Mycorrhizas in Tropical Forests

Workshop UTPL, September 22-25

Place: Octogono, Campus UTPL

www.utpl.edu.ec
# PROGRAM

**Monday September 22**

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<tr>
<td>8:00</td>
<td><strong>Registration</strong></td>
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<td>Centro Convenciones</td>
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<tr>
<td>9:30</td>
<td><strong>Opening of the workshop</strong></td>
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<td>Octagon, lecture room 1 (basement)</td>
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<td>10:00-11:00</td>
<td><strong>Opening lecture</strong></td>
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<td></td>
<td>Ian Alexander, Institute of Biological and Environmental Sciences, University of Aberdeen, Scotland, UK</td>
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<td></td>
<td><strong>Mycorrhizas in tropical forests – looking backwards and forwards</strong></td>
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<td>11:00-11:30</td>
<td><strong>Coffee break</strong></td>
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<tr>
<td>11:30-13:00</td>
<td><strong>Lectures on arbuscular mycorrhizas of tropical forest trees</strong></td>
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<td>11:30-12:00</td>
<td>Scott Mangan, Smithsonian Tropical Research Institute, Panama</td>
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<td>The importance of the species composition of arbuscular mycorrhizal fungi to tropical tree seedlings</td>
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<td>12:00-12:30</td>
<td>Claudia Diez, Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, Medellín, Colombia</td>
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<td>Ecological considerations on the mycorrhizal associations in soils of Andean highlands</td>
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<td>12:30-13:00</td>
<td><strong>General discussion</strong></td>
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<td>13:00</td>
<td><strong>Lunch in Octagon</strong></td>
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<td>15:00-17:00</td>
<td><strong>Lectures on ECM of tropical forest trees</strong></td>
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<td>15:00-15:30</td>
<td>Leho Tedersoo, Institute of Ecology and Earth Sciences, Tartu University, Tartu, Estonia</td>
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<td>High diversity of resupinate thelephoroid fungi in the Southern Hemisphere</td>
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<td>15:30-16:00</td>
<td>Bernard Moyersoen, Laboratoire d’Algologie, de Mycologie et de Systématique expérimentale, Université de Liège, Belgium</td>
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<td>Biodiversity and specificity of ectomycorrhizal fungi associated with Pakaraimaea dipterocarpacea in Guayana, Venezuela</td>
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<td>16:00-16:30</td>
<td>Jean Weber, Institute of tropical Forestry and Forest Products (INTROP), University Putra Malaysia (UPM), Selangor Malaysia</td>
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<td>Analysis of spatial relations between the distribution patterns of Shorea leprosula saplings and the ectomycorrhizal status of canopy trees</td>
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<td>16:30-17:00</td>
<td><strong>General discussion</strong></td>
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<td><strong>Welcome dinner in Casa Lojana</strong></td>
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<tr>
<td>9:00-13:00</td>
<td>Lectures on mycorrhizas of Ericaceae, myco-heterotrophic plants and</td>
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<td>green orchids in tropical forests</td>
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<td>9:00-9:30</td>
<td>Markus Göker, Systematic Botany, Mycology and Botanical Garden,</td>
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<td>Eberhard-Karls-University, Tübingen, Germany</td>
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<td>Clustering optimisation techniques to define biologically meaningful</td>
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<td>molecular operational taxonomic units (MOTUs)</td>
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<td>9:30-10:00</td>
<td>Sabrina Setaro, Systematic Botany, Mycology and Botanical Garden,</td>
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<td>Eberhard-Karls-University, Tübingen, Germany, currently: Wake</td>
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<td>Forest University, Biology Department, Winston-Salem, US</td>
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<td>Sebacinales in Andean ericads</td>
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<td>10:00-10:30</td>
<td>Juan Pablo Suarez, Biologia Molecular y Celular, Universidad Técnica</td>
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<td>Particular des Loja (UTPL), Loja, Ecuador</td>
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<td>Phylogeny and notes on ecology of Tulasnellales forming mycorrhiza</td>
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<td>with orchids: a comprehensive overview</td>
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<td>10:30-11:00</td>
<td>Ingrid Kottke, Botanical Institute, Systematic Botany and Mycology,</td>
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<td>Eberhard-Karls-University Tübingen, Germany</td>
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<td>Verification of orchid mycobionts by ultrastructural characteristics</td>
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<td>11:00-11:30</td>
<td>Coffee break</td>
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<td>11:30-12:00</td>
<td>Marc-André Selosse, Centre d’Ecologie Fonctionnelle et Evolutive,</td>
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<td>CNRS, Université Montpellier II, France</td>
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<td>Tropics change the rule! Heterotrophic plants using carbon from soil</td>
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<td>fungi (myco-heterotrophy): Contrasting strategies in fungal association</td>
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<td>and carbon source between temperate and tropical regions</td>
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<td>12:00-12:30</td>
<td>Stephan Imhof, University Marburg, Germany</td>
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<td>Cryptic biodiversity: AM colonization pattern in tropical myco-</td>
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<td>heterotrophic plants</td>
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<td>12:30-13:00</td>
<td>Tupac Otero, Departamento de Ciencias Biológicas, Universidad</td>
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<td>Nacional de Colombia, Sede Palmira, Colombia</td>
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<td>Coevolution in an orchid mycorrhizal Interaction</td>
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<td>13:00</td>
<td>Lunch in Octagon</td>
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<td>15:00-17:00</td>
<td>Poster sessions</td>
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<td>Octagon lecture room 2</td>
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<td>Amadou M. Bâ, Université des Antilles et de Guyane: ECM of Coccoloba</td>
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<td>Ingeborg Haug, University Tübingen, Germany: ECM of Nyctaginaceae in</td>
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<td>the pristine Andean cloud forest</td>
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<td>Ingrid Kottke, University Tübingen, Germany: Three functionally</td>
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<td>different mycobiont groups associated with Graffenrieda emarginata</td>
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<td>(Melastomataceae) in the tropical mountain rain forest of Southern</td>
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Mycorrhizas in Tropical Forests

Ecuador
Ingrid Kottke, Juan Pablo Suárez, Paulo Herrera, Dario Cruz, University Tübingen, Germany and UTPL, Loja - Ecuador. Symbiotic fungi of epiphytic and terrestrial orchids in the pristine forest and regenerating gaps of the Andean cloud forest.

