Determining the role of edaphic factors in structuring *Inga* (Fabaceae) diversity in a tropical forest

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BACKGROUND: One goal of tropical forest ecology is to describe the drivers that allow for the maintenance and generation of plant diversity. A variety of edaphic factors are thought to play a significant role in allowing for the persistence of biodiversity in plant communities in general, and in tropical forests in particular. For example, a recent pan-tropical analysis found that soil resources and topography explain ~30% of the variation in plant community composition over a forest.[2] Similarly, an analysis of the Yasuní Forest Dynamics plot in Ecuador (hereafter "Yasuní") found that the distribution of ~40% of the tree species significantly correlates with soil nutritional profiles.[3] Soil biotic factors are also thought to contribute to forest structure. Pathogenic soil microbes may drive coexistence of dissimilar plant species by limiting the recruitment of seedlings near conspecific adults.[4,5] Conversely, arbuscular mycorrhizal fungi, which colonize roots of plants in mutualistic symbioses, are thought to promote the recruitment of seedlings near conspecific adults.[6] The net impacts of these competing drivers in allowing species coexistence remains unclear. Using a clade-based approach to understanding coexistence is especially interesting, as competition between closely related species is expected to be stronger than between distant species.[7] Such an approach may also yield insight into the development of ecological differences between sister species.

The family Fabaceae (i.e. legumes) is remarkably diverse and abundant in Neotropical forests.[8] For example, at Yasuní, legumes top the list of families for basal area, number of individuals, and number of species.[9] The legumes are also ecologically distinct: in attempting to classify tropical plants into functional groups for analyzing ecosystem impacts of biodiversity, a recent study[10] found that legumes in a Costa Rican forest have a unique suite of functional traits relative to all other plants in the forest. The difference was not explained by the legumes' high Nitrogen (N) concentrations or their compound leaves. The remarkable diversity and ecology of legumes, along with their functional relationship with N fixing rhizobacteria, make them an ideal clade in which to study the relationship between edaphic factors and plant biodiversity.

I propose to focus on the genus *Inga,* which is the second most abundant genus in Yasuní in terms of basal area, number of individuals and number of species. *Inga*, which has its largest center of diversity in the Andean foothills and Western Amazonia near Yasuní, is a member of the Mimosidae subfamily, which commonly exhibits root nodulation.[11] The genus has recently been the subject of much attention, including a complete taxonomic revision.[12] Although *Inga* species clearly segregate based on morphological characters, the phylogenetic signal in DNA sequences is relatively weak, suggesting a recent and rapid diversification.[11] Chemical analyses of *Inga* anti-herbivory compounds also showed little phylogenetic signal, but revealed that co-occurring species tend to have more disparate anti-herbivory compound profiles than expected by chance. This suggests that interactions with pathogens may be a potential axis of niche differentiation within the genus.[13]

PROPOSED RESEARCH: My objective is to describe how edaphic factors impact diversification and coexistence in the genus *Inga* in Yasuní. I will have two specific goals – ***Goal 1:*** *Determine the structure of the microbial community occupying Inga spp. rhizospheres*; and ***Goal 2****: Determine the relationship between Inga phylogenetic diversity, biotic and abiotic edaphic factors, and plant functional traits*.

APPROACH TO GOAL 1: I will sample at least ten individuals from each *Inga* species to generate a robust estimate of their microbiome. I will extract genomic DNA from the rhizosphere

and sequence the 16s rRNA gene region using universal primers and published next-generation sequencing protocols[14] at the Smithsonian Laboratory of Analytical Biology. If a large proportion of the sequences fail to match to a known bacterium (as might be expected, given that the microbial diversity of the tropical rhizosphere is largely undescribed), sequences will be organized into Operational Taxonomic Units. I will use the UniFrac method[15] to make pairwise comparisons of microbiomes to test the hypothesis that (**H1**) the difference between the average rhizosphere microbiomes of two species positively correlates with the phylogenetic distance between them. I will also compare microbiomes of multiple individuals of a species to quantify the variation within a species. In order to determine the direct impact of host phylogeny on rhizosphere bacterial community, I will grow *Inga* spp. in a common garden setting at the Yasuní Scientific Station and compare the rhizosphere using the procedure described above. Additionally, I will attempt to sequence bacterial 16s rRNA from root nodules and the ITS region of root fungal endophytes using published protocols.[14]

