21st New Phytologist Symposium

The ecology of ectomycorrhizal fungi

Centre for Evolutionary & Functional Ecology (CEFE-CNRS)
Montpellier, France
10–12 December, 2008

Programme, abstracts and participants

New Phytologist

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Organizing committee

Ian Alexander (*University of Aberdeen, Aberdeen, UK*)
Marc-André Selosse *(CEFE-CNRS, Montpellier, France)*
Holly Slater (New Phytologist, *Lancaster, UK*)

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Programme, abstracts and participant list compiled by Jill Brooke.
Ecology of ectomycorrhizal fungi illustration by A.P.P.S., Lancaster, U.K.

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Speaker Abstracts

Session 1 **Individuals**
Chair, Ian Alexander

1.1. The genetics and life cycle of *Tuber magnatum*

ANDREA RUBINI, C. RICCIONI, B. BELFIORI, V. PASSERI, S. ARCIONI, F. PAOLOCCI
Institute of Plant Genetics, National Research Council, Via Madonna Alta 130, 06128 Perugia, Italy

Tuber magnatum and *T. melanosporum* are the two most appreciated truffle species, producing the finest white and black truffles, respectively. Both species show a restricted geographical distribution and an apparent low level of genetic diversity, likely as result of a population bottleneck suffered from these fungi during the last ice age. Furthermore, a strict selfing has been accounted to explain the lack of heterozygotic profiles exhibited by the putatively diploid/dikaryotic structures of truffle ascocarps when genotyped with codominant markers. By using SSR markers and appropriate sampling strategies here we show that *T.magnatum* populations are genetically structured, with the southernmost and north-westernmost populations different from all the others, its life cycle is prevalently haploid and, finally, that this species outcrosses. These findings allow us to extensively revise the life cycle and mating strategies of *Tuber* spp.

1.2. Lessons learned from *Armillaria*

JAMES B. ANDERSON
Department of Cell and Systems Biology, University of Toronto, 3359 Mississauga Road North, Mississauga, Ontario L5L 1C6, Canada

The life histories of *Armillaria* and other basidiomycetes, including ectomycorrhizal fungi, share many important features. Individuals of these fungi arise in mating events, proliferate mitotically to occupy spatially discrete territories, and are embedded within populations that are highly recombined over scales ranging from regional to continental. Such individuals can also be treated as populations of ‘cells’, but almost nothing is known about evolution at this level of organization. I will show how individuals of *Armillaria* can be used as well-controlled natural experiments in which evolutionary change, including mutation, mitotic recombination, and selection, can be measured over time intervals of up to 1000 years in a defined spatial context. I will also describe several examples of non-standard genetic recombination in *Armillaria* and will discuss the evolutionary implications of these life-cycle ‘anomalies’ for other fungi with extended diploid or dikaryotic phases.

1.3. Resource allocation in mycelial systems

MARK FRICKER
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Transport networks are vital components of multicellular organisms, distributing nutrients and removing waste products. Animal cardiovascular and respiratory systems and plant vasculature are fractal-like branching trees whose architecture determines universal scaling laws in these organisms. In contrast, transport systems in multicellular fungi are not expected to fit into this conceptual framework, as they have evolved to explore the environment rather than ramify as a three-dimensional organism. Many fungi grow as a foraging mycelium, formed by the branching and fusion of threadlike hyphae. This process gives rise to a complex network that continuously adapts to its environment. However, the properties of the network and its dynamic behaviour have not yet been characterised. Using a range of woodland saprotrophic basidiomycetes, we have examined network development and its nutrient transport characteristics over a range of scales, using a combination of imaging, modelling, gene expression profiling and metabolomics.

We have found that fungal networks can display both a high transport capacity and high resilience to damage. These properties are enhanced as the network grows, while the relative amount of material used to build the network decreases. Thus, mycelia achieve the seemingly competing goals of efficient transport and resilience, with decreasing relative investment, by selective reinforcement and recycling of transport pathways. The fungal network demonstrates that indeterminate, decentralised systems can yield highly adaptive networks.

To test the predictions from the theoretical analysis of transport, we have mapped the distribution of non-metabolised, radiolabelled amino-acid and sugar analogues during mycelial development in spatially heterogenous resource environments using photon-counting scintillation imaging. These studies have revealed a number of novel phenomena, including a marked pulsatile transport component superimposed on a rapid underlying flux, preferential resource allocation to C-rich sinks, abrupt switching between different pre-existing transport routes and organization of the network into well demarcated domains differing in phase or frequency of oscillations. Furthermore, fusion between compatible individuals leads to rapid nutrient re-distribution and formation of a fully synchronised super-colony. Overall the spatial organisation of these mycelial systems provide an almost unique opportunity for any eukaryotic system to directly correlate metabolite levels, nutrient fluxes, gene expression patterns and morphological development.

Session 2 **Populations**
Chair, Marc-André Selosse

2.1. Gene flow and cryptic species in ectomycorrhizal fungi

GREG W. DOUHAN
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Much progress has been made in the study of population genetics of ectomycorrhizal (EM) fungi within the last decade. Somatic incompatibility reactions, based on pairing individuals on artificial agar media, provided early insights in how individual fruiting bodies were structured in natural ecosystems. However, these studies were limited to the few EM species that could be cultured *in vitro*. More powerful and efficient molecular methods to identify EM 'individuals' and 'species' are now readily available and have been used in recent years. Many of these studies have generally been descriptive and used to indirectly make inferences concerning the reproductive biology of these fungi but have also shown that many morphological species are actually species complexes composed of cryptic taxa structured on spatial scales ranging from centimeters to continents. Our ability to now more efficiently identify EM species using molecular methods makes the next challenge to understand the ecological function of these important symbiotic fungi with respect to how reproductive isolation and speciation occurs because these processes are directly related to how forest ecosystems evolve and function.

2.2. Population genetics of *Rhizopogon*

ANNETTE M. KRETZER[1], **R. MOLINA**[2], **S. M. DUNHAM**[3], **J. W. SPATAFORA**[3]
[1]SUNY College of Environmental Science and Forestry, 1 Forestry Drive, Syracuse, NY 13210; [2]USDA Forest Service; 620 SW Main St., Suite 400, Portland, OR 97205; [3]Oregon State University, Department of Botany and Plant Pathology, 2082 Cordley Hall, Corvallis, OR 97331

We have used *Rhizopogon vinicolor* and *R. vesiculosus* (Boletales, Basidiomycota) as model taxa to study genetic structure and gene flow in two ectomycorrhizal fungi with hypogeous sporocarps that are thought to be primarily animal-dispersed. *R. vinicolor* and *R. vesiculosus* are sister species that associate exclusively with Douglas-fir (*Pseudotsuga menziesii*) and form large tuberculate ectomycorrhizas that are comparatively easy to sample in the field. Despite their close relationship, *R. vesiculosus* forms significantly larger genets than *R. vinicolor i*n second-growth stands where both species co-occur. Interestingly, populations of *R. vesiculosus* appear more structured as evidenced both by spatial autocorrelation analysis within plots as well as measures of genetic differentiation between plots. If we take genet size as a proxy for longevity, these results are contrary to trends observed in the plant literature where ephemeral annual plants have generally been found more structured than woody perennial plants due to greater fluctuations in population size and densities. Other biological and ecological differences between *R. vinicolor* and *R. vesiculosus* that might explain the pattern are currently being investigated and will be discussed. The largely sympatric nature of the two sister species raises questions with respect to niche partitioning and mode of speciation.

2.3. The biogeography of ectomycorrhizal fungal species

ANDY F. S. TAYLOR
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Twenty-five years ago, Pirzynski pointed out the misconception that fungi are unreliable as biogeographical indicators due to their edaphic versatility and the physical adaptations of their propagules for long range dispersal. However, in the intervening period there have been remarkably few biogeographic studies on fungi and most of these have focused on saprotrophic or pathogenic taxa. A small number of studies on ectomycorrhizal (ECM) fungi have revealed both ancient distribution patterns at continental scales and recent post-glacial migrations at more local levels. This presentation will summarise these studies, placing them in a spatially scaled framework and will highlight some of the factors suggested as influential in shaping the observed geographic patterns. Recent investigations into the geographic distribution of bolete taxa will also be used to illustrate a number of issues relating to ECM fungal biogeographic studies including the influence of host migration and problems with morphological stasis, recent introductions, synonymy and the lack of conspecificity between wide geographic regions.

