# Comparison of the Phylloplane Microbiota of Invasive *Delairea* and Native Plants


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## Abstract

Terrestrial leaf (phylloplane) surface area is about $6.4 \times 10^8$ km$^2$, yet there is little research on this large habitat. We aimed to apply a metabolic footprint approach to investigate microbial colonization of the phylloplane. We address the question of whether the phylloplane of native species and invasive *Delairea odorata* plants are different. The similarities and differences in the phylloplane microbiota may give new insight to the success and control of invasive species. Our hypothesis is that the phylloplanes of plants in a given habitat do not differ significantly, regardless of native or invasive status. Culturable bacteria from *D. odorata* leaves and native California coastal scrub plants were enumerated using aerobic heterotrophic plate counts of leaf washings and leaf impressions on nutrient agar. Metabolic characteristics of the phylloplane microbiome were assessed using Biolog Ecoplates. The number of culturable bacteria on *Delairea* ($8.75\times10^3\pm2.02\times10^4$ CFU/mm$^2$) was midway within the range found on the native plants ($0.79\pm1.46$ CFU/mm$^2$). The metabolic Shannon diversity index of the *Delairea* microbiota is 1.39, which is greater than that of native plants ($1.28\pm0.03$). More different microbial enzymes were found on *Delairea* leaves (richness: 23) compared to native plants (richness: $17.8\pm3.2$). We are currently classifying the bacteria. Our results suggest that understanding of plant microbiota could provide new ways to use plant-associated microorganisms to control invasive plants.


## Hypothesis

The phylloplanes of plants in a given habitat do not differ significantly, regardless of native or invasive status.

## Background

- The phylloplane is the part of the phyllosphere consisting of terrestrial leaf surfaces. This large, $6.4 \times 10^8$ km$^2$ , habitat houses an ecosystem of bacteria and fungi (7).
- Biotic factors influencing microbiota of the phylloplane include plant exudates and competing microorganisms.
- Bacteria introduced to the phylloplane from contaminated water exhibited increased growth during winter rather than any other season indicating that the phyllosphere is influenced by abiotic factors such as water, light, temperature (6).
- *Delairea odorata*, Cape Ivy (Asteraceae, **Figure 1**), is an invasive vine originating in South Africa, *D. odorata* is invading natural areas in Australia, Europe, and the United States. It is a serious pest that currently occupies over 500,000 acres of California (5).
- Recent studies have been shown that *D. odorata* causes a decrease in diversity of native plants In California (2) It is threatening 12 rare native plant species, two endangered butterfly species, and compromising the habitats of freshwater shrimp and Coho salmon (1).
- It quickly regrows after mechanical removal or treatment with herbicides (3). *Parafreutreta regalis*, a tephritid fly, is one possible biological control (4).
- Understanding the microbiota of the Cape-Ivy phylloplane may give us new insight into biological control of invasive species.
- By using the metabolic footprint approach, we can measure the microbial richness and diversity of the phylloplanes of native and invasive plants, which may provide new insight on biological control of *D. odorata.*

## Methods

- Collection sites were on Sweeney Ridge, Golden Gate National Recreation Area .
- Plants were collected aseptically using gloves, sterile collection cups and autoclaved forceps.
- Surface area of the leaves was determined using ImageJ (imagej.nih.gov/ij/).a 2D rendition of each leaf was made using photographs of the leaves.
- Biolog Ecoplates containing 31 different substrates were used. Leaves were diluted $10^{-1}$ [CC1] by weight in phosphate-buffered saline (PBS), vortexed, and diluted again in PBS. Each well on the plate was inoculated with 100 µL of the $10^{-3}$ dilution. The rate of utilization of the carbon sources was identified by reduction of tetrazolium violet redox dye. Color change was recorded at 590 nm. Absorbance of the control (water) wells was subtracted from the absorbance of each test well.
- Culturable bacteria were quantified by heterotrophic plate counts.1 g leaf was diluted in sterile distilled water and plated on nutrient agar. Plates were incubated at 36°C.
- Isolated colonies were subcultured and tested for gram reaction, catalase, and oxidase. The oxidase test was performed using oxidase test strips (Hardy Diagnostics).