Paulo Herrera, Juan Pablo Suárez, Ingrid Kottke, University Tübingen, Germany and UTPL, Loja - Ecuador. First information on Ascomycetes in the tropical rain forest of RBSF: Isolates from roots of epiphytic orchids identified by molecular methods

Darío Cruz, Juan Pablo Suárez, Ingrid Kottke, University Tübingen, Germany and UTPL, Loja - Ecuador. Sexual states of Tulasnellales forming mycorrhiza with epiphytic orchids in the RBSF

Leho Tedersoo et al. Institute of Ecology and Earth Sciences, University of Tartu.
Towards the global community of ectomycorrhizal fungi: missing links from Tasmania

Evening lecture 18:00-19:00 Open to public, Octagon, room

Dr. Jürgen Homeier, University of Göttingen, Germany, The South-Ecuadorian Biodiversity Hotspot: Aspects of a unique ecosystem

20:00 Dinner in Mar Y Cuba

Wednesday September 24

Whole day excursion to Estación Scientífica San Francisco (1820 m; half way to Zamora) and forest sites of Reserva Biológica San Francisco (up to 2100m); bushy páramo at El Tiro pass (2700 m)

19:00 Dinner in Hotel Quo Vadis

Thursday September 25

9:00-11:00 Lectures on arbuscular mycorrhizas in different tropical regions, threats and afforestation efforts

9:00-9:30 Amadou M. Bâ, Laboratoire de Biologie et Physiologie Végétales, Université des Antilles et de la Guyane, Guadeloupe (West Indies), France Occurrence and importance of arbuscular mycorrhizas in the wetland tree Pterocarpus officinalis Jacq. (Fabaceae)

9:30-10:00 Tesfaye Wubet, Helmholtz Center for Environmental Research, Department of Soil Ecology (UFZ) Halle, Germany Diversity of arbuscular mycorrhizal fungi in the Afromontane forest ecosystems of Ethiopia

10:00-10:30 Ingeborg Haug, Systematic Botany, Mycology and Botanical Garden, Eberhard-Karls-University, Tübingen, Germany
Arbuscular mycorrhizal fungal communities in degraded rehabilitation plots and a neighbouring pristine mountain rain forest in southern Ecuador

**10:30-11:00** Laura Aldrich-Wolfe, Department of Biological Sciences, North Dacota State University, USA

**Diversity and composition in arbuscular mycorrhizal fungal communities of forest and pasture: implications for re-establishment of tropical forest trees**

**11:00-11:30** Coffee break

**11:30-13:00** Poster session

Oscar Vivanco, Verónica Cueva, Ingrid Kottke, Juan Pablo Suárez, Centro de Biología Celular y Molecular, Universidad Técnica Particular de Loja, Loja – Ecuador: **Morphologic and molecular characterization of arbuscular mycorrhizal fungi of wild tree tomato (Solanum cajanumensis) and cultivated tree tomato (Solanum betaceum).**

Narcisa Urgiles, Paul Loján, Arthur Schüßler, UNL and UTPL, Loja, Ecuador; LMU München, Germany: Application of arbuscular mycorrhizal fungi for the growth of tropical trees

Claudia Krüger, Arthur Schüßler, LMU, München, Germany: Molecular characterisation of South-Ecuadorian AMF.


Herbert Stockinger, Arthur Schüßler, LMU, München, Germany: DNA-barcoding, molecular identification and detection of AMF at the species-level.

Adela Beck and Ingrid Kottke, University Tübingen, Germany: Linking morphotypes with phylotypes of arbuscular mycorrhizas

**13:00** Lunch in Octagon

**15:00-17:00** Round table discussion

**17:00-18:00** Guided Tour UTPL

**19:00** Farewell dinner Centro Convenciones UTPL (cultural program)
From over a century of research at temperate and boreal latitudes, we know that mycorrhizas play an essential role in the carbon and nutrient cycles of natural vegetation, and we are beginning to be convinced that mycorrhizal fungi are important in determining the structure and dynamics of higher plant communities. So what are the priorities for mycorrhizal research in tropical forest? I believe that these should be those aspects of mycorrhizal biology and evolution that tropical forests are uniquely able to reveal. The first is the question of diversity. Do tropical forests support greater mycorrhizal fungal diversity than their temperate counterparts, in line with the greater above ground diversity? There are many challenges here, and the taxonomic infrastructure will have to evolve rapidly if this is not to turn into just a sequence collecting exercise. There are important things to find out about the biogeography and evolution of mycorrhizal fungi, and probably the evolution of the mycorrhizal habit itself, by looking in tropical forest. But perhaps it may be more important to determine if and how this newly found diversity of mycorrhizal fungi plays a role in promoting and maintaining the complexity of the forest vegetation. Secondly, the nutrient cycles in many tropical forests are uncommonly fragile. What role do mycorrhizal fungi play in sustaining these forests, and can we turn that knowledge to good effect in forest conservation and restoration? Thirdly, in tropical forest AM and ECM trees co-exist in a manner unfamiliar to temperate mycorrhizal ecologists. That suggests to me that a re-appraisal of the conventional nutrient capture roles of these two mycorrhizal types is called for. Fourthly, we have to make mycorrhizal research attractive to local funders. And that means going beyond our understandable fascination with the novelty and diversity of mycorrhizal fungi in tropical forest, and showing why understanding their role in ecosystem processes is important, while at the same time being cautious about promising a quick fix with inoculation programmes.
Scott A. Mangan, Smithsonian Tropical Research Institute, Panama, smangan37@gmail.com

Most Neotropical trees are dependent on mutualistic associations with arbuscular mycorrhizal fungi (AMF) for improved growth and survival. These soil-born fungi colonize plant roots and provide their hosts with increased abilities to uptake scarce resources in exchange for carbohydrates. It is becoming clear that ecological differences among members of AMF communities are important to the composition of plant communities in temperate grasslands; however, in tropical systems, the importance of the AMF composition to forest dynamics is less studied. In this talk, I will discuss studies conducted in Panama that reveal that members of the AMF community cannot be lumped together has ecological equivalents, but species-specific differences among AMF may be fundamental in shaping the species composition of tree seedlings. Specifically, these studies demonstrate that AMF communities are diverse and that the community composition of AMF differs across spatial scales. Furthermore, these studies show that both growth and allocation of tree seedlings are dependent on the species identity and life history of the fungus colonizing its roots. For example, AMF species comprising the AMF community of Panama differ in dispersal strategies; whereas the majority of species are restricted to slow dispersal through the soil, a subset of the species have evolved the ability to be dispersed by rodents. Such differences in life history have important implications to seedling performance. Finally, I will discuss a study that demonstrates that when seedlings of different species of tropical trees are provided with identical communities of AMF, host-dependent divergence of AMF communities occurs relatively quickly. Such divergence is strong enough to differentially influence performance of the next generation of tree seedlings. Taken as a whole, this research provides strong evidence that plant-AMF interactions in the tropics are species specific, and such interactions are most likely important in shaping both tree and AMF communities.
A series of experiments were carried out to evaluate the potential use of arbuscular mycorrhizal fungi (AMF) on soil management in forests of Andean-highlands in the Eastern Antioquia Region, Colombia. To this purpose the mycorrhizal dependency of some promissory forest species of this ecosystem has been studied. We detected that Retrophyllum rospigliosii, Chamaesenna colombiana Britton & Killip, and Ocotea sp. were moderately dependent on the mycorrhizal association, while Callophyllum brasilense Cambers was independent. Also, the mycorrhizal effectiveness of native mycorrhizal fungi population associated with different types of vegetation (from forest to pastures and agronomic crops) has been also investigated. We found that soils under forest, conifer plantation, fallow, and fern-land exhibited a low density of mycorrhizal propagules (100-650 kg-1) and, consequently, low mycorrhizal effectiveness. Soils from a grassland, potato, common bean, and vegetable crops showed high density of mycorrhizal propagules (>3950 kg-1) and high mycorrhizal effectiveness. Soil from a cut flower crop did not have mycorrhizal propagules and, consequently, exhibited low mycorrhizal effectiveness. The differences are explained in terms of soil acidity conditions and plant nutrient availability and soil management.