Session 3 **Communities**

Chair, Roger Koide

3.1. Community assembly in ectomycorrhizal fungal communities

PETER KENNEDY
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There has long been interest in understanding the factors affecting the spatial and temporal dynamics of ectomycorrhizal (EM) communities. Much of the recent progress in this area can be attributed to the greater ability to identify and quantify EM fungi in natural settings, but it also reflects a growing interest in expanding ecological principles beyond the macroorganisms from which most were developed. This talk will examine the ways in which ecological theory may inform current thinking about EM community dynamics. Emphasis will be placed on understanding community assembly patterns by assessing the relative importance of factors such as dispersal, niche partitioning, and species interactions. The talk will also discuss on how the explicit consideration of scale may help reconcile different ideas about the dominant factors controlling EM community composition.

3.2. Host effects on ectomycorrhizal fungal community composition

KAZUHIDE NARA
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Ectomycorrhizal fungi (EMF) live in symbiosis with host plants in nature. Thus, it is reasonable to propose that hosts affect EMF communities in a variety of ways. Here I want to think about two different host effects: host species (taxonomic distance) and host communities (diversity). To understand the effect of host species, we compared EMF communities among eight codominant tree species (belonging to three families: Fagaceae, Betulaceae, and Pinaceae) in temperate conifer-broadleaf mixed forests in Japan. A Mantel test showed a significantly negative correlation between EMF community similarity and host taxonomical distance, i.e., EMF compositions were more similar in congeneric pairs than in conifer-broadleaf pairs. To examine the effect of host diversity, EMF communities were compared among some EMF forests from monospecific conifer forests to tropical rainforests, where host species richness is among the highest in the world. Irrespective of forest type, Russulaceae and Thelephorales are often dominant in many forests. The diversity of EMF in the tropical rainforests was not higher than that in many temperate forests, indicating that host community diversity is not necessarily a surrogate of EMF diversity. Some possible mechanisms that explain the observed pattern of EMF community composition will be discussed further in relation to hosts.

3.3. Functional diversity in ectomycorrhizal fungal communities

JEAN GARBAYE
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Communities of ectomycorrhizal fungi are central to many aspects of bio-geochemical cycles of interest for ecosystemic services: carbon fixation, water flow, preservation of soil fertility, emission of greenhouse-effect gases, etc. Ectomycorrhizal communities are very diverse, taxonomically as well as functionally. However, until recently, nothing was known of their structure-function relations and of their response to environmental disturbance, and more especially to sylvicultural practices. Recent methodological developments have made it possible to decipher the complex structure of these specialized fungal communities. More specifically, high throughput enzymatic assays applied to individual excised ectomycorrhizas, and the comparison of the activity profiles thus obtained, reveal the contribution of each ectomycorrhizal population within the community to mobilize nutrients from soil organic matter and therefore to ensure the mineral nutrition of the trees. Five examples of the impact of treatments such as thinning, soil amendment or past land use will be presented. The results will be discussed from the point of view of fungal ecology, stressing how ectomycorrhizal fungi collaborate to the overall functioning of the soil–tree interface.

Poster Abstracts

Listed alphabetically by first author, presenting author is underlined

1. High global diversity of *Tomentella* and *Thelephora* (Thelephorales, Basidiomycota)

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Institute of Ecology and Earth Sciences, Tartu University, 40 Lai Street 51005 Tartu, Estonia

Tomentella and *Thelephora* are two paraphyletic sister genera in the order Thelephorales. They are highly diverse and common as fruit-bodies and on root tips in boreal forests of the Northern Hemisphere and also in different forest types of the Southern Hemisphere. We have sampled fruit-bodies and ectomychorrizal root tips from boreal and temperate forests of the Northern Hemisphere and wet sclerophyll forests in Australia, miombo woodlands in Zambia, rain forests in Cameroon and Seychelles. The rDNA ITS sequences of these ectomycorrhizal fungi were obtained and analysed together with exhaustive dataset from INSD. Software packages Modeltest and PAUP were used for the phylogenetic placement of unknown sequences among known species of *Tomentella* and *Thelephora.* Perl scripts were created to analyse host-preference of certain species, to assess species limits (DNA barcodes) in these genera based on molecular data, and to obtain a rough estimate on the total number of species in these genera. We demonstrate a high species richness of resupinate thelephoroid fungi world-wide. 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.

2. Mycorrhizal synthesis between *Boletus edulis* species complex and rockroses (*Cistus* sp.)

ÁGUEDA, BEATRIZ, PARLADÉ, JAVIER, FERNÁNDEZ-TOIRÁN, LUZ MARINA, CISNEROS, ÓSCAR, DE MIGUEL, ANA MARÍA, MODREGO, MARÍA PILAR, MARTÍNEZ-PEÑA, FERNANDO, PERA JOAN
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Sporocarps of *Boletus edulis* Bull. are regularly observed and collected in some areas of Central Spain, which are dominated exclusively by *Cistus ladanifer* L. after abundant fires. The natural sporocarp production of *B. edulis* in association with *C. ladanifer* offers an alternative economic resource in these marginal and inland areas with low incomes. The aim of this work was to obtain the ectomycorrhizas formed by *B. edulis* species complex with *Cistus* sp. under controlled conditions and to provide their anatomical descriptions. Identification of fungal isolates was confirmed by amplifying their ITS rDNA region and further sequencing. Ectomycorrhizas of *Boletus aereus*, *B. edulis* and *B. reticulatus* with *Cistus albidus* L. and *C. ladanifer* were obtained under laboratory conditions in synthesis tubes filled with a mixture of sterilized peat-vermiculite and nutrient solution. The ectomycorrhizas formed were described based on standard morphological and anatomical characters. The three ectomycorrhizas were rather similar, with white monopodial-pinnate morphology, a three-layered plectenchymatous mantle on plan view and boletoid rhizomorphs. *Boletus pinophilus* failed to form mycorrhizas with either host plants. Controlled mycorrhization with *B. edulis* on *Cistus* and outplanting inoculated seedlings for sporocarp production might be a feasible and promising way to exploit this symbiosis providing economic benefits.

3. Growth and phosphorus mobilization by ectomycorrhizal fungi associated with *Pinus pinaster* cultivated in rhizoboxes on forest soil samples.

ALI, MUHAMMAD A., LAJOINIE, VIVIEN, LEGNAME, ELVIRA, CLAIROTTE, M., PLASSARD, CLAUDE
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The *Pinus pinaster* forest in south west of France covers 0.9 million hectares, mainly on acidic sandy, nutrient-poor spodosols. It produces the fifth of French wood and is characterized by a large variation in productivity. We aimed at linking these variations to plant growth, phosphatases activities of ectomycorrhizae (ECM) and soil phosphorus (P) status. Soil samples were taken from different contrasting situations and stored at 4°C. It includes unfertilised soils (Control), fertilized with NPK with or with out irrigation (NPK-Irr and NPK) and very particular soils (Baudes) from 93 years old *P. pinaster* trees stands. These soils were used to grow young *P. pinaster* seedlings in controlled conditions. After 6 months, roots presented numerous indigenous ECMs morphotypes. Fertilization affected negatively the phosphatase activities secreted by ECMs compared with those measured in control soils. Plant dry biomass and N uptake were less variable between soil types than P uptake by plants, which was extremely low in control soils. Surprisingly, a significant decrease of organic P fraction was only observed in NPK-Irr soils. Simultaneously, fungal biomass estimated with ergosterol was greatly enhanced in rhizosphere soil from fertilized soils, suggesting a possible role in P mobilization by soil fungi.

4. Nitrogen uptake in *Cenococcum geophilum*

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Chair of Tree Physiology, University of Freiburg, Georges-Köhler-Allee 53/54, 79110 Freiburg, Germany

Cenococcum geophilum was found to be the most dominant ectomycorrhizal fungus at our field site in Tuttlingen, Swabian Alp, Germany, where we investigate competitive mechanisms of water and nitrogen partitioning in beech-dominated deciduous forests. To complement ongoing work on N metabolism in European beech trees (*Fagus sylvatica* L.), we investigate nitrogen uptake in *C. geophilum* in feeding experiments with ^{15}N labelled organic and inorganic nitrogen compounds (i.e. ammonium, nitrate, glutamine, and arginine) in liquid fungal cultures. Fungal tissue is analysed using stable isotope analysis. In a current study, we identify the time intervals required for the uptake of these compounds. Furthermore, we investigate the impact of substrate concentrations and incubation temperature on N uptake as well as gene expression patterns by real time PCR and micro-arrays. In addition, we identify fungal genes that are involved in the uptake of nitrogen compounds. Our work is expected to yield insights into the role of mycorrhizal fungi on N uptake and metabolism in forest trees on a molecular level.

