine differences in soil microbial communities between the two different study areas.

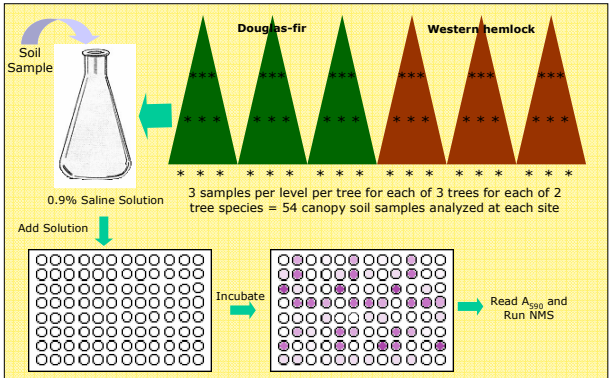
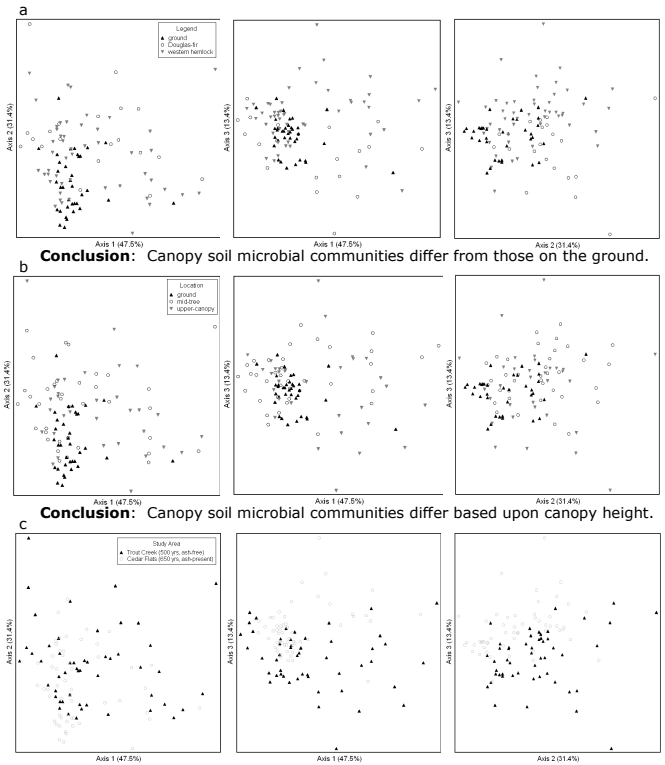


Figure 2. Culture-based, biochemical techniques investigate metabolic characteristics of the soil communities



Conclusion: Canopy soil microbial communities differ from those on the ground.

Conclusion: Canopy soil microbial communities differ based upon canopy height.

Conclusion: Canopy soil microbial communities differ between sample sites.

Figure 3. Non-metric multidimensional scaling ordination results explaining 92.4% of the variation and displaying comparisons of (a) ground samples versus tree species, (b) ground samples versus height in the canopy and (c) different study areas. Axis 1 contains almost half (47.5%) of the variation seen.

Experimental Methods:

- Community level metabolic activity was observed using BioLog EcoPlates consisting of 96 wells containing 31 different carbon sources in triplicate (Figure 2).
- The wells were inoculated with the soil samples in a 0.9% Saline solution.
- Metabolism caused wells to turn purple due to a reduction in a tetrazolium dye.
- Color development was monitored using a μQuant Universal Microplate Spectrophotometer from Bio-Tek Instruments over a ten day period.
- Data from day seven were charted based on relative absorbance values at 590 nm wavelength.
- Non-metric multi-dimensional scaling (NMS) was used to analyze the data.

Summary of Results (Figure 3):

- The forest floor communities were more similar to each other than to the canopy communities.
- There was variation in the canopy samples.
- Tree species and height within the tree contributed to differences in biochemical community structure.
- Sample location and ash composition highly affect the community capabilities.
- Itaconic acid (r^2=0.652) and β-methyl-D-glucoside (r^2=0.566) are the carbon substrates that accounted for the most variability among the samples.

Discussion:

NMS was used for analysis of the samples because there are no underlying assumptions about the normality of the data. Comparing carbon source utilization patterns using Biolog EcoPlates followed by NMS statistical analysis, differences among samples can be noted. 92.4% of the variation observed can be explained using three axes (Figure 3).

There are significant differences between ground and canopy samples. This is understandable because the microbes have a continuous environment from the ground below one tree to the ground below the next. The results of the NMS analysis for varying heights within the canopy as well as between tree species show a greater amount of variation. The samples at a specific height appear to be slightly more similar to each other than to samples at different heights. Tree species also appears to have an effect on carbon source utilization patterns, but there is variability here as well. Most significant is the analysis of microbial communities based on location, where distinct clustering of samples based upon sampling site was observed. This may be due to the effects that the ash had on the pH and the other characteristics of the soil.

Each of the 31 carbon sources was analyzed for the amount it contributed to variation among the samples. Those providing the most variation were itaconic acid and β-methyl-D-glucoside (Figure 4). Itaconic acid is excreted by fungi and thought to increase the solubility of metals and to decrease the soil pH inhibiting the growth of other microbes (Magnuson and Lasure). Production is similar to that of citric acid; itaconic acid is made from cis-aconitate by the enzyme cis-aconitate-decarboxylase (Bressler and Braun), and breakdown of itaconic acid is also accomplished by this enzyme. β-methyl-D-glucoside is produced in the leaves of many plants and is broken down by β-glucosidases (Aubert et. al.). These glucosidases, a large group of enzymes found in a variety of organisms, break down a variety of carbohydrates. Few β-glucosidases are able to cleave the side chain of β-methyl-D-glucoside. Both of these carbon sources, that are selectively broken down, appear to be good indicators of biochemical community structure.

This work provides evidence that carbon source utilization patterns in canopy soils differ from ground soils, suggesting that forest canopy soils harbor different microbial communities than the ground below. Thus, the forest canopy provides an unexplored frontier for the discovery of novel microbes.

Future Studies:

- Examine canopy soils from sites in other parts of the Pacific Northwest
  - Different tree ages
  - Different seasons

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