

## Intro:

The intensity and types of environmental challenges facing plants vary at all spatial scales, from landscape-level scales down to variability among different regions of individual plant bodies. Temporal changes in environmental challenges to plants also range from hourly, to seasonal or even decadal fluctuations in climate, pathogen loads, nutrient availability, and exposure. Plant genomes contain many solutions to these challenges, especially to environmental challenges which have acted as long-term selective pressures on plants. In these times of rapid change, however, the abundance and rhythm of all types of plant stressors has increased, and plant genomes alone may not evolve quickly enough to ensure the success of ecologically or socially important plants in the face of increased pathogen loads, decreased climatic stability, and decreased habitat availability.

Some have proposed that the plant microbiome, with its collective abundance of microbial genetic resources, may represent an important and adaptive addition to the host plant genome. Together, it is proposed, the microbiome and the plant sum to make up the plant “hologenome”, which is more extensive and more dynamic than the plant genome alone. It remains a mystery how historically important hologenomes were in shaping plant populations, even prior to the current era of rapid anthropogenic change. However, evidence has mounted rapidly that microbiomes are important determinants in plant distributions and ecosystem health. If microbiomes are to be harnessed in any way to help plant health in times of rapid change, their spatial and temporal patterns must be better understood.

## Methods:

Here we propose to undertake sampling for the functional microbiome of a host tree species, *Eugenia nesiotica*, across the extent of the BCI ForestGEO plot. Endophytes from both leaf and root tissue of *Eugenia* will be surveyed for the presence of biosynthetic gene clusters, using two survey methods:

## Laboratory:

1) A metabarcode study will broadly target polyketide synthases (PKS) and nonribosomal peptide synthases (NRPS) with degenerate primers. Though plants show much-reduced capacity for these families of biosynthetic gene clusters, primer design will attempt to include plant-blocking nucleotide sequences. These data will be engaged to get a general picture of some broad classes of secondary metabolites that tree microbiomes are predicted to be producing differentially in response to environmental challenges. These enzymes encode backbone molecules for two of the broadest families of microbial secondary metabolites (polyketides and non-ribosomal peptides), and exist in diverse homologs that can be used to infer medium-to-fine grain portrait of the secondary metabolome of the plant microbiome.

2) This metabarcode will be augmented with more targeted PCR surveys for the presence of biosynthetic pathways of select microbial products. For example: trees challenged by herbivory may show upregulation of alkaloids, such as ergotamines, and trees challenged by drought stress will show increased fungal antioxidants and osmolytes. Trees challenged by pathogen loads, including those that reside close to a conspecific (see below) may show upregulation of fungal antifungals (echinocandins, polyenols) and fungal chitinases. Existing literature and public databases will be used to develop pathway-specific primers for each of these enzyme synthesis pathways.

## Sampling scheme:

In both survey methods, spatial analysis of the presence of “useful” metabolites produced by plant-associated microbes will be coupled with microsite data, estimates of disease and herbivory load, and the proximity of conspecific trees (see below). Leaf and root tissue will be collected at numerous locations within the plot according to a spatial grid with a grain of 10m. Additionally, aerial plant tissues will be sampled at inner and outer, upper and lower (2 x 2) sites, to test microsite variability in microbiome expression. (See figures 1 and 2, which I haven’t made yet...) In all cases, bulk DNA will be purified from host plant tissue and selectively amplified with above primers. Plant pathogen loads and damage from herbivory will be scored visually at the site of each collection.

## Statistical Analyses:

Metabarcoding results will be subject to standard bioinformatic pipelines to remove sequencing artefacts and to agglomerate reads into enzyme pathways. Resulting matrices of enzyme pathways present in our samples will follow standard numerical ecology-based methods for modeling large matrices as dependent objects of environmental data (“regression” of metabarcoding results against environmental data and physical distances using redundancy analyses, including variation-partitioning methods). Read libraries will also be mined for specific biosynthetic pathways of interest (alkaloids, antibiotics, antioxidants, osmolytes, etc), to the level of specificity allowed by the homology of the polyketide and peptide backbones. and these will be treated as point patterns, along with the results of the targeted PCR tests. Point patterns of secondary products of interest will be analyzed for cooccurrence with environmental challenges, herbivory, pathogen loads, and proximity of conspecifics.

## Pertinence to research on-going at Smithsonian Institute (SI) and Smithsonian Tropical Research Institute (STRI):

One of the greatest contributions to tropical ecology in recent memory by SI and STRI is the empirical testing of the Janzen-Connell hypothesis (JCH), and exploration of the mechanisms behind the negative density dependence of seedling success as predicted by the JCH. This work will add to this ongoing effort to exploring this natural pattern in tropical forests. Mangan et al. (2010) found that soil-born enemies may be main culprits in the negative density dependence patterns of rare trees in the tropics, but noted that highly abundant trees do not seem to display the same vulnerabilities to pathogens from congeners that rare tree do. The mechanisms at work in abundant tree species in overcoming the limiting of the JCH are still largely unexplained, though one group has found that small population sizes of rare trees may be subject to inadequate R gene diversity and gene flow (Marden 2017) to keep up in the pathogen arms race. Here we suggest another, complementary mechanism - if functional microbiomes are key to pathogen resistance (as suggested here by us and elsewhere by numerous others), **then recruitment of beneficial microorganisms into the microbiome may represent a positive-density-dependent feedback.** Abundant organisms may be able to transmit their microbiomes to seedlings more successfully (see Christian et al. (2017)) causing positive-density-dependent feedbacks in seedling success, and clusters of abundant species may represent larger “target” areas not only for pathogens but for environmental recruitment of beneficial microbial partners. As just one example, Rock-Blake et al. (2017) found hypersensitivity by host plants to availability of symbiotic soil fungal partners on the landscape. This indicates that the availability of mutualistic symbioses may be important to overcoming the inhibitory effects of soil-born enemies and the negative density: both

plant and fungal mycorrhizal partners presumably benefit by the presence of the other. This would therefore represent a possible positive-density-dependent feedback that could counter the effects of soil-born fungal pathogens.