5. Spatial patterns and cohort associations of *Rhizopogon vesiculosus* and *R. vinicolor* genets in a mixed-age interior Douglas-fir forest

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We investigated the spatial ecology of the ectomycorrhizal species *Rhizopogon vesiculosus* and *R. vinicolor* (Basidiomycota, Villosuli-group sensu Kretzer et al.) within a mixed-aged interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) forest. Tree and fungal individuals were distinguished based on microsatellite analysis of DNA obtained from *Rhizopogon* tuberculate mycorrhiza samples and from aboveground reference trees. Our results indicate that *R. vesiculosus* and *R. vinicolor* are contiguous in the soil through diffuse rhizomorphs and patches of dense mycelium, are indiscriminant with respect to species of vegetation cover and soil substrate types, and they colonize all Douglas-fir tree cohorts from seedlings to mature trees. *Rhizopogon vesiculosus* genets were significantly larger and associated with significantly more trees (1–19 trees) than *R. vinicolor* genets (1–10 trees). We also found that these species co-occur in horizontal space but are partitioned vertically in the soil, with *R. vesiculosus* occurring across a significantly broader depth range than *R. vinicolor*. Together, these species colonized roots from 43 of the 65 trees in a 900 m^2 plot and an additional 14 trees with boles outside the plot, thus linking these trees in a potential mycorrhizal network that was highly interconnected and easily traversed.

6. The ectomycorrhizal fungal community associated with alpine and arctic populations of *Dryas octopetala* along a latitudinal gradient

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In this project, the ectomycorrhizal (ECM) fungal community associated with the root systems of the arctic alpine dwarf shrub *Dryas octopetala* will be investigated along a latitudinal gradient from Southern Norway to the high arctic in Svalbard (13 populations and 26 plants), using molecular analyses. DNA has been extracted from bulk root samples from each plant and 24 ITS fragments have been cloned and sequenced from each root system. The sequences have been submitted to Blast to detect their taxonomic affiliation and then grouped into operational taxonomic units (OTUs) based on a 97% sequence similarity criterion. ITS sequences obtained from basidiocarps will be compared to the environmental sequences. The genotypes of the plants will be analyzed using microsatellite markers and related to the ECM community composition. Preliminary results show that a high phylogenetic diversity of ECM fungi occurs in the root systems of *D. octopetala.* From the 624 analyzed sequences, a total of 273 unique ITS genotypes were obtained. Most genotypes had highest similarity to well-known ECM genera. Noteworthy, the *D. octopetala* plants in the high arctic (Svalbard) seem to have a high diversity of ECM symbionts. In the southernmost locality (an aberrant Norwegian coastal *D. octopetala* relict population occurring on calcareous bedrock) one ITS genotype clustering within Chantarellales was highly dominant.

7. *Bistorta vivipara* and its mycobionts – a model system for ectomycorrhizal research

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The mycorrhizal symbiosis is crucial for the composition, functioning and evolution of terrestrial ecosystems. As one of few herbaceous plants, Bistorta vivipara forms ECM relationships with various fungi. The small and condensed root systems makes *B. vivipara* an ideal model organism for ECM community studies since the entire fungal assemblage associated with the roots easily can be analyzed simultaneously. Using the herb B. vivipara as a model system for ECM associations may overcome many of the challenges we face when working with the large root systems of slow-growing cultivated trees. One main focus will be to analyze which factors that determine and influence on the structure and composition of ECM fungi in the B. vivipara root system. Other main tasks will be to evaluate the spatial and temporal variation in the ECM fungal community associated with B. vivipara. We will conduct both observational field studies and more experimental studies as common garden and re-synthesis experiments. New high-throughput sequencing technology (454 sequencing) will be used to analyse the ECM root community and will yield both quantitative and qualitative measures of the ECM community.

8. Class II peroxidases in ectomycorrhizal fungi

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Fungal peroxidases (Class II) play a key role in degrading recalcitrant polyphenolic compounds of leaf litter and humus in the low productive soils of boreal forests. They have mainly been studied in model 'white rot' fungi, but in ecological studies, peroxidase activity is commonly measured in humus and soil where typical 'white rotters' are absent. The humus layers and mineral soil of boreal forests are instead dominated by ectomycorrhizal fungi, which live in symbiosis with tree roots and receive photosynthetic products directly from their tree hosts. Here, we investigate if peroxidase encoding genes are present in the genomes of ectomycorrhizal fungi. Degenerated PCR primers were applied on representative ectomycorrhizal homobasidiomycetes distributed over a wide phylogenetic range. Subsequent cloning and sequencing showed that ectomycorrhizal taxa from several different genera possess peroxidase genes. In all taxa, active sites are well conserved. Furthermore, there is evidence of late gene duplication, as several isoforms were detected in some species (*e.g. Cortinarius* spp). However, genes to ectomycorrhizal fungi show a high resemblance to well established wood decomposers (*Hypholoma fasciculare*). Thus, Class II peroxidases can be considered an ancient and common feature of most homobasidiomycetes, including ectomycorrhizal fungi.

9. Truffles in the environment: The molecular ecology of *Tuber*

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Truffles belonging to the genus *Tuber* are mycorrhizal fungi characterized by hypogeous fruitbodies. Molecular-based studies of mycorrhizal community composition in various habitats have documented *Tuber* mycorrhizae in many environments including floodplains, clearcuts, tree nurseries, and orchards. However, it is often difficult or impossible to identify the particular species of interest because of a lack or biased sampling of genetic diversity within this genus and taxonomic confusion residing in public databases. Work in our lab to resolve the phylogenetic relationships within *Tuber* has resulted in DNA sequencing from a large number of identified *Tuber* species representing a most of the known phylogenetic diversity of this genus. In this study we use a phylogenetic framework to place and identify a large number of 'unidentified' *Tuber* sporocarps and mycorrhizae using ITS sequences from our own studies, Genbank, and colleagues. Our results show that the most commonly found mycorrhizas from *Tuber* species are from non-economic species in the Rufum, Puberulum, and Maculatum clades. Using molecular-based identification, we were able to taxonomically place 39 unrepresented phylotypes of *Tuber* including 9 known only from mycorrhiza.

10. Ecophylogenetics of ectomycorrhizal fungi and extreme environments

BRANCO, SARA
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Ecophylogenetics is an incipient field of study that integrates ecological and evolutionary theory to explain community assembly and species coexistence. One of its main predictions is that strong environmental filtering will result in phylogenetic clustering (i.e., co-occurring species are on average more closely related to each other than expected by chance) of species within communities, due to the conservation of niche preferences within clades. Serpentine soil is an extreme habitat rich in heavy metals and poor in nutrients that hosts depauperate floras with high rates of endemism. It is believed to act as a strong environmental filter, but its effect on the phylogenetic structure of serpentine communities is yet to be studied. Here, I document the phylogenetic structure of ectomycorrhizal fungal communities in adjacent serpentine and non-serpentine habitats in northeastern Portugal. I found strong phylogenetic clustering on serpentine and the opposite (overdispersion) on non-serpentine soil at the genus level. However, this trend was phylogenetically scale-dependant and reversed at the species level. Serpentine soil seems then to act as an environmental filter only at the genus level.

11. Mapping fungal communities to fine scale soil phosphatase activities

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Compared to the large amount of research on spatial distributions of ectomycorrhizal fungi, almost nothing is known about the fine-scale distribution of their functions. We used a soil imprinting technique to visualize phosphomonoesterase activity at root windows installed in birch/Douglas-fir stands in the southern interior of British Columbia. These sites comprise a chronosequence from four stages of development, with three replicates per age. Shortly after imprinting, small soil samples (0.25 g) were collected from three locations where enzyme activity was detected, and three where it was not detected, in each organic and mineral horizon at each window. Using terminal restriction fragment length polymorphisms, we compared the fungal assemblages associated with the two types of functional soil micro-sites, between organic and mineral horizons, and across stand age. Multi-response permutation procedure detected broad differences in the total fungal assemblages among stand ages, but variability among sites made fine-scale effects more difficult to interpret. A database of 60 ectomycorrhizal fungi was insufficient to detect differences in ectomycorrhizal fungal assemblages amongst sampling locations, especially for older, more diverse sites. While micro-site sampling has the potential to connect in-situ soil function to ectomycorrhizal fungal species, our study indicates that considerable sampling intensity is required.

