NUTRIENT CONTENT AND BIOTA OF EPIPHYTIC AND TERRESTRIAL SOILS IN A TROPICAL FOREST IN MOOREA, FRENCH POLYNESIA

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Abstract. Despite epiphytes constituting only up to two percent of a rainforest’s biomass, they significantly contribute to keeping mineral nutrients locked and sequestered in the ecosystem. Epiphytes have the unique ability to capture and mineralize airborne nutrients. These nutrients are not only absorbed and integrated into the tissues of the epiphytes, but they also become a part of the soil that accumulates beneath the mats of moss. This arboreal or epiphytic soil may be different from terrestrial soils in primary nutrient content and invertebrate biota. This study aims to better understand epiphytic communities and their soil biota. Specifically, it seeks to better understand epiphytic host-tree preference between the two dominating trees in the Hibiscus-Inocarpus lowland rainforest in Moorea, French Polynesia and the possible differences in soil biota between epiphytic soil invertebrate communities and terrestrial soil invertebrate communities. Additionally, soil samples were tested for primary nutrient content, N, P, K and pH. While there were no significant differences in all primary nutrients between epiphytic and terrestrial soils, pH between the two soils differed significantly, suggesting a difference in other essential cations such as Ca, Mg, and Fe. These elements were not detectable with the soil testing kit that was available at the time. Epiphytic diversity on Inocarpus fagifer had a significantly higher Shannon-diversity index than on Hibiscus tilliaceus. Epiphytic soils in general have a higher diversity index of invertebrates compared to that of terrestrial soils. Inocarpus soils had the highest invertebrate diversity followed by Hibiscus soils.

Key words: epiphyte communities; epiphytic soil; terrestrial soil; substrate biota; invertebrates; primary nutrient; Inocarpus fagifer; Hibiscus tilliaceus;

INTRODUCTION

In tropical rainforest ecosystems, epiphytic tissues can hold up to 45 percent of the nutrient capital held in the foliage of their host trees (Rains 2003, Nadkarni 1984). They are responsible for a considerable amount of the sequestration and immobilization of minerals in a rather labile system (Nadkarni 1984). Nutrients that have been integrated into their bodies come from primarily airborne minerals which epiphytes accrue to create a substrate under the mats of their leaves (Benzing 1981, Benzing 1984, Nadkarni 1984). Studies on mineral nutrient pool and flux dynamics have been important in the development of theories of community stability and ecosystem resilience (Nadkarni 1984). This has crucial implications for nutrient cycling in a forest system when considering management plans for rainforest soil, secondary succession after clear-cutting, and studying the resilience of deforested areas.

Ecosystem-wide mineral nutrient studies have led to the formation of fundamental mineral cycling concepts (Rodin and Bazilevich 1967). Understanding the formation and differences between soils of different origins in an ecosystem is crucial to understanding a specific ecosystem’s mineral nutrient cycle because soils are large pools for these nutrients. An important actor in soil formation in forests are arboreal epiphytes, namely mosses and lichens (Nadkarni 1984). However, the role epiphytes play as an accumulator of soil and as a generator of a nutrient pool is an understudied topic in ecosystem ecology (Woodwell and Whitaker 1968, Nadkarni 1984).

Because their biomass has been considered insignificant in comparison to other major forest components (Nadkarni 1984), the magnitude of the ecosystem services that epiphytes provide has been downplayed in proportion to the magnitude of the services of other forest parts. Consequently, while soils as a general field of science are a well studied topic in ecosystem ecology, soils that form through association with epiphytes, on the other hand, have not been studied as much as
other ecosystem parts that contribute to soil formation (Zotz and Hietz 2001). Epiphyte studies have generally been limited to morphological and taxonomical investigations (Benzing 1981). Their contributions to nutrient cycling on an ecosystem-level, especially with respect to impounding nutrients into the soils they create, have mostly only been examined in neotropical cloud forest canopies, and even these studies are sparse (Pike 1972, Nadkarni 1991).

