



UEFS

Fungal endophytes of native rubber trees in the Brazilian Amazon



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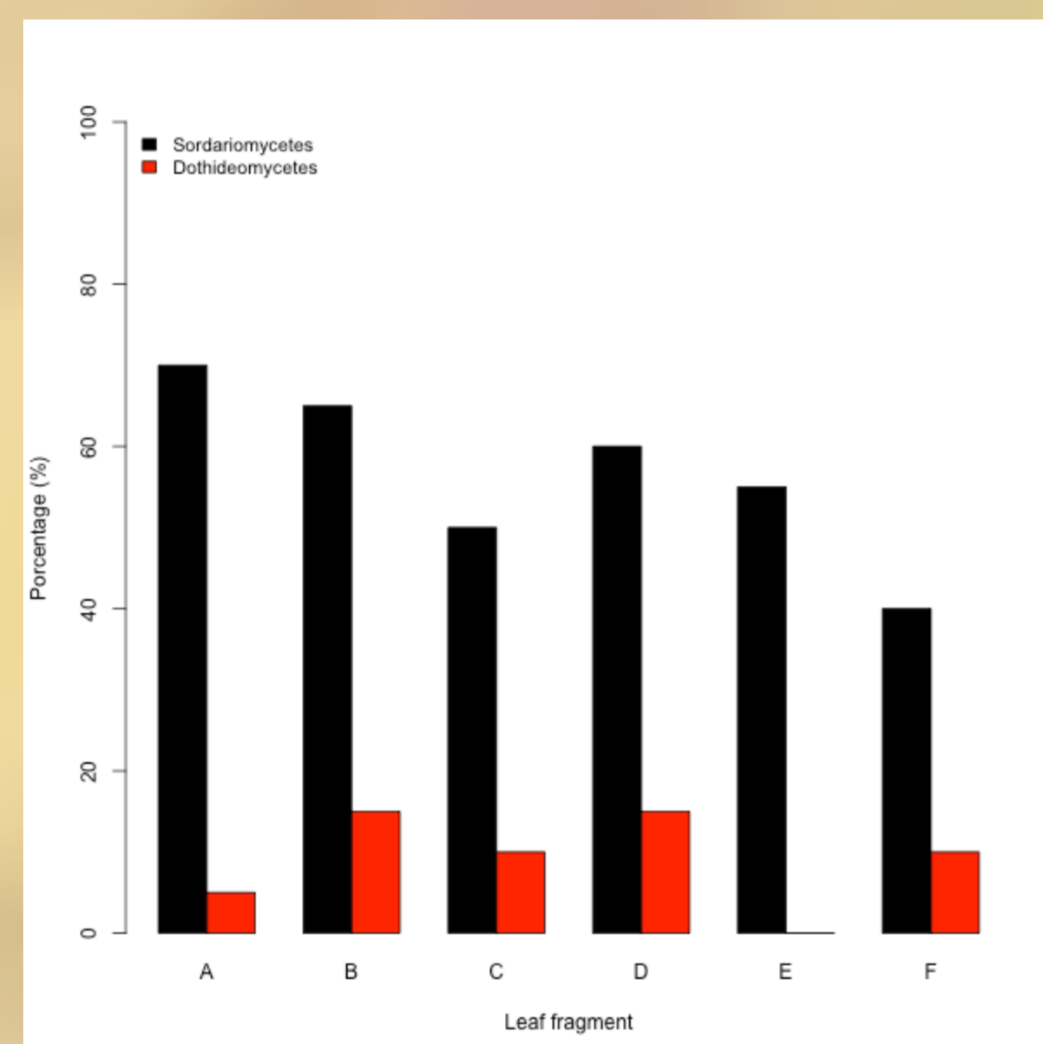
INTRODUCTION