12. Characterization of ectomycorrhizal communities in a pine forest affected by wildfire

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In this study we analysed the species richness and composition of ectomycorrhizal fungi colonizing seedlings of *Pinus pinaster* Aiton in a Mediterranean ecosystem, naturally regenerated after wildfire. We tested the hypothesis that the composition of the ectomycorrhizal community in different areas of the open forest would affect the performance of the regenerating pine seedlings. One hundred and thirty-five seedlings were harvested in nine areas characterised by a variable seedling regeneration presence, both in terms of cover percentage and seedling height. Ectomycorrhizal fungi colonizing the roots were characterised with molecular techniques (ITS and DNA sequence analysis).The association between the fungal communities of the different study areas and the natural processes of regeneration of maritime pine were investigated through the use of ordination techniques.

13. Molecular detection of fungal root symbionts of *Bistorta vivipara* in a grass land site: Comparing 454 pyrosequencing with cloning and Sanger sequencing

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In order to compare the utility of traditional Sanger sequencing to 454 pyrosequencing, we have analyzed the diversity and composition of fungal symbionts associated with roots of four plants of the perennial herb *Bistorta vivipara* (L.) Delarbre using both techniques. The entire fungal assemblage associated with the roots can easily be analyzed simultaneously due to the small root systems of the plant. *Bistorta vivipara* is forming both ecto and arbuscular mycorrhizal relationships, in addition to having root endophytes. Most of the ITS genotypes detected with cloning and Sanger sequencing had best BLASTn matches against well-known ectomycorrhizal genera such as *Cortinarius, Hebeloma, Inocybe, Peziza,* and *Tomentella*. Only a small fraction of the Sanger sequences were considered to be of chimeric origin. A higher taxonomic coverage was obtained using 454 pyrosequencing. For example, a higher frequency of BLASTn matches against arbuscular mycorrhizal fungi was recorded with 454 pyrosequencing.

14. The functional role of the ectomycorrhizal fungus *Lactarius quietus* in an oak forest: an eco-physiological and eco-genomic approach

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The functions of the ectomycorrhizas formed by *Lactarius quietus* in a mixed oak forest were studied using a combination of enzymatic, isotopic, transcriptomic and immunological approaches. We hypothesized that the peak of secreted enzymatic activities in spring, when oak cambium is already active while leaves are not yet functional, corresponded to the saprotrophic behaviour of the fungus in response to the carbon shortage in the trees.

This hypothesis was further supported by the $\delta^{13}C$ content of *L. quietus* ectomycorhizas, revealing that the major part of the carbon originated from the soil during bud break, while it solely derived from photosynthates during the tree vegetative period.

Among the litter-degrading, nutrient-mobilizing enzyme activities secreted by *L. quietus* ectomycorrhiza, laccases have been studies at the gene expression level and by localizing laccase activity in the different compartments of the ectomycorrhizas.

15. Taxonomic and functional diversity of ectomycorrhizal fungal communities and associated eukaryotic microorganisms: their role in nutrient cycling

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In microbial ecology, recent metagenomic approaches have revealed the microbial communities structure (species richness and distribution) and the functional potential of these communities in various environments. In temperate forest ecosystems, fungi play key roles in nutrient cycling. Among these fungi, ectomycorrhizal species contribute to nutrient cycles by the mobilisation of nitrogen, phosphorus and other minerals from organic matter. Taxonomic community structure of ectomycorrhizal fungi has been essentially studied and is generally characterised by a high taxonomic diversity. Much less is known about their functional diversity and the activities they express in soil. We explore the taxonomic and functional diversity of ectomycorrhizal fungi in different forest ecosystems, one thanks to a new approach, the metatranscriptomic one. Taxonomic diversity is studied by ITS typing of ectomycorrhizal tips DNA and by 18S ribosomal typing of soil DNA. The first results show very different fungal community structure and composition depending on forest type (coniferous *versus* deciduous). The 'metatranscriptome' corresponds to all the genes expressed by the different microorganisms in a common environment. DNA and RNA are extracted from forest soils and polyadenylated mRNA are converted into cDNA to constitute soil cDNA libraries. These libraries can be screened by large sequencing and by heterologous expression of the genes in yeast. This approach allows the study of activities performed by microorganisms directly in environmental samples without cultivation and gives access to the extraradical mycelia of ectomycorrhizal fungi. Results concerning functional diversity are under progress.

16. Diversity and P-solubilizing ability of mycorrhizosphere bacteria associated with *Pinus pinaster* in the Landes forest ecosystem

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Ectomycorrhizal fungi (ECM) and their associated mycorrhizosphere bacteria (MB) play a major role in phosphorous (P) nutrition of maritime pine, particularly in the Landes forest ecosystem considering the low bioavailability of P in its sandy-acidic soils. In order to characterize the MB associated to ECM, three collection campaigns of ECM root tips were performed from autumn 2005 to autumn 2006 throughout three forest stations. Five MB and nine ECM genera were identified overall through sequencing of 16S rDNA and mitochondrial large subunit rDNA fragment respectively. Dramatic seasonal changes were observed in the different MB-ECM associations especially in *Burkholderia–Lactarius* and *Bacillus–Russula* that were the most abundant ones. P-solubilizing ability of MB was shown to be taxon-dependent with a large majority of P-solubilizing isolates among *Burkholderia*, *Pseudomonas* and *Paenibacillus* contrary to *Bacillus*. Relationships between functional diversity of the MB-ECM associations and bioavailability of soil P remain to be investigated.

17. Effects of ozone stress on exoenzyme activities and the composition of ectomycorrhizal communities

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There is increasing concern that environmental pollution and global climate change will affect the relation between ectomycorrhizas and trees. In the frame of the FUNDIV project different stresses on ectomycorrhizas are studied. In our part of the project, we focus on the influence of ozone on the structure and function of ectomycorrhizal communities. Elevated atmospheric ozone is one of the most serious air pollutants damaging vegetation, and may indirectly affect ectomycorrhizas via altered rhizodeposition and root physiology. In a 70-year-old mixed stand of spruce (*Picea abies* (L.) Karst.) and beech (*Fagus sylvatica* L.) at Kranzberger Forst (near Munich, Germany), a free-air O_3 fumigation system installed around mature trees was set to double the ambient ozone concentration (< 150 O_3 l^{-1}) during the vegetation period (May till October, 2000-2007). Our sampling campaigns were in June and October 2007 and addressed the community structure and composition of ectomycorrhizas of spruce by morphotyping, sequencetyping of the ITS region and tip counting. In addition, we measured eight different exoenzyme activities relevant for nutrient turnover processes in forest soils from each morphotype to assess whether functional changes at the community level were affected by ozone stress. The corresponding data will be presented at the meeting.

18. Elevated CO_2 and nitrogen influence the exudation by ectomycorrhizal roots

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Root and mycorrhizal exudation contributes significantly to soil carbon fluxes, and is likely to be altered by elevated atmospheric CO_2 levels and nitrogen deposition. We quantified low molecular weight (LMW) organic compounds exuded by ectomycorrhizal (ECM) plants in relation to increased CO_2 levels and different nitrogen (N) sources. Scots pine seedlings, either colonised by ECM fungi or non-mycorrhizal (NM), were exposed to ambient (360 ppm) or elevated (710 ppm) concentrations of CO_2, and received either ammonium or alanine as the N source in a liquid growth medium. Exudation of LMW organic acids (LMWOAs), dissolved monosaccharides and total dissolved organic carbon (DOC) was determined. Both N and CO_2 had a significant impact on exudation, especially of LMW organic acids. Exudation differed between ECM seedlings and NM seedlings. In addition, there were ECM species-specific differences. The N treatment significantly affected exudation of total LMWOAs and all individual organic acids. The CO_2 treatment significantly affected exudation of individual organic acids (except for oxalate), DOC and shoot N content. This may impact on nutrient mobilization under changing environmental conditions.