Epiphytic soils are not only important in the cycling of primary nutrients (N, P, K), but are also crucial in that they house a rich biota (Giller 1995). They are responsible for housing organisms that decompose nearly 60-90% of terrestrial primary production, terrestrial insects, most of which are soil dwellers at some point in their lives, and microbiomes whose phylogenetic biodiversity is surpassed perhaps only by coral reefs (Giller 1995). The aforementioned biotic components of epiphytic soils, as well as its abiotic counterparts (mineral nutrients) are both topics that need more investigation for a thorough understanding of the relationships between epiphytes, the nutrient content of soil they create, the biota within their soils, and terrestrial soils adjacent to their host trees (Giller 1995, Nadkarni 1991).

Despite the lack of ecological knowledge on the roles epiphytes play within an ecosystem, the mechanisms by which epiphytes accumulate soils are known. Epiphytic soils form by epiphytes accumulating minerals and expanding the nutrient capital of their host trees primarily in three ways (Nadkarni 1984, Rains 2003). First, their mats increase the surface area of their hosts by extension of their leaves, allowing for more contact with rain, mist, and dust, which provide essential minerals (Benzing 1981, 1984). Second, negatively charged sections of their mats retain cations (Ca, Mg, Fe) provided by throughfall and stemflow (Nadkarni 1984). Third, physiological and morphological characteristics such as absorptive trichomes are highly efficient in capturing and retaining airborne nutrients (Nadkarni 1984). After the sequestration of these mineral nutrients in their tissues and soil, epiphytes, their accompanying substrate, and other organic matter from the canopy of the host tree can fall to the forest floor, contributing litterfall that adds a significant portion of primary nutrients and carbon to the forest floor (Nadkarni and Matelson 1992).

Thus, terrestrial soil nutrient levels are affected by epiphytes. However, terrestrial soils do not have the same capabilities to accrue the same nutrients. For example, ground soils do not have the same means by which epiphytes capture airborne nutrients and protect nutrients from leaching by providing rhizoidal anchors for soil substrates. For these reasons, we might expect primary nutrient content from ground soils to be lower than primary nutrient content from epiphytic soils, but this has yet to be explored.

The aforementioned qualities that distinguish epiphytic soils from ground soils lead to several questions regarding the nutrient and biotic content in arboreal soils compared to that of ground soils. Here I tested three hypotheses. First, I hypothesized that epiphytic soils contain higher levels of primary nutrients, nitrogen (N), phosphorus (P), and potassium (K), than in ground soils. Second, I hypothesized that epiphytic soils are higher in species richness and abundance than the soils that are directly below their host trees; organisms in epiphytic soils and their biota could have a dynamic relationship with their host tree species and are protected by the epiphyte cover from excessive wind, sunlight, rain, and various environmental factors that can contribute to turbulence and disturbance in soils. Third, I predicted that epiphyte species richness will be higher on Hibiscus tilliacus than on Inocarpus fagifer.

**METHODS**

Sampling took place in the *Inocarpus-Hibiscus* lowland rainforest near the Moorean Maraes (Figure 1, 17°31'59.6"S 149°50'04.0"W), religious temples used by the ancient people of Mo'orea.

**FIG. 1.** Satellite image of Mo’orea, French Polynesia. Sampling site indicated in the box. The forest is dominated by *Inocarpus fagifer*, and the next dominating species is *Hibiscus tilliacus* (Figure 2).
Fig. 2. Tree on the left is **Hibiscus tilliaceus**, tree on the right is **Inocarpus fagifer**.

**Field site and sampling**

The boles of both tree species are covered with various species of moss. The epiphytic soil that was examined accumulates between the mats of rhizoids of these epiphytes and the bark of **Inocarpus** and **Hibiscus** trees. Sampling took place on October 19th, 22nd, 27th, November 3rd, 6th, 8th, and 10th. Field days were consistent in weather and sampling time of start and end.