Fungal endophytes inhabit healthy plant tissues during at least one stage of their life cycle without causing any apparent symptoms of disease or negative effects on the hosts (Petri, 1992). Little is still known about the ecological role or the outcome of the interactions between most endophytic species and their tree hosts, and a wide spectrum of interaction types, ranging from antagonism to mutualism can be expected. Therefore, they can be latent pathogens, latent saprotrophs or even mutualists (Sanchez Marquez, 2012). *Hevea brasiliensis*, a native neotropical tree species of the Amazon Basin, is the main source of natural rubber in the world. Today, rubber is primarily produced in large-scale plantations in Southeast Asia and Africa, and millions of workers in Africa and Asia rely on rubber plantations for employment. In the Americas, rubber plantations have failed largely because of South American Leaf Blight (SALB), caused by the phytopathogenic fungus *Pseudocercospora ulei* (Hora-Júnior et al., 2014). Because SALB is presently endemic to the Americas, many are concerned about the proliferation of SALB to plantations in Asia and Africa, where SALB could result in significant socioeconomic losses. The endophytic fungi associated to native rubber trees occurring in the Brazilian Amazon region can be potentially utilized in biological control of *Pseudocercospora ulei*, as those endophytes isolated from plantation *H. brasiliensis* trees, outside the natural range of the plant species, which have already been tested by our research group (Rocha et al., 2011).