19. Evidence of high genetic diversification in neotropical ectomycorrhizas

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Community ecology of ectomycorrhizas has been studied mainly in boreal and temperate forests. Mexican neotropics are a main center of diversification for *Pinus* and *Quercus*. We evaluated the genetic diversity of ECM in a wide area of pine-oak forests in the state of Michoacan, Mexico. We sampled ectomycorrhizas and fruit bodies and obtained their ITS sequence by conventional direct sequencing of PCR products. We found high alpha and beta diversity, 253 genetic species grouped at 97% of similarity and 623 unique sequences. The diversity of Thellephoraceae and Sebacinaceae was particularly high. However when sequences were blasted with NCBI and UNITE the most common scheme was a poor similarity. Phylogenetic and non phylogenetic methods such as UNIFRAQ were used to evaluate the genetic distance of Mexican sequences for selected groups as *Tomentella*. All estimations found that Mexican ECM fungi have a considerable genetic distance with their North American and European relatives. Finally we discuss the need of better methods for comparing the structure of ECM communities when sequences are used as raw data.

20. Nickel tolerance in *Cenococcum geophilum* from serpentine soils: evidence for an adaptive trait

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Selection for metal-tolerant ecotypes of ectomycorrhizal fungi has been reported in instances of metal contamination of soils as a result of human activities. However, no study has yet provided evidence that natural metalliferous soils, such as serpentine soils, can drive the evolution of metal tolerance in ectomycorrhizal fungi. We examined *in vitro* Ni tolerance in isolates of *Cenococcum geophilum* from serpentine and non-serpentine soils to assess whether isolates from serpentine soils exhibited patterns consistent with adaptation to elevated levels of Ni, a typical feature of serpentine. Biomass production in liquid media was assessed for isolates from both soil types exposed to increasing Ni concentrations so that EC_{50} could be determined. Isolates of *C. geophilum* from serpentine soils exhibited significantly higher tolerance to Ni than non-serpentine isolates: the mean EC_{50} value for serpentine isolates was 7-times higher than the estimated value for non-serpentine isolates. Although there was still a considerable variation in Ni sensitivity among the isolates, none of the serpentine isolates had EC_{50} values for Ni within the range found for non-serpentine isolates. These results suggest that Ni tolerance arose among serpentine isolates of *C. geophilum* as an adaptive response to Ni exposure in serpentine soils.

21. Functional fungal bridges linking pine trees and ericaceous shrubs

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Fungi from the *Rhizoscyphus ericae* aggregate (Ascomycetes, Helotiales) have repeatedly been found in the roots of both ectomycorrhizal (ECM) trees and ericoid mycorrhizal (ERM) shrubs. One isolate has been shown to simultaneously form ECM and ERM structures *in vitro*. However, the suggestion that a ubiquitous group of fungi may act as shared symbionts between trees and shrubs has so far remained hypothetical, due to the lack of convincing functional evidence. Here, we demonstrate for the first time, significant reciprocal resource transfer between ericaceous shrubs and ascomycetes isolated from ECM pine roots. In synthesis experiments, these fungi formed typical ERM intracellular coils, and some strains also formed patches of sheath on the surface of hair roots. The same fungi colonised pine roots *in-vitro*, and one strain formed typical ectomycorrhizas. We also show that identical fungal genotypes (ISSR-types) can be isolated from co-occuring pine and ericaceous roots in the field. Our study demonstrates that certain ECM ascomycete fungi can also form functional ERM mycorrhizas. Hyphal connections that act as a bridge between overstory ectomycorrhizal trees and understory ericoid mycorrhizal shrubs are, therefore, a strong possibility.

22. Nitrogen isotopes ($\delta^{15}N$) in ectomycorrhizal mushrooms correspond to belowground exploration types and carbon demands

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We suggest that fungal $\delta^{15}N$ reflects sequestration of fungal nitrogen to build fungal biomass, and should accordingly reflect fungal exploration strategies. To test this, we compared $\delta^{15}N$ to exploration types and hyphal hydrophobicity in ectomycorrhizal species from comprehensive surveys at four sites (Taylor et al. 2003 and Trudell et al. 2004). Fungi with rhizomorphs and exploration types of high carbon demand [long-distance (e.g., *Suillus*), medium-distance mat (e.g., *Hydnellum*), and medium-distance fringe (e.g., *Cortinarius*)] were 4-7‰ enriched in ^{15}N relative to fungi with exploration types of low carbon demand [medium-distance smooth (e.g., *Amanita*), short-distance (e.g., *Inocybe*), and contact (e.g., *Hygrophorus*)]. High-carbon demand types comprised 79% (Åheden, northern Sweden), 65% (Deer Park, Pacific Northwest, USA), 45% (Stadsskogen, central Sweden), and 38% (Hoh, Pacific Northwest, USA) of ectomycorrhizal species, with high carbon demand more prevalent at sites of low N availability. Species with hydrophobic hyphae or with rhizomorphs were enriched by 3-4‰ relative to taxa with hydrophilic hyphae or without rhizomorphs. The consistency of these patterns suggest that $\delta^{15}N$ measurements could provide insights into belowground functioning of poorly known taxa of ectomycorrhizal fungi.

23. Effect of the initial colonization level and the inoculated strain in the field persistence of the edible ectomycorrhizal fungus *Lactarius deliciosus*

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Pinus pinea plants were inoculated with the edible ectomycorrhizal fungus *Lactarius deliciosus* to determine the effect of the initial colonization level and the inoculated strain on fungal persistence in the field. The mycorrhizal plants obtained were established in six experimental plantations located in two sites in the Mediterranean area. The ectomycorrhizal root colonization and extraradical soil mycelium biomass were monitored at different times using both morphological and molecular approaches based on ectomycorrhizal counting and TaqMan® real-time PCR, respectively. The results obtained indicate that the field site played a decisive role in the persistence of *L. deliciosus* after outplanting. The initial colonization level and the selection of the suitable strain are also significant factors but their effect on the persistence and spread of *L. deliciosus* may be conditioned by the physical-chemical and biotic characteristics of the plantation soil and, possibly, by their influence in root growth. Molecular techniques based in real-time PCR allowed non-destructive and precise quantification of extraradical mycelium of *L. deliciosus* in the field which may be correlated with ectomycorrhizal development. However, the accuracy of the technique will ultimately depend on the development of appropriate soil sampling methods because of the high variability observed.

24. Effects of irrigation on the functional and structural diversity of ectomycorrhizal fungi

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Several thousand fungal species live in ectomycorrhizal symbiosis with trees. What is the functional significance of this diversity for the host plant? It has been shown that the community structure of ectomycorrhizal fungi (ECM) can change when exposed to drought. What is the functional significance of this change for the host tree? To study these questions, various root samples are collected in the context of an irrigation experiment which is conducted at one of the driest valleys in Switzerland. The ECM community structure is investigated by morphotyping and ITS sequencing. Furthermore, the activities of eight exoenzymes on these ECMs are analyzed. A microplate fluorimetric assay which can be applied on individual ectomycorrhizal tips is used. Preliminary findings on the community structure indicate that the species richness is reduced by irrigation. The enzyme activities vary among the different morphotypes. E.g., *Cantharellus lutescens* shows a strong increase on the chitinase acitvities for the irrigated sites. The differences regarding the global activities when comparing control and irrigated sites are dependent on the enzymes; phosphatase and xylosidase activities are decreased in the irrigated sites whereas glucuronidase acitivities show an increase. Thus, the functional structure of the ectomycorrhizal community as well as the community itself seem to change.