The sampling process was standardized on a per-tree basis. Eight **Inocarpus** trees were randomly selected every 30m starting from the beginning of the Three Pines Trail. However, **Hibiscus** trees were distributed very sparsely compared to **Inocarpus**, and the first eight **Hibiscus** that appeared on the trail were sampled. For every tree, five items were collected: epiphytic soil sample collected at breast height, ground soil sample collected from 0.5m from the trunk of the sampled tree, and three 4cm² patches of epiphytes and their accompanying substrates along the circumference of the tree at breast height for in-substrate community analysis.

Epiphytic soils and ground soils were collected at varying quantities, but each sample’s dry weight exceeded 6g, which was the amount required for soil chemistry tests (LaMotte Co., Code 3-5880).

**Epiphyte community analysis**

The epiphytic community on each tree was characterized using standardized circumferential area, which was calculated by multiplying circumference by 30cm, an arbitrary value. The community was characterized by assessing percent cover of different species of epiphyte. One group was collectively characterized as leafy liverworts. Certain epiphytes consistently dominated the surfaces of each tree and many epiphytes covered less than 10% of the area; Only mosses that had equal to or greater than approximately 10% cover were identified. Epiphytes were identified using illustrative and dichotomous keys (Whittier 1976). Epiphyte richness and abundance of each species were used to calculate diversity using Shannon-Weiner index (Oxford, A Dictionary of Ecology 2014).

**In-substrate community analysis**

There were two levels to in-substrate community analysis, and the following applies to both epiphytic and ground soils. The first level was to identify large organisms that can be observed under the dissecting microscope. These organisms include large arthropods and large nematodes. The second level was the detection of small nematodes, soil mites, and rotifers under the compound microscope at 10x magnification.

For the first level, three grams of wet soil were examined for large invertebrates by thinly spreading wet soil on an 89mm petri dish. The sample was observed for 10 minutes. Afterwards, I diluted the same sample in 30ml of water and used the solution for small invertebrate analysis.

For the second level of analysis, I took two drops from the 30ml solution to look under the compound microscope at 10x and 40x magnification. The soil was agitated before extraction to obtain a relatively homogenous mixture. 10x magnification is enough to detect soil animals and distinguish them from plant tissues. 40x magnification was used to verify what an organism was at the phylum level. Slides under the compound scope were analyzed for 10 minutes each.

**Soil nutrient analysis**

Soils were tested for three primary nutrients: nitrogen, phosphorus, and potassium. I used a LaMotte N, P, K tablet-based soil testing kit (LaMotte Co., Code 3-5880). I followed the instruction manual provided for all soil chemistry tests.

The following was applied to both epiphytic and ground soils. The only difference between them was that epiphytic soils had to be separated from from the rhizoids they were attached to. A general soil solution mix was
created with 6g of dried soil sample, 30mL of deionized water and a FLOC-EX tablet (LaMotte Co.), a floculent, which causes fine particulates to clump and settle to the bottom of the solution, creating a clearer solution.

With this general solution, I was able to create three solutions of smaller volumes to measure N, P, and K levels. The levels of primary nutrient content corresponded to a specific kg/Ha value (Figure 3).

<table>
<thead>
<tr>
<th>PN Levels</th>
<th>N</th>
<th>P</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>22.4</td>
<td>4.5</td>
<td>22.4</td>
</tr>
<tr>
<td>L-M</td>
<td>78.4</td>
<td>12.3</td>
<td>56.0</td>
</tr>
<tr>
<td>M</td>
<td>112.1</td>
<td>15.7</td>
<td>67.3</td>
</tr>
<tr>
<td>M-H</td>
<td>224.2</td>
<td>34.7</td>
<td>112.1</td>
</tr>
<tr>
<td>H</td>
<td>269.0</td>
<td>47.1</td>
<td>134.5</td>
</tr>
</tbody>
</table>

Fig. 3. Primary nutrient (PN) levels (low, medium, high) corresponding to kg/Ha values for nitrate, phosphorus, and potassium.