These studies have allowed us to predict the need for mycorrhizal inoculation in a given soil combination, which has been probed in some experiments. For instance, a soil with low number of mycorrhizal propagules (<100 kg-1) required the addition of an effective mycorrhizal inoculum when a highly mycorrhizal dependent plant was grown. Conversely, a soil with high number of native mycorrhizal propagules (>3950 kg-1) did not require mycorrhizal inoculation because there was enough propagules to colonize the roots and enhance plant P uptake.
HIGH DIVERSITY OF RESUPINATE THELEPHOROID FUNGI IN THE SOUTHERN HEMISPHERE

Urmas Kõljalg, Kessy Abarenkov, Triin Suvi, Teele Jairus, Irja Saar, Leho Tedersoo*
Institute of Ecology and Earth Sciences, Tartu University. 40 Lai Street 51005 Tartu, Estonia; * corresponding author: leho.tedersoo@ut.ee

The order Thelephorales comprises both stipitate tooth-fungi and resupinate fungi. The latter are termed resupinate thelephoroid fungi and they are highly diverse and common both as fruit-bodies and on root tips in the Holarctic realm of the Northern Hemisphere. The aim of this ongoing project is uncover the diversity of resupinate thelephoroid fungi in the Southern Hemisphere. Three independent fruit-body and ectomycorrhiza surveys were performed in a wet sclerophyll forest in Tasmania; 2) Vateriopsis seychellarum and Intsia bijuga communities in Seychelles; 3) in miombo woodland of Zambia. These surveys included sampling of ectomycorrhizal (EcM) tree roots and fruit-bodies following slightly different sampling design and efforts. Sampled EcM were segregated into different morphotypes and further into anatomatypes based on the structure of mantle, cystidia, emanating hyphae and rhizomorphs. One or two root tips of each anatomatypes were subjected to DNA extraction and sequencing. The ribosomal DNA internal transcribed spacer (rDNA ITS) sequences of the EcM fungi were obtained for all and rDNA LSU sequences for most analysed root tips and fruit-bodies using ITS1F, ITS0F-T or LR0R combined with a newly designed primer LR3-Tom (5´CTACCGTAGAACCGTCTCC3´) that is strongly taxon-specific to Thelephora and Tomentella. Software packages Modeltest, Paup, Mr. Modeltest and MrBayes were used for the phylogenetic placement of unknown EcM sequences with sequences of known species of resupinate thelephoroid fungi. We also created species accumulation curves for the EcM resupinate thelephoroid fungi to demonstrate species richness vs sampling effort at different sites and to estimate the number of unseen species. We demonstrate a high species richness of resupinate thelephoroid fungi in the Southern Hemisphere and their different relations to taxa in the Northern Hemisphere. We conclude that filling gaps in the Southern Hemisphere and tropical ecosystems provide an invaluable source for assessing the biogeography and phylogeny of these fungi.
**BIODIVERSITY AND SPECIFICITY OF ECTOMYCORRHIZAL FUNGI ASSOCIATED WITH PAKARAIMAEA DIPTEROCARPACEA IN GUAYANA, VENEZUELA**

**Bernard Moyersoen,** Université de Liège, Laboratoire d’Algologie, de Mycologie et de Sytématique expérimentale, Bd du Rectorat, 27, 4000 Liège, Belgium, bmoyersoen@hotmail.com

*Pakaraimaea dipterocarpacea*, Pakaraimoideae, is a neotropical endemic tree related with the mostly SE Asian Dipterocarpaceae. The recent discovery of *P. dipterocarpacea* ectomycorrhizal (ECM) status suggests that ECM evolved in the ancestors of Dipterocarpaceae before the splitting of S America from Gondwana, 130 Ma. Whether *P. dipterocarpacea* vicariant distribution is also reflected below ground in ECM fungal associates is not known. Only few ECM fungal taxa have been reported to date in association with *P. dipterocarpacea*. A better knowledge about *P. dipterocarpacea* ECM fungal diversity and specificity is a preliminary step for the understanding of the biogeography of ECM fungi associated with this Dipterocarpaceae. To describe *P. dipterocarpacea* ECM fungal diversity and to test whether there is a specific ECM fungal flora associated with this endemic, ECM fungi have been surveyed in a 8.5 x 10 m² forest plot where *P. dipterocarpacea* co-occurs with the ECM tree Aldina, Papilionoïdeae. Up to 20 species belonging to the fungal groups Sebacinaceae, Clavulinaceae, Cortinariaceae, Thelephoraceae, Hymenochaetales, Amanitaceae, Cantharellaceae, Russulaceae and Boletaceae have been identified. Several host-fungus associations with interesting biogeography will be described.
ANALYSIS OF SPATIAL RELATIONS BETWEEN THE DISTRIBUTION PATTERNS OF SHOREA LEPROSULA SAPLINGS AND THE ECTOMYCORRHIZAL STATUS OF CANOPY TREES

Jean Weber 1, Thorsten Wiegand 2, Su See LEE 3

1 Institute of tropical Forestry and Forest Products (INTROP), University Putra Malaysia (UPM), 43400, UPM Serdang, Selangor, Malaysia jean_weber31@hotmail.com
2 Helmholtz Centre for Environmental Research – UFZ, Department of Ecological Modelling, Permoserstr. 15, 04318 Leipzig, Germany, e-mail: thorsten.wiegand@ufz.de
3 Forest Research Institute Malaysia (FRIM), Kepong, 52109 Selangor Darul Ehsan, Malaysia, e-mail: leess@frim.gov.my

Point patterns of Shorea leprosula saplings 1-2 cm dbh (dipterocarpaceae) from a 50-ha demographic plot of a lowland dipterocarp forest in Peninsular Malaysia were classified according to their level of aggregation. Spatial relations between S. leprosula saplings 1-2 cm dbh and ectomycorrhizal trees >25 cm dbh as well as all trees >25 cm dbh were described using Ripley’s K function and compared to the respective null-models of random labeling of the ectomycorrhizal status of trees >25 cm dbh and random toroidal shifts of the positions of all trees >25 cm dbh. S. leprosula saplings showed aggregated distributions in zones where the ectomycorrhizal status of trees >25 cm dbh is less dense. Levels of saplings aggregations correlated better with the distributions of the ectomycorrhizal status of trees >25 cm dbh than with the density variations of all trees >25 cm dbh. Higher aggregations of saplings in zones where the ectomycorrhizal status of trees >25 cm dbh is less dense suggest that S. leprosula regeneration dynamics prevents the monodominance of ectomycorrhizal species. The approach used in this study illustrates that hypotheses about possible links between ectomycorrhizal functions and the dynamics of trees might be advantageously addressed in dipterocarp forests where the mixed distribution of ectomycorrhizal and non-ectomycorrhizal species permits to test spatial correlations.
CLUSTERING OPTIMISATION TECHNIQUES TO DEFINE BIOLOGICALLY MEANINGFUL MOLECULAR OPERATIONAL TAXONOMIC UNITS (MOTUs)