25. Trophic relationships in the rhizosphere of woody plants: effect of the communities of fungi, bacteria and nematodes on mineral nutrition of *Pinus pinaster*

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In terrestrial ecosystems, and more particularly forest ecosystems, the availability of macro nutrients such as N and P is often limiting the plant growth. Generally, plants take up these macronutrients as mineral ions from the soil solution. During plant growth, these mineral nutrients are assimilated into complex, organic molecules that come back to the soil to be mineralized by the soil microbial populations. This alternance of mineral and organic forms constitutes the bio-geo-chemical cycle of a given element. In natural conditions, complex interactions between microbial populations and microfaunal grazers create the actual biogeochemical environment of roots. So far, the responses due to microfloral grazers (protozoa, nematodes, microarthropods) have been studied in herbaceous plants and they are increased plant growth, increased N uptake by plants, increased N and P mineralization, and increased substrate utilization. By contrast, there is very little data available on woody plants such as gymnosperms that always form symbiotic associations with fungi on most of their root apices through the formation of ectomycorrhizal roots (ECM). In the field, ECMs live with specific bacterial populations called BAMs (Bacteria Associated with Mycorrhizae). BAM abundance can be regulated by trophic factors such as the availability of carbon and mineral nutrient, and biological factors such as predation by bacterial nematodes that will result in positive and negative effects, respectively. In this study, the effects of microbivorous nematodes on nutrient cycling, plant growth, and nutrient uptake were examined with reference to activities within the rhizosphere of *Pinus pinaster* seedlings. Two-month old seedlings, whether or not associated with the ectomycorrhizal basidiomycete *Hebeloma cylindrosporum* were grown in gnotobiotic microcosms containing agar medium with insoluble mineral P and nitrate. An additional source of N was supplied as labelled ^{15}N in the bacterial biomass of *Pseudomonas fluorescens*, whether or not with nematodes. After 35 d of co-culture, the treatments containing nematodes and bacteria increased shoot biomass and root surface area compared to treatment without nematodes. Non mycorrhizal plants growing with bacteria and bacteria-feeding nematodes took up more N from nitrate than plants grown alone or with only bacteria, because of increased N mineralization from bacteria, NH_4^+-N excretion by nematodes, and greater initial exploitation of media by plant roots. Addition of fungal treatment did not increase plant growth or N uptake because the N taken up by the fungus alone was sufficient for plant growth.

26. Germination and infectivity of ectomycorrhizal fungal spores in relation to their ecological traits during primary succession

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Ectomycorrhizal fungal (EMF) spores play critical roles in population and community development of EMF. Here, we examined the germination and infectivity of EMF spores with reference to the ecological traits of the EMF species. Spores were collected from 12 EMF species, whose successional patterns have been studied in the volcanic desert on Mount Fuji, Japan. Spore germination experiments were conducted with host plants (*Salix reinii*), with non-host plants (*Polygonum cuspidatum*), and without plants. The mycorrhizal formation ability of spores was also examined in seven EMF using spore inoculation experiments. To determine the effects of the spore preservation period, both experiments were repeated up to 1 year after spore collection. Spore germination was very low in the absence of host plants. In the presence of hosts, even 30 days after spore collection, spore germination was significantly enhanced in all pioneer EMF (*ca.* 20%) but less so in late-stage EMF (<5%), except in *Hebeloma* species. Mycorrhizal formation from spores was also greater in pioneer EMF but was significantly reduced by 1-year spore preservation. High spore germination and infectivity of pioneer EMF should enable these species to colonize disturbed and isolated areas in accordance with their ecological traits.

27. Substrate exploration by ectomycorrhizal fungi of Scots pine

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It has been shown that ectomycorrhizal fungi colonise decomposing substrates in soil and can mobilise nutrients for plant uptake. Such colonisation has been demonstrated using a few culturable ectomycorrhizal fungi in microcosms and little information is available in field settings with a variety of plant materials. We tested the hypothesis that different substrates with diverse chemical compositions may harbour distinct ectomycorrhizal mycelial communities. Meshbags were prepared with Scots pine needles, bark, roots and moss collected from a 120 year old plantation. A total of 240 meshbags (60 bags in each substrate) were buried in the organic layer of five randomised plots in May 2004 and were recovered every two months for two years. The ectomycorrhizal fungi present in each bag at each harvest were determined by molecular methods based on matching T-RFLP patterns of PCR-amplified ITS sequences to a local reference database of TRFs from fruit bodies and mycorrhizas. There were some differences in fungal colonisation between substrates and with the passage of time.

28. Susceptibility of ectomycorrhizal fungi to forest fire - an experimental approach

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Due to climate change, forest fire frequency is predicted to increase in the dry, inner-alpine valleys such as the Valais or the Aosta valley. The question arises whether and how trees recolonize the burnt sites. It is well known that ectomycorrhizal fungi enhance seedling growth by improving water and nutrient uptake. But mycorrhizal propagules may be destroyed by fire as well. Literature of field studies investigating the resistance of ectomycorrhizal fungi to fire is contradictory, probably because uncertainties relate to high variation of fire intensity and duration. We therefore chose a reductionistic approach by heating forest soil samples in a drying oven to 50, 65, 75 °C and assessing surviving propagules with a bioassay. We used *Pinus sylvestris* because this tree species dominates forests on the shallow, southerly-exposed slopes in the regions mentioned above. The number of fungus species decreased significantly in all three treatments compared to the unheated controls. Community composition was altered, with stress-tolerant species such as *Cenococcum geophilum* becoming more abundant. These results provide indications on how fire changes ectomycorrhizal fungi communities. However, the question whether the lower diversity and the lack of certain mycorrhizal species hinder tree recruitment still remains open.

29. The ectomycorrhizal community changes over a short nitrogen deposition gradient

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Within forests exposed to high atmospheric nitrogen deposition, short N-deposition gradients may form – the N-throughfall is highest at the forest front and decreases towards the interior of the forest. A spruce associated ectomycorrhizal community has been analyzed over such a short N-deposition gradient and significant changes in the species composition are correlated with a 100-fold change in the production of external mycelia in the soil. Ongoing studies are investigating the ectomycorrhizal community preference of nitrate, ammonium and amino acids across the same gradient.

30. Heavy metal tolerant fungi play a key role in the protection of pines against combined Zn-Cd toxicity

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In this study with ectomycorrhizal Scots pine (*Pinus sylvestris*) seedlings we considered toxicity of the individual metals Zn and Cd, as well as toxicity caused by a combined Zn-Cd treatment. A *Paxillus involutus* isolate and three *Suillus luteus* isolates with different degrees of Zn- and Cd tolerance were used to inoculate pine seedlings. Inoculated pines were exposed to Cd, Zn or a combination of both metals. Nutrient uptake was monitored, along with the growth of the symbiotic partners, as well as Cd and Zn accumulation in the host. Plants colonized with a Zn- and Cd-tolerant isolate performed best under Zn-Cd stress. A competitive interaction between Cd and Zn could be observed on growth of the fungi and on nutrient uptake capacities of the root systems. Cd toxicity is clearly reduced in the presence of elevated Zn. Zn and Cd concentrations in needles and roots give also an indication of such an interaction between both metals. Because of the key role of the ectomycorrhizal symbiosis for plant fitness, the evolution of both Zn- and Cd tolerance in an ectomycorrhizal partner such as *S. luteus* can be of major importance for the persistence or establishment of vegetation on heavy metal contaminated soils.

31. The development of simple sequence repeat markers in the ectomycorrhizal fungi *Laccaria bicolor* and *L. amethystina* for molecular ecology applications

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The ectomycorrhizal fungi *Laccaria bicolor* and *L. amethystina* form mutualistic symbiotic associations with a wide variety of tree species in the Northern Hemisphere. Populations of these fungi have been studied with various types of molecular markers (Roy *et al.* 2008). However, these markers are adapted for fingerprinting, but they are not adapted to measure most of the classical population genetic parameters. Only a limited set of simple sequence repeat (SSR) markers is available for *L. bicolor* (Jany *et al.* 2006). The genome sequence of *L. bicolor* has been released recently (Martin *et al.* 2008). We have used this genomic resource to develop hundreds of new SSR markers to construct a genetic map (Labbé *et al.*, 2008). Part of these SSR markers was used in a preliminary assay to evaluate their polymorphism within *L. bicolor* and *L. amethystina* populations. Our objective then was to develop a unique set of markers adapted to different types of studies (population diversity and evaluation of gene flow) from micro- to macrogeographical scales (from local sites to European continent). Based on their large range of polymorphism, this set of SSR markers is expected to be useful for different kind of population studies at different geographical scales.