Each cylinder for each nutrient had its own specific tablet provided by the soil kit. All three primary nutrients were analyzed visually with a color chart. Nitrate levels corresponded to a pink hue gradient, where low levels of nitrogen reflected a clear pink solution and high levels of nitrogen reflected a solution closer to red. Phosphorus levels were detected in the same way as nitrate levels, but on a skyblue-indigo gradient. Potassium was detected by levels of clarity, where the solution with the dissolved K-tablet would reflect different degrees of cloudiness. Furthermore, I measured the pH of each soil sample.

**Data analysis**

Ten categories of epiphytes were found (see Appendix 1): five species of moss, three species of filmy ferns, leafy liverworts, and lichen. Response variables for both epiphytic and ground soils across two species of trees included primary nutrient content, pH, in-subsitet diversity, and epiphyte diversity on trees. ANOVA, t-tests, and chi-squared tests were used to examine the differences and relationships between the means of each category of data.

**RESULTS**

**Epiphyte community analysis**

The Shannon-Weiner index was used to calculate the diversity of the epiphyte communities on *Inocarpus* and *Hibiscus*. *Inocarpus* had a diversity index that is more than double that of *Hibiscus* communities (t-test, degrees of freedom, statistic, p < 0.0007) (Figure 4).

Fig. 4. Difference between the Shannon-Weiner indices of epiphytic communities on *Inocarpus* and *Hibiscus*. Error bars represent 95% confidence intervals.

Additionally, an NMDS ordination plot demonstrates a much stronger clustering of points representing epiphytes on *Inocarpus* than points representing epiphytes on *Hibiscus*. Even after data transformation, the NMDS ordination plot did not show a consistent pattern with epiphytes on *Hibiscus* (Figure 5).

Fig. 5. NMDS ordination plot exhibits clustering of points. Each point represents the epiphyte community on a specific sample.
This was expected because *Hibiscus* had lower richness than *Inocarpus*. Additionally, total percent cover of epiphytes on each sample was higher on *Inocarpus* than on *Hibiscus*.

**In-substrate community analysis**

For soil biota analysis, I used the Shannon-Weiner index to calculate the diversity of in-substrate communities in *Inocarpus* epiphytic soil, *Hibiscus* epiphytic soil, ground soil 0.5m from the trunk of *Inocarpus* and *Hibiscus*. (Figure 6).

![Graph showing Shannon-Weiner diversity index for different substrate types.](image)

**Fig. 6.** Difference between the Shannon-Weiner indices of *Inocarpus*, *Hibiscus*, and their respective nearby ground substrates. Error bars represent 95% confidence intervals.

I used ANOVA to compare the means of each of the aforementioned substrate variables and found significant differences (p < 0.001). Subsequently, I performed a Tukey test to find out the specific pairs of the variables whose differences generated significance. All pairs (a total of 6) except for the comparison between ground soil near *Inocarpus* and ground soil near *Hibiscus* had significant differences (p < 0.05).

**Soil nutrient and pH analysis**

For primary nutrient analysis, I used chi-squared tests on each primary nutrient (N, P, and K) for each soil type mentioned in the previous section (for a total of 12 tests). Eleven of the tests failed to show significance, and only one of the tests for phosphorus content in ground soil near *Inocarpus* showed significance (p < 0.003). This may be an outlier.

For soil pH analysis, I used ANOVA, and four types of substrate were analyzed (Figure 7).

![Graph showing pH differences between different substrate types.](image)

**Fig. 7.** Difference in pH between substrate types. Error bars represent 95% confidence intervals.

Four out of six of the comparisons showed significance (p < 0.01). As expected, *Hibiscus* ground substrate and *Inocarpus* ground substrate comparison failed to show significance. Comparison between *Hibiscus* epiphytic substrate and *Inocarpus* epiphytic substrate also failed to show significance.

**DISCUSSION**

Moss epiphytic diversity was much higher on *Inocarpus* than on *Hibiscus*. Possible explanations include differences in bark structure and texture, growth rate and structure of the trees, or different ecosystem services provided by different invertebrate biota. One possible explanation is differences in bark chemistry which has been demonstrated to alter epiphyte composition in other species. For example, it is known that epiphytic preference on different locations on *Populus tremula* (quaking aspen) strongly depends on exchangeable Ca, Mg, and Na fixed in the bark (Gustafsson and Eriksson 1995).