**Figure 1.** Cape Ivy forms large monocultures that block light from other plants.

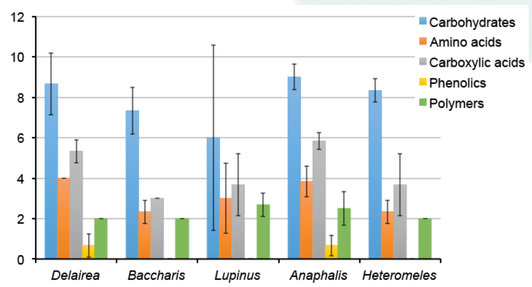


**Figure 2.** Functional diversity of bacteria on *Delairea* averaged 70.96% ± 5.58% and on Native plants averaged 59.99% ± 16.58%. Error bars = 1 S.D.

## Results

- *D. odorata* communities used 23 of 31 carbon sources compared to an average of 16.75 used by microbes on native plants (**Figure 2**).
- This resulted in higher metabolic diversity and richness of *D. odorata* microbiota than native plants although native plants have a greater evenness of microbial enzymes. (**Figure 3**).
- The number of culturable bacteria ($5.4 \times 10^7 \pm 1.3 \times 10^8$- CFU/g) on *D. odorata* was midway within the range found on native plants. The highest number ($8.6 \times 10^7$ CFU/g) was on *Heteromeles* [CC2] and the lowest number ($3.9 \times 10^3$ CFU/g) was on *Baccharis* (**Figure 4**).
- Enterobacteriaceae were the predominant culturable bacteria in all the samples.
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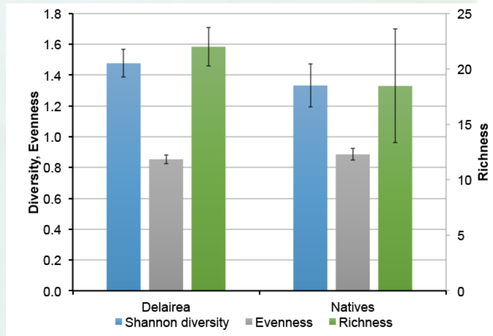


**Figure 3.** Microbial communities on *D. odorata* showed more diversity and richness. Microbial species were more evenly represented on native plants. Error bars = 1 S.D.

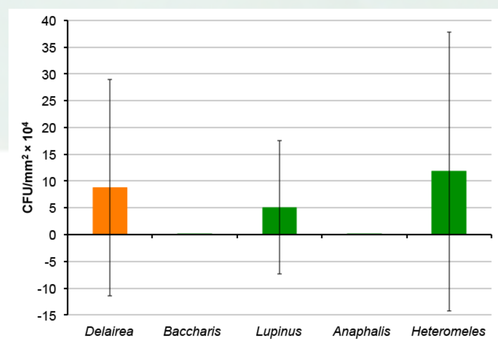


**Figure 4.** Culturable bacteria from *D. odorata* exhibited significant growth compared to native plants with *Heteromeles* being the exception. Error bars = 1 S.D.

## Discussion & Conclusion

- *D. odorata* scored higher regarding richness and diversity of substrates used by the bacterial communities on their leaves.
- Native plants showed more evenness and abundance of bacterial species. The decreased evenness on *D. odorata* may indicate an effect of this invasive on microbial communities.
- However, the similarities between *D. odorata* and native plants suggest that the phylloplane of leaves is similar for habitat rather than host species and, therefore, most affected by abiotic factors.
- **Future studies** include
  - Determining whether microbes are genuine phylloplane inhabitants, and not transients.
  - Fungal identification.
  - The effect of abiotic factors on the phylloplane.
  - Further identification of bacteria of the phylloplane.

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