Markus Göker, Systematic Botany, Mycology and Botanical Garden, Eberhard-Karls-University, Tübingen, Germany; info@goeker.org

Reliable taxonomy is crucial for the assessment of biodiversity and for the comparison of habitats based on their species composition. Determining taxon boundaries is challenging in case of organisms for which only molecular data are available, such as bacteria, fungi, and many unicellular eukaryotes. Even in the case of organisms with well-established microscopic characteristics, molecular taxonomy is necessary to determine misidentified and mislabelled GenBank sequences, to identify incompletely known specimens and cryptic species, and last but not least to analyze sequences directly sampled from the environment as in metagenomics studies. In molecular taxonomy, researchers mostly use a predefined threshold for pair-wise genetic distances in clustering algorithms to assign sequences to molecular operational taxonomic units. However, thresholds applied differ in literature, even if applied to the same organisms and molecular markers, and are often based on subjective criteria or just on tradition. Furthermore, the clustering algorithm applied also has a profound impact on the clustering outcome, but it is seldom addressed which algorithm is appropriate for molecular taxonomy. Finally, the calculation of the distance matrices may cause considerable methodological problems because of alignment ambiguity, rate heterogeneity between sites, and other potential sources of biases. To address these issues, we have designed and implemented a simple yet effective and flexible clustering optimization method. Using biologically sensible reference partitions, it automatically distinguishes between within-taxon and between-taxon sequence heterogeneity in the course of identifying optimal thresholds, clustering algorithms, and distance methods. Usage examples for clustering optimization with alternative types of biological data are provided. The method is discussed as a general method for improving molecular taxonomy.
Sebacinales are mycorrhizal fungi associated with different plants in various habitats. Especially in Ericaceae they occur abundant as found in ericoid and arbutoid mycorrhizas. Ericaceae have a global distribution with a diversity centre in the Neotropics, particularly in the Northern Andes. This region harbors the so called “Andean-clade” of Ericaceae, which is a recently defined, monophyletic subgroup of Ericaceae, endemic to the Neotropics. The respective plants grow mostly epiphytic or hemiepiphytic in tropical cloud forests and are believed to have radiated in the northern Andes. During the last five years, we studied mycorrhizas of Andean-clade species and other Ericaceae in South Ecuador and identified the associated fungi. Our morphological investigations, based on light and transmission electron microscopy, revealed, that Sebacinales are highly abundant in mycorrhizas of neotropical Ericaceae. They are the dominant mycorrhizal fungi in Andean-clade Ericaceae, forming a particular association, which we called cavendishiod mycorrhiza. The fungi form a hyphal sheath around the finest rootlets as well as inter- and intracellular hyphae in the cortex. Other Ericaceae in south Ecuador are typically ericoid mycorrhizal. Molecular phylogeny as well as a novel cluster optimization analysis revealed a high diversity of Sebacinales in Andean Ericaceae. So far there are no hints for host specificities as nearly every investigated mycorrhizal sample results new Sebacinales species. Comparison of Sebacinales isolated from mycorrhizas of Ericaceae in Meso- and North America shall yield more information about distribution and evolutionary patterns of Sebacinales associated with Andean Ericaceae and of Sebacinales in general.
Orchids produce tiny seeds that are dispersed mostly by wind. Seed germination and further protocorm development require the association with a suitable mycorrhizal fungus. Our recent investigations have shown Tulasnellales as the dominant mycorrhizal fungi associated to epiphytic orchids in a mountain rain forest. Our finding of constant colonization of roots in contact with the bark supports the view that the role of the fungi may be crucial also for the adult epiphytic orchids. The most important benefit for the orchid may be to retain the fungus in order to assure further seed germination. Saprotrophic capabilities, documented for several Tulasnella species, could explain the growth of Tulasnella on tree bark, including the capacity of fruiting close to colonized roots and promotion of orchid seed germination.

Tulasnellales were confirmed as an order with a range of orchid mycorrhiza forming species, including green terrestrial and epiphytic orchids, ectomycorrhiza tripartite associations of mixotrophic orchids and myco-heterotrophic orchids. The Tulasnellales with quite different trophic strategies are displayed as closely related in the nucLSU phylogenies challenging the interpretation of the molecular data. Specificity is still controversial for this group. Tulasnella calospora was proposed as a universal orchid symbiont considering the capacity to establish in vitro symbiotic associations with a broad range of orchid species. There are, however, taxonomic problems concerning the species concept in Tulasnellales and these earlier findings may be connected to diverse Tulasnella species. Previous research showed already Tulasnellales associated to liverworts (Aneuraceae) and a tropical mountain rainforest tree (Graffenrieda emarginata, Melastomataceae). Molecular data may help to clarify the phylogeny of this Order.
Ingrid Kottke, Botanical Institute, Systematic Botany and Mycology, Eberhard-Karls-University Tübingen, Germany; Ingrid.Kottke@uni-tuebingen.de

Orchids depend on fungi for germination and protocorm development and maintain mycorrhizas in their adult forms. Mycorrhizal fungi are, thus, a driving force in orchid evolution and speciation. Identification of the true mycobionts by isolation of mycelia or DNA extraction is, however, biased by fungi colonizing only the velamen of orchid roots. Sequencing of mycelium isolates and of DNA extracted directly from orchid roots yielded predominantly ascomycetes, whereas re-infection experiments found basidiomycetes as true mycobionts. Active orchid mycobionts can be verified in situ by transmission electron microscopic studies (TEM). Distinctive ultrastructural features discern the basidiomycete groups of orchid mycobionts from each other and from ascomycetes. Homobasidiomycetes, found in terrestrial orchids of ectomycorrhizal forests, display dolipores with perforated caps and sometimes clamps in situ. Tuberales, the only ascomycetes so far proven as orchid mycobionts, display electron translucent walls and simple septa with Woronin bodies. Sebacinales are characterized by fine hyphae (2.5 µm) with electron opac walls and dolipores with straight or slightly dish shaped, imperforate pore caps. Tulasnellales and Ceratobasidiales have coarser hyphae (4-5 µm), the cell walls of Tulasnellales include slime bodies. Tulasnellales have dolipores with dish shaped, imperforate caps with recurved ends. Ceratobasidiales display dolipores with dome shaped caps containing large perforations. Recent investigation of orchid mycorrhizas sampled in the Reserva Biológica San Francisco, Loja, Ecuador, revealed Tulasnellales, Sebacinales, and Ceratobasidiales in living cortical root cells of epiphytic and terrestrial orchids. Totally unexpected and so far not documented elsewhere is the occurrence of a basidiomycete with simple septa and no membrane cap in cortical cells of a terrestrial orchid from this place.
Heterotrophic plants using carbon from soil fungi (myco-heterotrophy): Contrasting strategies in fungal association and carbon source between temperate and tropical regions