In addition to bark chemistry, the study on quaking aspen also found that the height of the tree and the direction at which epiphytes face sunlight also affect epiphyte habitat location (Gustafsson and Eriksson 1995). Future studies...
that have access to a more rigorous nutrient testing kit may be able to detect differences in levels of exchangeable cations and correlate the differences with epiphyte habitat preference. Another feature which may influence epiphyte diversity is the morphology of *Inocarpus* that allows for the development of unique mini-biomes in the crevices and catchments created by the giant buttresses of the tree.

These mini-biomes include shaded, moist anthills at the base of the tree, spider webs that use the buttresses as anchors for their webs, and small bowls of soil that act as nurseries for larger, vascular epiphytes such as orchids. Epiphyte communities differ significantly in different localities on a single *Inocarpus* tree and the average abundance of epiphytes on trunks is double that of buttresses (Fok 2007). This complex ecosystem is one that the *Hibiscus* does not provide and may be responsible for the difference seen in epiphyte diversity across the two tree species. Future studies could investigate the relationship between the morphology of different tree species and its effects on epiphyte diversity on the trees. Additionally, physiological factors of the trees should be considered; *Inocarpus fagifer* is part of the legume family and is a nitrogen fixer, and this may contribute to the differences in epiphytic diversity (Gustafsson and Eriksson 1995).

In previous studies, diversity has been shown to be significantly higher in arboreal soils than in ground soils (Paolleti 1991). The same pattern was found in this study as well. Within arboreal soils, *Inocarpus* had a higher diversity index than *Hibiscus* and more than twice the in-substrate diversity of terrestrial soils. Possible explanations could include the same reasons mentioned in the previous section for epiphyte community analysis. However, ground soil is affected by other unique factors such as runoff, stream erosion, and receiving higher organic matter contributions from litterfall (Nadkarni and Matelson 1991). Another unique input for ground soil includes mineral nutrients that have been eroded by streams and carried by rain; Epiphytic soils, on the other hand, accumulate mineral nutrients by sand and dust carried by winds (Hsu 2002). Invertebrate diversity might be lower for ground soils because of the aforementioned factors. Epiphytes may act as protective cover for these invertebrates.

This study found no significant difference in the three primary nutrients, N, P, and K, between epiphyte and ground soil. However, pH was significantly higher in the ground soils than the epiphytic soils. While this could be a result of higher organic matter content in epiphytic soils, it can also be a result of the possible higher capacity for epiphytic soils to retain cationic nutrients than ground soils can (Nadkarni 1984). Future studies could investigate the relationship between soil nutrient chemistry and in-substrate biota for a causal link.

Another mini-biome that may show unique nutrient levels and community structure are the bowls of *Inocarpus* trees. Water tree holes in Neotropical zones have been found to house communities with unique predator-prey dynamics (Yanoviak 2001). Increased litter in water filled tree holes coincided with increased species richness and growth rate of predators (Yanoviak 2001). The same pattern may appear for *Inocarpus fagifer*. *Inocarpus* morphology creates small catchments throughout its trunks that capture litterfall, water, and epiphytic soil. Small orchids and ferns grow in these “plant pots”. The nutrient content in these pots can be compared with the epiphytic soils examined in this study and arboreal soil structures engineered by ants, which are prevalent throughout the *Hibiscus-Inocarpus* rainforest.

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**LITERATURE CITED**


APPENDIX A

Photographs illustrating the epiphytes found in this study:

Rectolejeunea sp.

Crepidomenes bipunctatum

Calympere moorei
Orthorrhynchium cylindrium

Ectropothecium sodale

Juvenile lichen

Crepidomanes minutum

Didymoglossum tahitense

Two leafy liverworts

Calymeres pseudopodianum