32. Is diversity of mycorrhizal community related to the diversity of tree species?

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There is evidence for grassland ecosystems that the below-ground mycorrhizal diversity drives above-ground diversity and productivity. Little is known about these relationships in mature deciduous forest ecosystems. We aim at investigating whether diversity of mycorrhizal communities is correlated with the diversity of deciduous tree species.
For this purpose six plots characterised by similar edaphic and climatic conditions in three different mixtures of tree species were chosen: (a) two plots only stocked with *Fagus sylvatica*, (b) two plots with three tree species (*Fagus sylvatica, Fraxinus excelsior, Tilia sp.)* and two plots with five tree species (*Fagus sylvatica, Fraxinus excelsior, Tilia sp., Acer sp., Carpinus betulus*) in the Nationalpark Hainich (Germany). Roots samples were collected in two year in different seasons and used to classify mycorrhizal fungi using morphotyping, ITS-markers and sequencing. We found a total 145 different morphotypes at roots of *Fagus sylvatica, Tilia sp., and Carpinus betulus* of which the majority of further analysed by ITS sequencing. Beech and lime harboured higher EM-species richness than hornbeam. *Fraxinus excelsior and Acer sp.* were colonized by AM fungi. Colonisation with EM fungi was always high and not significantly fluctuating between April and November. Diversity and evenness were not affected by season and only little by the tree diversity gradient.

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33. Catalytic properties of differents extracellular acid phosphatases secreted by ectomycorrhizal fungi under different phosphorus availability

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Mycorrhizal symbiosis has a beneficial effect on the host plant phosphorus nutrition. This effect is attributed, partly, to the release of fungal phosphatases which will mineralise the organic phosphorus substrates in the soil solution. In spite of the importance of this phenomenon in P-deficient soils, few data are available on biochemical properties of these enzymes. To gain new insights in this important biological question, we studied the catalytic properties of acid phosphatases released in the external medium by a model ectomycorrhizal basidiomycete fungus, *Hebeloma cylindrosporum,* selected for its strong capacity of enzyme secretion in the culture medium. Three isoforms of acid phosphatases were separated by column chromatography. Phosphatase activity of each isoform was strongly increased with phosphorus deficiency. Values of optimal pH ranged between 5-6 for the three isoforms. However, these isoforms presented different catalytic properties. This could help the fungus to hydrolyse a large variety of substrate in different soil conditions and consequently, a better adaptation to soil containing a low P availability.

34. Mycorrhizal fungi of chestnut ecosystems in the northeast of Portugal

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The biodiversity of macrofungi in the majority of the ecosystems in Portugal is of limited knowledge and inventories are necessary to contribute for a more complete list of the native species. The Project AGRO 689 contributed for a better understanding of the species associated with chestnut (*Castanea sativa*) ecosystems in the north east of Portugal. The results of the inventory made in a chestnut orchard from 2004 to 2007 will be shown. The relative abundance of mycorrhizal vs. non-mycorrhizal and of edible vs. non-edible species is one of the main tasks of this work. The field work was made from October of 2004 until December of 2007, comprising 7 collecting seasons: Fall of 2004, Spring and Fall of 2005, Spring and Fall of 2006 and Spring and Fall of 2007. Three collecting areas of 100m^2 were delimited in chestnut orchards near Bragança. All macrofungi specimens were collected weekly during Fall and Spring and monthly during the rest of the year. Identification and quantification and herborization of the carpophores were made in the laboratory according to identification manuals. During the period of study in *C. sativa* ecosystem, 53 species of macrofungi belonging to 23 genera were collected. The list of the gathered species will be shown and results will be discussed in terms of biodiversity associated with this ecosystem in terms of mycorrhizal vs. non mycorrhizal species.

35. Elucidating the trophic dynamics of fungi using stable isotopes

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Mycorrhizal and saprotrophic fungi are essential components to terrestrial element cycling due to their uptake of mineral nutrients and saprotrophic decomposition of detritus. Linking these ecological roles to specific fungi is necessary to improve our understanding of global nutrient cycling, fungal ecophysiology, and forest ecology. Using discriminant analyses of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope values from 940 fungi across 26 sites, we verified collector-based trophic categorizations as either ectomycorrhizal (ECM) or saprotrophic (SAP) in >91% of the fungi, and provided probabilistic assignments for an additional 27 fungi of unknown ecological role. As sites ranged from boreal tundra to tropical rain forest, we also tested if isotopic variability in fungi could be predicted by climate or latitude, as previously shown in plant and soil meta-analyses. Using a combination of linear regression and mixed effect models, we demonstrated that these proxies could predict the variability among fungal $\delta^{13}C$ (26 sites) and $\delta^{15}N$ (32 sites) values. Fungal $\delta^{13}C$ values are likely reflecting universal trophic differences in C-source, whereas a consistent enrichment in ^{15}N in ECM fungi relative to SAP fungi suggests that ECM fungi are globally important for tree N nutrition.

36. The common ectomycorrhizal guild of pine and oak growing in a mixed forest

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Several species of fungi are present on individual tree roots in a mixed forest. Some are highly specialized but others have broad partner range. The aim of the present study was to check to what extend the roots of oak and pine, co-occuring frequently, are connected by ectomycorrhizal fungi. We collected root samples in the oak-pine forest in two subsequent years. Ectomycorrhizas were isolated and taxa discrimination was done by combined morpho-anatomical and molecular methods. Over 35 fungal species were identified. More than half was connected to one tree species. The number of fungi found exclusively on pine was higher than those typical for oak. Ectomycorrhizal population of oak was dominated by the common species (82% and 93% in subsequent years). Pine community was characterized by higher percentage of exclusive pine symbionts (24% and 47% in subsequent years). Ectomycorrhizal communities on both trees were dominated by limited number of fungi, mostly by *Cenococcum geophilum,* boletoid, russuloid and corticioid species. This case study indicates that common ectomycorrhizal communities may dominate the root systems of oak and pine, and may be characterized by high diversity.

37. Dynamics of microbial communities associated with ectomycorrhizal mats

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Ectomycorrhizal (EcM) mats are prominent features in old-growth Douglas-fir forests of the Pacific Northwest. We have been studying the structure and function of microbial activities associated with two EcM mat types: those formed in the organic horizon by *Piloderma* spp. and by *Ramaria* spp. in the upper mineral soil horizon. Non-mat soils served as controls. We examined the dynamics of bacterial and fungal communities it two studies: one focused on seasonal changes and one focused on temporal change associated with the death or formation of EcM mats. We used T-RFLP fingerprints to assess the composition of the bacterial and fungal communities. As expected, microbial communities differed between EcM mat and non-mat soils but did not vary seasonally. Death of EcM mats was studied by severing mats from their host trees and following shifts in microbial communities; birth, or formation, of EcM mat communities was studied by implanting non-mat soil into EcM mats. Microbial communities shifted during the two years following the establishment of the birth and death treatments; however, the rate and direction of community change varied by EcM mat type and between treatments.

38. Fungal carbohydrate nutrition: Sugar transporters and hexose uptake by *Laccaria bicolor*

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An important aspect of mycorrhizal symbiosis is the supply of the fungal partner by plant-derived photo assimilates. We have investigated the first step of fungal carbon nutrition, the uptake of sugars. Fifteen potential hexose transporter proteins, of which ten group within three clusters, are encoded in the genome of the ectomycorrhizal model fungus *Laccaria bicolor*. For 14 of them transcripts were detectable. Compared to carbohydrate supported hyphae, carbohydrate starvation resulted in a significant higher transcript level for 10 genes. Compared to the extraradical mycelium, the expression of six genes was enhanced upon ectomycorrhiza formation, and transcript levels of four of these genes revealed their highest observed transcript levels in symbiosis A function as hexose importer was proven for three of them. Only three genes, of which just one was expressed at a considerable level, revealed a reduced transcript content in mycorrhizas.

From gene expression patterns and import kinetics, the *Laccaria bicolor* hexose transporters could be divided into two groups: those being responsible for uptake of carbohydrates by soil growing hyphae for a) improved carbon nutrition and b) to reduce nutrient uptake competition by other soil microorganisms, and those responsible for efficient hexose uptake at the plant/fungus interface.