APPROACH TO GOAL 2: I will collect topographic information (elevation, slope, convexity and aspect) following published protocols[2] at each corner of a 1m x 1m box around each individual sampled for Goal 1. I will use elemental analysis to determine soil nutrient profile and gravimetrically determine soil moisture content. Plant functional traits related to edaphic factors will be collected as required. These data will be combined with published *Inga* phylogenetic and functional trait data[11,16] to test the hypothesis that (**H2**) *Inga* spp. separate regularly over one or more of the edaphic factors measured. I will employ nonmetric multidimensional scaling and other multivariate statistics to synthesize across edaphic factors and plant traits.

INTELLECTUAL MERIT: The 16s rRNA sequences from soil bacteria will be deposited to the Ribosomal Database Project.[17] Herbarium vouchers of the plants will be submitted in duplicate to the National Herbarium of Ecuador and the US National Herbarium. This study will synthesize a variety of published information with novel data to develop a mechanistic explanation to previous results showing that the structure of the plant community at Yasuní is determined by species-level ecological strategies.[16] Finally, there are concerted international efforts to study legumes, which have been suggested as a model group of plants in which to test ecological theories.[18,19] The proposed study represents a novel approach to study this important clade. I will present results from my studies at the Association for Tropical Biology and Conservation annual conference and through peer-reviewed publications.

BROADER IMPACTS: The National Museum of Natural History (NMNH) attracts more than 7.5 Million visitors per year, making it the most visited science museum in the world. I have developed strong relationships at the museum during an NSF-REU internship, and will continue to collaborate with NMNH scientists while a student at the University of Maryland. I plan to develop a project incorporating molecular and herbarium-based data collection for the NMNH Youth Engagement in Science program, which recruits Washington D.C. area high school students from minority groups. Social media is another powerful avenue on which to bring science outside the laboratory. I plan to write a blog about tropical ecology for a non-technical audience, with a focus on describing a tropical ecologist's lab and field activities. I will invite other scientists at both the University of Maryland and the Smithsonian to contribute to this blog. Finally, I have a demonstrated commitment to international collaboration in ecology (see *Personal Statement*), which I will continue to develop during my PhD research.

References: 1. Bever, J.D. 2002. New Phytol. **2.** Baldeck, C.A. 2013. Pros. R. Soc. B. **3.** Robert, J. 2007. PNAS. **4.** Mangan, S.A. 2010. Nature. **5.** Comita, L.S. 2010. Science. **6.** Mangan, S.A. 2010. Ecology. **7.** Darwin, C. "On the Origin of Species", Ch. 3. **8.** Gentry, A.H. 1988. Ann. Miss. Bot. Gard. **9.** Valencia, R. 2004. In "Tropical forest diversity and dynamism." **10.** Powers, J.S. & Tiffin, P. Funct. Ecol. **11.** Richardson. J.E. 2001. Science 293(5538). **12.** Pennington T.D. 1997. "The Genus Inga." **13.** Kursar, T.A. 2009. PNAS 106(43). **14.** Oh, Y.M. 2012. Microb. Ecol. **15.** Lozupone, C., & Knight, R. 2005. App. Environ. Microbiol. **16.** Kraft, N.J.B., & Ackerly, D.D. 2010. Ecol. Monogr. **17.** http://rdp.cme.msu.edu/ **18.** Yahara ,T. 2013. Taxon. **19.** LPWG. 2013. Taxon.