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Most plant ecophysiology is shaped by symbioses in which plant roots associate with soil fungi to form mycorrhizae. Fungi exploit soil mineral nutrients that are shared with the plant, and receive carbon as a reward. Moreover, several forest-understorey achlorophyllous plants, once thought to be saprophytic, are now shown to receive carbon from their mycorrhizal fungi and are therefore called mycoheterotrophs (MH). In the many investigated temperate MH species, including many orchids, the associated fungi in turn form mycorrhizae with surrounding plants that are the ultimate carbon source of the entire system. Direct and indirect evidences support the latter point, especially isotopic methods (13C contents are similar to mycorrhizal fungi and 15N contents suggest a high position in trophic chains, above fungi). The ability of mycorrhizal fungi to link different plants therefore allows sufficient inter-plant carbon transfers to support MH growth. Furthermore, all temperate species studied so far indicate a high specificity of each MH species to a narrow fungal clade. However, most MH plants live under the tropics. Here, we investigate tropical MH orchids to (i) identify their mycorrhizal partners, using molecular methods and (ii) confirm their carbon source, using stable isotopes.

We studied six tropical orchid species from Asia, Africa and the Caribbean islands, including several populations for each of them. They revealed contrasting features as compared to the ‘temperate rule’. Species from Asian Dipterocarpaceae forest
shared diverse ectomycorrhizal species with the later trees; their isotopic content clearly indicated that, exactly as temperate models, they received carbon from this source. However, they were not specific, and had up to four different partner species per individual (and eight per population). The other models from Africa and the Caribbean islands, from forests devoid of the mycorrhizal fungal species usually colonizing MH orchids, associate with saprobic fungi. Together with the identity of the fungi, isotopic studies showed that the ultimate carbon source was, respectively, dead wood and dead leaves. These examples, obtained in the same phylogenetic context as the models studied in temperate regions, are unlikely to represent specific idiosyncrasies, but rather emphasize functional differences of tropical ecosystems, especially for fungi. We hypothesize that (1) a wetter climate allows saprophytic fungi to be sufficiently active over the rain season to provide carbon to MH plants and (2) a higher availability of photosynthates for tropical mycorrhizal fungi reduce arm race with exploiting MH orchids, and thus evolution toward specificity.

**CRYPTIC BIODIVERSITY: AM COLONIZATION PATTERN IN TROPICAL MYCOHETEROTROPHIC PLANTS**

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Arbuscular mycorrhiza (AM) is still largely considered as a structurally rather uniform association, at best differentiated in *Arum*- and *Paris*-type. However, especially the tropical mycoheterotrophic plants (MHP) have developed sophisticated AM colonization patterns which are highly divers: in thirteen so far investigated AM-MHP eleven different colonization patterns were found. Related MHP may have similar patterns but differ in details which can be interpreted as evolutionary steps towards an efficient and sustainable benefit from the fungus. For example, most MHP have functionally distinct tissues, designed to either digest, preserve or otherwise manipulate the colonizing hyphae. Several examples from the Burmanniaceae, Triuridaceae, Polygalaceae and Gentianaceae will be addressed in this presentation, including the presumably most complicated mycorrhizal
colonization pattern in the plant kingdom. These complex AM patterns can only be detected through consistent serial sectioning combined with a notional three-dimensional reconstruction if the structures seen. This contribution emphasizes the relevance of the third dimension of roots, rhizomes or shoots in anatomical investigations of mycorrhizas. Squeezed preparations or single anatomical sections are not sufficient for a comprehensive recognition of an association.

**COEVOLUTION IN AN ORCHID MYCORRHIZAL INTERACTION**

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All orchids depend on mycorrhizal fungi (MF) for germination, but some species also depend on MF for nutrient absorption though their whole life. Such situation suggests that some orchid species may have co-evolve with their MF to optimize the mycorrhizal interaction. In Australia, Pterostylidinae orchids have a reduced root system with life cycles depending on MF for nutrients absorption and present specific mycorrhizal interaction. The main objective of this study was to compare the phylogenetic patterns of the Pterostylidinae orchids with their MF, to test for parallel cladogenesis and possible co-evolutionary processes. To address this question we compared the phylogenies of a group of representative orchids in the Pterostylidinae and their MF. Additionally we asked: are all orchids MF equally effective at inducing seed germination for all members of the Pterostylidinae? To address this question we compared the germination success of orchid taxa grown in the presence of their own mycorrhizal fungi vs. germination with fungal strains isolated from other host taxa. Pterostylidinae orchids germinated better with their own fungi than with those from different Pterostylidinae host. We also founded a significant phylogenetic congruence between Pterostylidinae and their MF, but differences in branch lengths between the two phylogenies suggest that this process was not the result of a co-cladogenetic process. Rather, the patterns of fungal evolution constrained Pterostylidinae orchid evolution based on their levels of specificity observed in their mycorrhizal interaction producing the observed phylogenetic similarities.
Mycorrhizas in Tropical Forests

OCCURRENCE AND IMPORTANCE OF ARBUSCULAR MYCORRHIZAS IN THE WETLAND TREE PTEROCARPUS OFFICINALIS JACP. (FABACEAE)