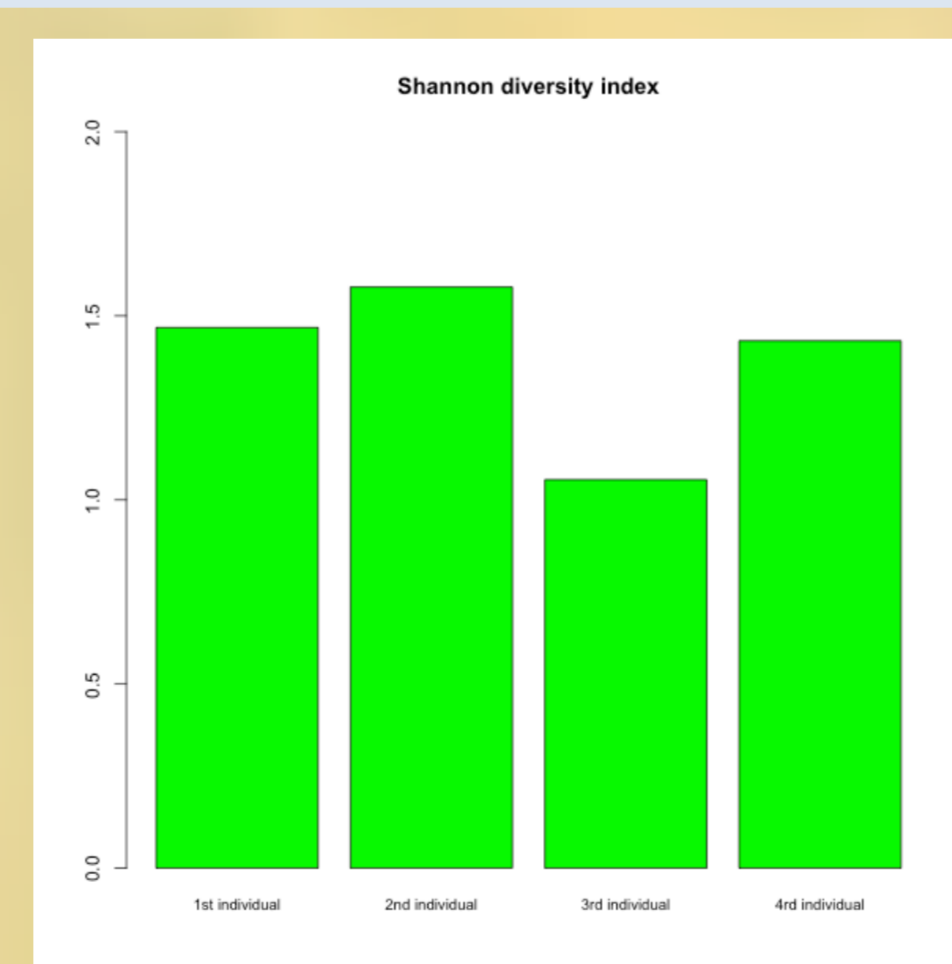
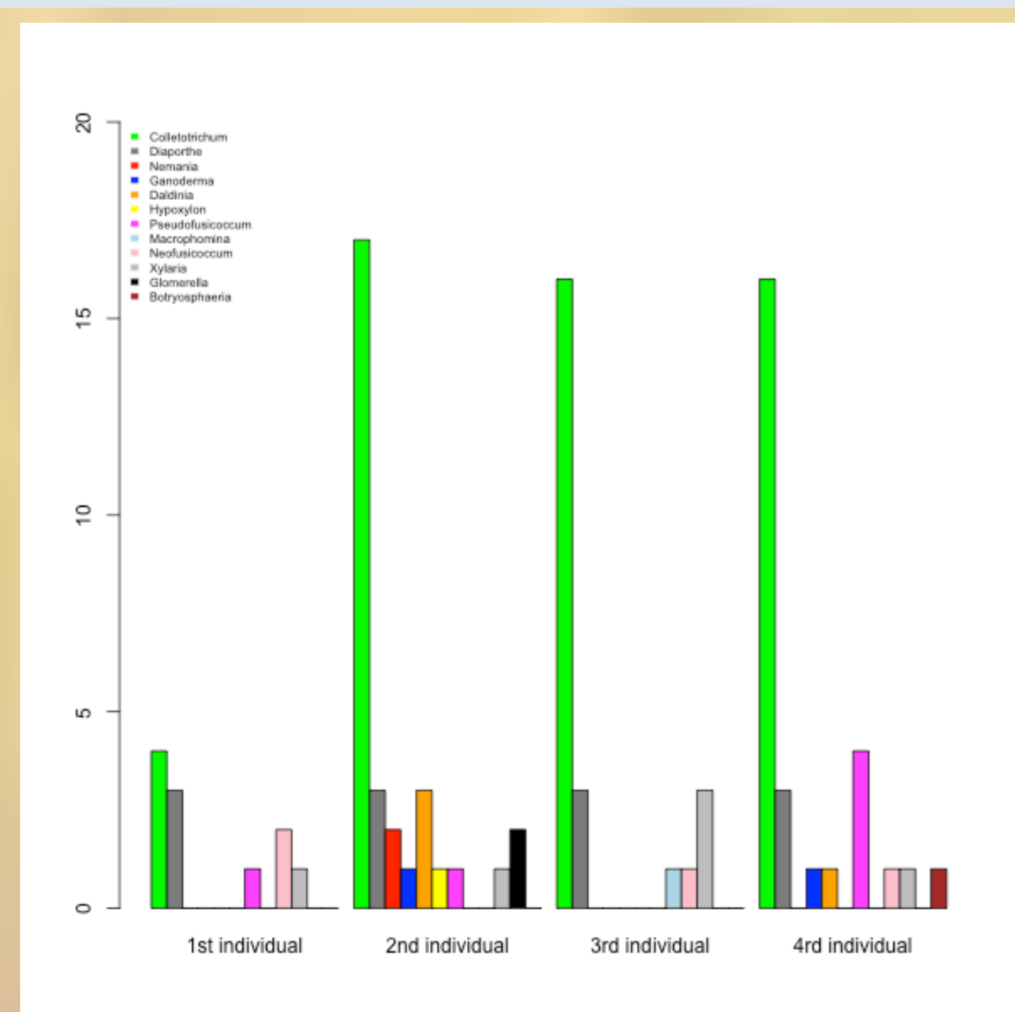
OBJECTIVES

The main objectives of this study are to characterize and compare the fungal community structures in leaves of rubber tree (*Hevea brasiliensis*) individuals from three well-preserved tropical rainforest areas in Eastern Amazon region (Anavilhanas National Park 02°03'S-03°02'S; 60°22'W-61°12'W, Caxiuanã National Forest 01°37'S-02°15'S; 51°19'W-51°58'W and Tapajós National Forest 2°45'S- 4°15'S; 54°45'W-55°30'W) to analyze the qualitative and quantitative components of diversity (taxonomic composition, richness and relative abundance).