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Pterocarpus officinalis (Jacq.) (Fabaceae) is the dominant wetland tree species of the seasonally flooded swamp forests in the Caribbean basin. It is found mainly behind mangrove area and covers areas of the coastal floodplain, and individual trees occur along rivers and in the mountains. The establishment and population maintenance of P. officinalis are affected by the variation in salinity and hydrology, and differences in soil microtopography in swamp forests. This tropical wetland tree species forms arbuscular mycorrhizas (AM) and bradyrhizobial nodules on lateral roots of buttresses. Here, we determined the occurrence and importance of AM in the wetland tree P. officinalis. Arbuscular mycorrhizal soil infectivity (MPN values) decreased along a salinity gradient when the salt levels increased in the dry and wet season in a stand of P. officinalis. AM colonization of P. officinalis was well developed (up to 50%) only within the low salinity levels (below 10‰) whatever the season. In thus, no spores were found in soil cores, suggesting that propagules were only mycelium pieces and/or root fragments of colonized Pterocarpus roots. In an experimental study, flooding of P. officinalis seedlings induced several morphological and physiological adaptive mechanisms, including formation of hypertrophied lenticels and aerenchyma tissue, and production of adventitious roots on submerged portions of the stem. Flooding also resulted in an increase in total dry weights, regardless of inoculation. However, under flooding, AM were well developed on root systems and adventitious roots compared with inoculated root systems under non flooding condition. AM made also noteworthy contributions to the flood tolerance of P. officinalis seedlings by improving plant growth and P acquisition in leaves. Further experimental investigations should be done to understand the mechanisms by which AMF increases AM colonization of P. officinalis seedlings in flooding and its adaptive significance.
Arbuscular mycorrhizal fungi (Glomeromycota) are the dominating mycobionts in the Ecuadorian tropical mountain forest. We studied for the first time arbuscular mycobiont richness and community structure of degraded pastures in tropical mountain rain forest area and compared it with our findings in the neighboring species rich pristine forest. Part of the fungal 18S rDNA was amplified and sequenced directly from the mycorrhizas of seedlings of four native tree species on degraded pastures, considered as trap plants under natural conditions. In the pristine forest mycorrhizal samples from 30 different tree species out of 18 families were investigated. Fungal phylotypes were identified by molecular phylogeny and fungal community structure analyzed by Ward clustering method. We found numbers of fungal phylotypes as equally large, but community composition was significantly different among the two habitats. Several wide spread phylotypes occurred on the reforestation plots, while phylotypes in the pristine forest were so far not shown from other areas. We conclude from these results that tropical climate may foster high species richness of arbuscular mycorrhizal fungi independent of host species richness, life history of hosts, and developmental state of the habitat. In contrast, fungal communities are either adapted to the established forest or to the open, degraded pastures.
SYMBIOTIC FUNGI OF EPIPHYTIC AND TERRESTRIAL ORCHIDS IN THE PRISTINE FOREST AND GENERATING GAPS OF THE ANDEAN CLOUD FOREST

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Orchids occur abundantly and in extraordinary high species diversity in the tropical mountain rain forest of the Northern Andeans as epiphytes on trees but also terrestrial. Substrates and climatic conditions in these habitats are frequently nutrient and water limited. Root associations with symbiotic fungi forming mycorrhizas are, therefore, expected important for survival and reproduction of the orchids. Our previous investigations in the tropical mountain rain forest of Southern Ecuador showed Tulasnellales and Sebacinales as mycorrhizal fungi of epiphytic, pleurothallid orchids. Molecular phylogeny revealed fungal genotypes as distinct from so far known fungi of terrestrial orchids of other regions. In a current scientific approach we aim to clarify species richness, community structure and potential specificity of the orchid mycorrhizal fungi by comparing epiphytic and terrestrial habitats in the pristine forest and regenerating gaps caused by man made disturbance. 56 permanent plots distributed in four different sites were installed in the Reserva Biológica San Francisco, Loja, Southern Ecuador, in cooperation with the German research group DFG FOR 816. Five plant individual from each plot were sampled, collecting three roots per plant individual. PCR amplification followed by cloning directly from the mycorrhizas using universal primers is performed. Preliminary results shown Tulanellales as the dominant fungal group. Sebacinales also are present but in a lower rate. Molecular phylogenetic analysis will show if a difference exists between sites or plots.
FIRST INFORMATION ON ASCOMYCETES IN THE TROPICAL RAIN FOREST OF RESERVA BIOLOGICA SAN FRANCISCO
Isolates from roots of epiphytic orchids identified by molecular methods

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Ascomycetes occur abundantly as saprophytes in soil and rotten wood. In ecosystems with low availability of nutrients as the tropical rain forest, ascomycetes principally, and other as bacteria are playing a key role in organic material decomposition. Scarce information is available on ascomycete diversity in tropical rain forest decomposing rotten wood close to orchids. Roots of 79 orchid individuals of \textit{Stelis hallii}, \textit{S. superbiens}, \textit{S. concinna} and \textit{Pleurothallis lilijae} were sampled in the RBSF. After the velamen was extracted cortical tissue was macerated and transferred into petri dishes containing Corn Meal Agar (CMA), Malt Extract Agar (MEA), and Melin Norkrans Modified Medium (MMNC). DNA was extracted from mycelia of isolates. PCR amplification and partial sequences of 5.8S-ITS and nrLSU were performed using primers ITS1/NL4 and ITS1F/LR5. DNA sequences in two directions were obtained using the primers ITS1, NL4, ITS4 and NL1. Phylogenetic calculations were used to determine the different groups of ascomycetes. Preliminary results showed that diverse ascomycetes, principally of the classes Sordariomycetes, Eurotiomycetes, Dothidiomycetes and Leotiomycetes are frequently associated to these four species of epiphytic orchids. Transmission electron microscopic investigations showed that acomycetes are not found in cortical cells. The ascomycetes are housed in the sheltered compartment of the velamen. Leotiomycetes (order Helotiales) were reported as forming mycorrhizas with Ericaceae. Results are of great value for understanding the ecological role of ascomycetes in the microhabitats of the tropical rain forest.
SEXUAL STATES OF TULASNELLALES FORMING MYCORRHIZAS WITH EPIPHYTIC ORCHIDS IN THE RESERVA BIOLOGICA SAN FRANCISCO

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Recent investigation showed Tulasnellales as frequently associated to epiphytic orchids in the Reserva Biologica San Francisco (RBSF), South Ecuador. Molecular phylogenetic studies revealed 12 sequence types of Tulasnellales based on proportional sequence differences. Morphological information on fruiting bodies was lacking and taxonomic clarification needed. Resupinate fruiting bodies near to epiphytic orchids were collected from pieces of decayed wood in the tropical mountain rain forest of RBSF. Fruiting bodies were morphologically characterized using light microscopy. Eight detailed illustrations were performed. PCR amplification and sequencing of partial 5.8S-ITS and nrLSU were performed using primers 5.8Tul/NL4. Molecular cloning was used for problematic PCR products. Phylogenetic calculations were compared with morphological results. We propose four different morpho-species defined by hyphal diameter, basidia and spore size, and appearance of hymenium. Molecular analyzes of the partial nucLSU showed sequences of Tulasnellales belonging to six distinct clades. Three clades included sequences previously reported from mycorrhizal fungi of pleurothallid orchids.
Ectomycorrhizal (EcM) symbiosis plays a key role in plant nutrient acquisition in the boreal and temperate forests of the Northern Hemisphere and in patches of rainforest and woodland vegetation in Africa and SE Asia. In Australia, EcM lifestyle is widespread especially in seasonal, semiarid regions. This study aims at determining the diversity, community structure and host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest that comprises elements of both temperate rain forest and evolutionally more recent sclerophyll vegetation. EcM fungi were identified based on anatomotyping and rDNA ITS-LSU sequence analysis using taxon-specific primers. Host tree roots were identified based on root morphology and length differences of the chloroplast trnL region. A total of 123 species of EcM fungi were recovered from root tips of Eucalyptus regnans (Myrtaceae), Pomaderris apetala (Rhamnaceae) and Nothofagus cunninghamii (Nothofagaceae). The frequency of two thirds of the most common EcM fungi from several lineages was significantly influenced by host species. The lineages of Cortinarius, Tomentella-Thelephora, Russula-Lactarius, Clavulina, Descolea and Laccaria prevailed in the total community and their species richness and relative abundance did not differ by host species. In contrast to previous research, this study demonstrates that strongly host-preferring, though not directly specific, EcM fungi dominate the belowground community. Apart from the presence of Descolea, Tulasnella and Helotiales and the lack of Suillus-Rhizopogon and Amphinema-Tylospora, the EcM fungal diversity and phylogenetic community structure is similar to that in the Holarctic realm.
DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN THE AFROMONTANE FOREST ECOSYSTEMS OF ETHIOPIA

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Arbuscular mycorrhizal (AM) fungi play a significant role in the functioning of tropical forest ecosystems. They are known to influence plant species composition and competitive ability of host plants, reduce recruitment limitations in forest ecosystems serving as a support system enhancing seedling survival, growth and establishment. Mycorrhizal investigations of co-occurring indigenous tree species in the degraded Afrotropical forest ecosystems in Ethiopia showed the dominance of arbuscular mycorrhizas, the presence of a high diversity of native AM fungal taxa and host species-specific interactions. Our results also suggest the potential of nursery growth conditions for mycorrhizal seedling production and ability to trap both native and widely distributed AM fungi. Thus, the potential role of native AM fungi in enrichment plantation and rehabilitation of these disturbed forest ecosystems will be discussed.
DIVERSITY AND COMPOSITION IN ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES OF FOREST AND PASTURE: IMPLICATIONS FOR RE-ESTABLISHMENT OF TROPICAL FOREST TREES

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Tropical forest conversion to pasture was first hypothesized to result in changes in the arbuscular mycorrhizal (AM) fungal community by Janos 25 years ago, yet our understanding of changes in AM fungal communities following tropical forest clearing and their role in limiting pasture recolonization by forest plant species remains incomplete. I characterized AM fungal communities of forest and pasture at three adjacent sites in Coto Brus, Costa Rica, using spores collected from the field, trap cultures in the greenhouse, and T-RFLP analysis of root samples from the forest canopy tree species Terminalia amazonia, the non-native pasture grasses Urochloa ruziziensis and U. decumbens, and seedlings of T. amazonia inoculated with forest or pasture soil and planted into experimental reforestation plots. Diversity of AM fungi did not differ between forest and pasture. However, community composition did. Three of the most common species observed as spores in pastures -- Entrophospora colombiana, Glomus hoi, and Scutellospora calospora-- were not observed in the forest. Seedlings inoculated with forest soil were more heavily colonized by AM fungi and experienced lower mortality than seedlings inoculated with pasture soil. After two years of growth, pasture seedlings of T. amazonia --regardless of inoculation treatment—were colonized neither by AM fungi typical of Urochloa nor by AM fungi typical of T. amazonia in forest, but rather by a species of Glomus rarely observed on the other plants. Results from the experimental reforestation plots indicate that changes in AM fungal community composition following forest conversion to pasture may slow pasture recolonization by T. amazonia. The role of particular AM fungal species early in seedling establishment, and the assumption that AM fungi associated with established vegetation colonize new arrivals to form a common mycorrhizal network, merit further examination.
APPLICATION OF ARBUSCULAR MYCORRHIZAL FUNGI FOR THE GROWTH OF TROPICAL TREES

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The mountain rainforest of Southern Ecuador is extraordinarily rich in tree species and more than 95% of these form arbuscular mycorrhiza (AM). Ecuador has one of the highest deforestation rates in South America and 12.3 million hectares of forest are lost each year by slash and burn.

The aim of our project is to help restoring a ‘close to natural’ forest, in collaboration with the forestry group of the DFG RU816. On the long term such a forest should also serve as sustainable income source. To increase the performance of nursery grown seedlings, we inoculate them with a native AM fungal (AMF) inoculum (we apply native organisms only) and try to improve concepts of nursery management practices. We also observe the effects of combined application of slow release fertilizer.

Trap-cultures were established with native tree seedlings and with Plantago lanceolata to initially culture associated AMF. Afterwards, the AMF spores are morphologically characterised and single spore isolates established, if possible. The first defined inoculum was produced in Germany (see poster of Krüger & Schüßler), and the isolates are now also cultured in the Ecuadorian nursery. To test if the inoculum increases seedling performance in the tree nursery experiment, we up to now worked with Alnus acuminate, Myrica pubescens, Cedrela montana, Heliocarpus americanus, and Tabebuia chrysanth. Later Junglas neotropica, an Inga sp., and potentially Podocarpus may follow. Mycorrhization rate and variables such as height of seedlings, root collar diameter, number of leaves, aboveground/underground biomass, and root system architecture are measured three and six months after inoculation. A molecular monitoring of AMF in the seedlings is performed in parallel (see poster of Krüger & Schüßler), to identify AMF useful for future reforestation attempts.
Mycorrhizas in Tropical Forests

**ISOLATION AND MOLECULAR CHARACTERISATION OF SOUTH-ECUADORIAN ARBUSCULAR FUNGI FOR REFORESTATION ATTEMPTS**

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Arbuscular mycorrhizal fungi (AMF) are obligate symbionts of >80% of all land plants. In this symbiosis the fungus delivers inorganic nutrients to the plant and regains carbohydrates in exchange. Arbuscular mycorrhiza (AM) is formed when the fungus colonises the roots, where it develops arbuscules for nutrient exchange. The fungal spores are the main character to identify AMF morphospecies. However this is difficult even for experts, often misleading because of hardly distinguishable characters, and in general restricted to the resting stages. Therefore, molecular methods are used and needed for phylogeny and also species recognition and tracing in roots.

In project B1.1 of the RU816 we intend to explore the potential of using AMF for reforestation in South Ecuador, with native trees, since AM can improve the survival rate and performance of nursery seedling. After defined inoculation of different tree species with indigenous AMF in the nursery, we observe which AMF are establishing and potentially useful for nursery application.

To produce fungal inoculum, several AMF species were isolated from native tree trap cultures. They were morphologically characterised in collaboration with Dr. Chris Walker (GB). Successful single spore cultures (isolates), multi spore cultures and root fragment cultures were used for inoculum production. Up to now, this inoculum was used for inoculating *Cedrela montana*, *Heliocarpus americanus* and *Tabebuia chrysantha* seedlings in the nursery at the UNL (Loja). Roots of the nursery grown trees are sampled after three month and before out-planting the seedlings (about six months) on reforestation plots. In these plots AMF will also be followed by long term tracing to investigate their establishment in the field. The monitoring of the AMF in the nursery and the field will show which AMF have the potential to improve the survival rate and establishment of the seedlings, finally resulting in an optimised inoculum.
Molecular Phylogeny and Evolution of AM Fungi – An Update

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The arbuscular mycorrhizal fungi are the most important symbionts of terrestrial plants; more than 80% of all land plants form this root/fungus association, called arbuscular mycorrhiza (AM). The plants obtain many inorganic nutrients via their obligate symbiotic fungal partners, which belong to the phylum Glomeromycota. In the last decade it turned out that morphological identification of the presently 206 described species of AM fungi is often misleading, due to few morphological characters and dimorphic spores and it was realised less then a decade ago that the genus Glomus was non-monophyletic. Since then molecular data gave insights into many new and unexpected phylogenetic relationships. Here we present the recent knowledge about the phylogeny, taxonomy and evolution of AM fungi. We sequenced the SSU, ITS-region and LSU rDNA of many described and several undescribed species, and by using ITS and LSU rDNA data we are able to resolve AM fungi down to the species level. These data are also needed for DNA barcoding that in future will make identification of AM fungi much easier.