RESULTS



GENUS	NUMBER OF ISOLATES	RELATIVE ABUNDANCE	PHYLUM	CLASS	ORDER	FAMILY
<i>Colletotrichum</i> sp.	84	0.672	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae
<i>Diaporthe</i> sp.	14	0.112	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae
<i>Xylaria</i> sp.	8	0.064	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae
<i>Dadinea</i> sp.	4	0.032	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae
<i>Pseudofusicoccum</i>	4	0.032	Ascomycota	Dothideomycetes	Botryosphaerales	Botryosphaeriaceae
<i>Glomerella</i> sp.	3	0.024	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae
<i>Ganoderma</i> sp.	2	0.016	Basidiomycota	Agaricomycetes	Polyporales	Ganodermataceae
<i>Nemania</i> sp.	2	0.016	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae
<i>Botryosphaeria</i> sp.	1	0.008	Ascomycota	Dothideomycetes	Botryosphaerales	Botryosphaeriaceae
<i>Hypoxylon</i> sp.	1	0.008	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae
<i>Macrophomina</i> sp.	1	0.008	Ascomycota	Dothideomycetes	Botryosphaerales	Botryosphaeriaceae
<i>Neofusicoccum</i> sp.	1	0.008	Ascomycota	Dothideomycetes	Botryosphaerales	Botryosphaeriaceae
TOTAL	125	1	2	3	5	5



CONCLUSIONS

Fungal endophyte communities in leaves of native *Hevea brasiliensis* of Anavilhanas National Park is mainly composed of few dominant ascomycotan genera of the classes 2 and 3 of non-clavicipitaceous fungi, exhibiting preference for tissue colonization, and they are probably latent pathogens and saprobes.

PERSPECTIVES

The next steps in our research program will be the completion of diversity analyses of endophytic mycobiota of rubber tree individuals recently sampled in Caxiuanã and Tapajós, and a comparison of all three studied areas in Eastern Amazon among them and with Western Amazon areas previously studied by the U.S.A partner group. In addition, fungal endophyte strains will be tested against *Pseudocercospora ulei*, the agent of South American Leaf Blight (SALB).

ABSTRACT

The main objective of our project is to study the diversity of the endophytic fungal communities of leaves of the socially important and economically valuable rubber tree (*Hevea brasiliensis*) in preserved rainforests of the Brazilian Amazon region. The methodology comprised the collection of visually healthy leaves of distinct rubber tree individuals, isolation and preservation of fungal endophytes, molecular identification of the isolates, and diversity analysis of fungal communities. A total of 12 fungal genera distributed in 5 families, 5 orders 3 classes and 2 phyla was identified in sampled trees collected in Anavilhanas National Park. Endophytic fungal communities varied in distinct trees, were primarily composed of latent pathogens and saprobes, and dominated by the genera *Colletotrichum* sp. and *Diaporthe* sp. Moreover, fungal endophytes of the classes Sordariomycetes and Dothideomycetes most likely exhibited some preference for leaf tissue colonization. After the completion of diversity analysis of sampled trees from Caxiuanã and Tapajós National Forests, the data of Eastern Amazon will be compared with those of Western Amazon (USA partner), and fungal strains will be tested against *Pseudocercospora ulei*, the agent of South American Leaf Blight (SALB), which is the most severe limitation of rubber tree development in neotropical region.

Keywords: Endophytic fungi, *Hevea brasiliensis*, Amazonian region, taxonomy, ecology.

METHODOLOGY

Three Macro-steps (workflow)

Sampling in the field: Collection of visually healthy leaves of rubber tree individuals in study areas



Processing of the samples in wetlab:

- isolation in pure cultures and preservation of fungal endophyte strains
- gDNA extraction, amplification of targeted segment (nrITS - fungal DNA barcode)
- DNA sequencing (Sanger)

Data analysis and interpretation in drylab:

- Pre-processing and quality control: Sequence edition, contig formation
- molecular taxonomic identification by comparative similarity analysis
- community ecology analyses

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ACKNOWLEDGMENTS

We thank all individuals and institutions that contributed directly or indirectly to this work and, especially, for NAS (National Academy of Sciences) and USAID (United States Agency for International Development) for grants PGA-2000003475 and AID-OAA-A-11-00012, respectively, which finance our study.