The oldest direct evidence for Glomeromycota in symbiosis with land plants derives from well-preserved plant fossils from the early Devonian (400 My old) Rhynie chert, which show well defined arbuscules. Moreover, fossilized fungal hyphae and spores were found in a 460 My old dolomite rock, therefore at a time when the land flora presumably consisted of plants on the bryophytic level. Molecular data moreover indicate that the AM fungal lineage is much older than the land plants. Therefore, the hypothesis that AM fungi played a crucial role in the colonisation of land by plants is supported on the one hand, but on the other hand interesting questions arise about how these important fungi and their symbiotic associations evolved before land plants existed; we will discuss some of these.
The most common symbiosis of plants in terrestrial ecosystems is the arbuscular mycorrhiza (AM), influencing plant nutrition, resistance, diversity, and also productivity in the vast majority of ecosystems. Only 206 AM fungal (AMF) species are described, usually mainly based on characters of their asexual spores, but recently also by means of molecular phylogenetic data. Species recognition is complicated by the fact that many AMF form spores with different morphotypes, and spore characters are very limited for many taxa. Obviously, the low species number is in contrast to the ubiquity of this fungal group. Also with molecular tools, the species level could usually not be resolved. Therefore, an important challenge is to establish molecular identification tools and DNA barcodes for these ecologically (and economically) important fungi.

Our aim was to test whether the ITS rDNA region can be reliably used for the discrimination of AMF species. Up to now it was unclear whether the high intraspecific variability (also within one multinucleated spore) allows the use of the ITS region as a DNA barcode for AMF.

We show that the ITS region is suited for AMF DNA barcoding and can also be used for ecological studies. We followed a cloning and sequencing (after RFLP) approach, to cover and define the intraspecific variability from AMF from the Diversisporaceae, Ambisporaceae, and Glomeraceae, also comprising frequently used “model species”. Moreover, we sequenced the LSU and SSU rRNA genes. It was possible to separate all investigated AMF species. No overlap of the sequences between different species was observed, and analyses of environmental data show that several (unknown) species are hidden behind the phylotype definitions often used in ecological studies. Our results demonstrate that, after a partial characterisation of intraspecific variability, AMF species can be identified also in field studies.
A new preparation technique, new structural features, three dimensional reconstruction and DNA sequencing of AMF of *Alzatea verticillata* (Alzateaceae) from the tropical mountain rain forest of Southern Ecuador

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The vast majority of the highly diverse trees in the tropical mountain rain forest of South Ecuador form arbuscular mycorrhizas and previous molecular investigations revealed a high diversity of fungi. Here we present a first trial to link fungal DNA-sequences with defined morphotypes characterized on the basis of partly new mycelial features obtained from field material of one tree species, *Alzatea verticillata*. Fine roots were halved lengthwise to study the mycelium anatomy on one half and to obtain fungal nuclear rDNA coding for the small subunit rRNA (nucSSU) of Glomeromycota from the other half. Light microscopy of thoroughly hand separated root layers revealed conspicuously large amounts of mycelium attaching to the surface of the rootlets. The mycelium formed fine or large branched appressoria-like plates, vesicles of regular or irregular shape, and very fine, multi-branched hyphal systems ensheathed by septate hyphae. These previously undescribed features of the supraradical mycelia combined with intraradical mycelium structures were used for distinguishing of four main morphogroups and subordinate 14 morphotypes. DNA sequences of *Glomus*-group A, *Acaulospora* and *Gigaspora* were obtained and linked to three morphogroups. Two sequence types within *Glomus*-group A could be tentatively associated to subordinate morphotypes.
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The tropical mountain rain forest of Southern Ecuador is extraordinary rich in tree species, the vast majority occurring scattered with few individuals. *Graffenrieda emarginata* (Ruiz&Pav.) Triana (Melastomataceae), however, is a frequent tree at 2000 – 2200 m in the macrophyll and microphyll ridge forest. The slowly growing tree roots in a pure humus layer acquiring nutrients from the organic soil fraction. Results obtained by use of transmission electron microscopy and molecular techniques indicate that special mycobiont associations might contribute to competitive advantage of this tree species. While all other surrounding trees form mycorrhizas solely with Glomeromycota, *G. emarginata* is simultaneously associated with members of Glomeromycota, *Rhizoscyphus ericae* aggr. (Ascomycota) and Tulasnellales (Basidiomycota). Light and transmission electron microscopy revealed that *G. emarginata* harbours Glomeromycota in the inner cortical cells, but forms ectomycorrhizas with a superficial, one layered hyphal sheath and Hartig net on the same individual roots with either an ascomycete or with a member of Tulasnellales. Tulasnella was proven by ultrastructure of dolipores with imperforate, dish shaped parenthesomes with recurved ends and by slime in the cell walls. This is the first clear structural evidence of mycorrhizal state of Tulasnellales with a tropical tree species. Saprophytic capabilities were previously shown for members of *Rhizoscyphus ericae* aggr. and presumed for Tulasnellales. These two fungi may, thus, improve nutrient acquisition from humus soil fraction promoting competitiveness of this slow growing tree species.
MORPHOLOGIC AND MOLECULAR CHARACTERIZATION OF ARBUSCULAR MYCORRHIZAL FUNGI OF WILD TREE TOMATO (Solanum cajanumensis) AND CULTIVATED TREE TOMATO (Solanum betaceum).

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The tree tomato (Solanum betaceum, SOLANACEAE) is an endemic species of Ecuador frequently cultivated in the Inter-Andean valleys. Mycorrhizal state and identity of the mycorrhizal fungi associated to this species was not previously reported.

Sampling was carried out in two locations near Loja city. Roots from five individuals of tree tomato (Solanum betaceum) were collected in Zamora Huayco UTPL’s Experimental Farm. Roots from 10 individuals of wild tree tomato (Solanum cajanumensis) were collected in Santiago forest under two conditions, five plants were collected in previously burned forest and five in non-burned forest. Root samples were analyzed by light microscopy to determine the colonization rate and to describe the structural features of the mycorrhiza. Molecular analyses were performed by PCR amplification of the partial nrDNA 18S region using Glomeromycota specific primers followed by molecular cloning. A BIONJ analysis was performed to produce a phylogenic tree.

Root colonization varied between species and sites. Molecular phylogenetic analyses indicated root colonization by diverse Glomerales and Diversisporales. Detailed analysis will show if differences exist between tree tomato species, sites and fire effect.
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