39. Growth on nitrate and occurrence of nitrate reductase-encoding genes in a phylogenetically diverse range of ectomycorrhizal fungi

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Ectomycorrhizal (ECM) fungi are often considered to be most prevalent under conditions where organic sources of N predominate. However, ECM fungi are increasingly exposed to nitrate from anthropogenic sources. Currently, the ability of ECM fungi to metabolise this nitrate is poorly understood. Here, we examined growth of one-hundred and six isolates, representing 68 species, of ECM fungi on nitrate as the sole N source. In addition, the occurrence of genes coding for the nitrate reductase enzyme (nar gene) in a broad range of ectomycorrhizal fungi was investigated. All isolates grew on nitrate, but there was a strong taxonomic signature in the biomass production, with the Russulaceae and *Amanita* showing the lowest growth. Thirty-five partial nar sequences were obtained from 43 tested strains comprising 31 species and 10 genera. These taxa represent three out of the four clades of the Agaricales within which ECM fungi occur. No nar sequences were recovered from the Russulaceae and *Amanita*, but Southern hybridisation showed that the genes were present. The results demonstrate that the ability to utilise nitrate as an N source is widespread in ECM fungi, even in those fungi from boreal forests where the supply of nitrate may be very low.

40. Friend or foe? Evolutionary history of genes encoding sucrolytic activity and its implication for fungal symbiosis

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Symbiotic fungi belong to a number of distinct ecological guilds that either share or differ in the benefit they confer to plant hosts. It is unknown whether functional trait conservation among fungi of similar interaction type explain similarity in symbiotic outcomes. One trait in which plant-associated fungi may vary is their ability to procure different forms of plant carbon. Culture-based studies suggest mycorrhizae cannot utilize sucrose but plant pathogens can. Is the incapacity to use sucrose a functionally conserved mutualistic trait? Many symbiotic fungi are unculturable; thus, to address this question I used the presence of glycosyl hydrolase family 32 (GH32) genes that encode sucrolytic enzymes as a proxy for sucrose utilization. Fungal genomes and a phylogenetically and ecologically diverse array of fungi were assayed for GH32. 7 GH32 clades were recovered; GH32 distribution showed a strong link to ecology: 91% of mycorrhizal taxa lacked GH32 genes, and 84, 54 and 43% of endophytes, pathogens and lichens possessed them. These data show functional diversity within ecological guilds, but also reveal a striking contrast between mycorrhizal and other symbiotic fungi in their potential to utilize sucrose, suggesting that carbon dynamics in mycorrhizal interactions are distinct from that of other plant–fungal mutualisms.

41. Changes in ectomycorrhizal communities structure under carbon limitation, into a beech forest

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Ectomycorrhizal fungal species present a wide range of morphological and functional varieties, especially with regard to root weathering capacity, development of extraradical mycelia - soil system exploration, or abilities for carbon uptake. Their survival may depend on capabilities to use different sources of carbon. Therefore species composition of an ectomycorrhizal community can change in response to perturbation of carbon flux. We studied changes of beech ectomycorrhizal communities after carbon flux was limited by stem girdling. Carbon content in phloem sap was decreased and a lower Shannon-Wiener Index of the ectomycorrhizal community was found although ectomycorrhizal colonization rate was not affected. Some ectomycorrhizal species disappeared. Since carbon pool of roots was not completely depleted, the reduction in species richness indicates that not all ectomycorrhizal species have the capacity to survive on limited carbon sources.

42. The role of external mycelium of ectomycorrhizal fungi in controlling of saprtrophic *Heterobasidion* fungi

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The annual economic losses due to root and butt rot caused by *Heterobasidion parviporum* and *H. annosum s.str.* in Europe are estimated to at least 790 million euros. Intensive human activities in the forests are considered to be the main reason for the increased incidence of this most severe forest disease in Northern Europe. We posed a hypothesis that the impaired competitive status of external mycelia of ectomycorrhizal (ECM) fungi due to forest cuttings is one of the mechanisms behind the dominance of saprotrophic fungi *Heterobasidion* in managed boreal forests.

In vitro, we have observed direct antagonism against *H. parviporum* and *H. annosum s.str.* by five of the tested 48 ECM pure cultures. The same strains belonging to the species *Paxillus involutus and Phialophora finlandia* were also able to protect young Norway spruce seedlings from lethal *Heterobasidion* infection in the laboratory. Currently we are analyzing ECM external mycelium in the seriously diseased Norway spruce stands. Aim is to compare the community composition of ECM external mycelia within aggressively expanding *H. parviporum* genets with that of *H. parviporum*-free patches.

43. Ectotrophic mycorrhizal symbioses dominate natural ultramafic ecosystems of New Caledonia

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In New-Caledonia, natural ultramaphic ecosystems were studied in the framework of the mine project of the Koniambo Massif, a large nickel deposit. After floristic inventories along a slope ranging from 700 to 900 m, 4 different floristic formations were clearly present along an altitudinal gradient. The ectomycorrhizal (ECM) status of the dominant endemic plants of the 2 lower formations were explored through molecular methods on sporocarps, mycorrhizas and soil mycelium. It allowed us to investigate a highly diversified fungal community in the basal formation, dominated by two *Nothofagus* tree species. Due to this high level of diversity the molecular phylogenies were established in a two-step procedure: a first analysis based on the 5.8S gene located within the ITS region of the nuclear ribosomal RNA was first established, revealing a high ratio of members of the Cortinariaceae. This fungal family was thus phylogenetically analyzed on the whole ITS. Such molecular characterization of unknown tropical ECM fungi is a first step towards a better analysis of phylogenetic inferences between tropical and temperate ECM basidiomycetes. With the increase in mining activity and the associated ecological restoration issues in New-Caledonia, these results are of prime importance for maintaining unique ecosystems biodiversities.

44. Defoliation effects on the ectomycorrhizal community of maritime pine (*Pinus pinaster* Ait.)

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We studied the existence of a negative feed-back of defoliation on diversity, abundance and community structure of the ectomycorrhizas hosted by maritime pines (*Pinus pinaster* Ait.) in NW Spain. We simulated two levels of defoliation (25%, 75%) by *Thaumetopoea pityocampa* in 90 5-years-old pines in two plots (Catoira (A) and Cotobade (B)). Ectomycorrhizal community was investigated by root tip morphotyping, molecular analysis (PCR and sequencing) and fruitbodies sampling. We found that defoliation did not significantly affect the variables studied. On the other hand, likely because site B had suffered a wildfire previously, we found that in this site species richness, diversity and ectomycorrhizal abundance was lower than site A. The commonest species in site A were *Tomentella sublilacina*, *Thelephora terrestris*, *Russula drimeia*, *Suillus bovinus* and *Paxillus involutus* while in site B were *Rhizopogon luteolus* and *Thelephora terrestris*. This is the first time that the diversity of the ectomycorrhizal community associated with *P. pinaster* is investigated using molecular techniques. We found six new genera (Tomentella, Pseudotomentella, Tomentellopsis, Tylospora, Phialophora and Meliniomyces) and 7 new species (*Tomentella sublilacina*, *Russula drimeia*, *Pseudotomentella tristis*, *Russula ochroleuca*, *Entoloma conferenda*, *Rhizopogon verii* and *Inocybe praetervisa*) that have not been previously described in symbiosis with *P. pinaster.*

45. Towards a standardised protocol for enzymatic profiling of excised ectomycorrhizas

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Enzymatic profiling is a method based on measurements of potential enzymatic activities of individual ectomycorrhizal tips (Courty et al. 2005; Pritsch et al. 2004). The enzymes hitherto addressed cover carbon, nitrogen and phosphate cycling enzymes that play a role in forest nutrient cycles. While applying the method in larger experiments and by different research groups in a broader scientific community, some disadvantages of the original method became apparent:

1. sieves for tip incubation had to be hand-made which is time consuming and hinders standardisation.
2. the original procedure comprised two series of enzyme assays requiring two identical sets of ectomycorrhizal tips which in addition of being time consuming is error prone since morphotyping very much depends on training and experience.

The new optimised protocol has overcome these two major restrictions by using purchasable equipment and by reducing the whole procedure to a single series of ectomycorrhizal tips. The optimised assay now saves time and chemicals, and more importantly is less error prone. The new protocol can be recommended as a standardised protocol for enzymatic profiling of excised ectomycorrhizas

Courty P-E, *et al*. 2005 *New Phytologist* **167**, 309–319.
Pritsch K, *et al*. 2004 *Journal of Microbiological Methods* **58**, 233–241.

46. Fungal diversity in decaying logs: proportion of ectomycorrhizal fungi revealed by DGGE fingerprinting and pyrosequencing

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Wood-decaying fungi have traditionally been explored through fruitbody inventories. Nowadays molecular techniques enable also investigation of cryptic species. In boreal forest soil, ectomycorrhizal fungi inhabit various habitats, including decayed wood. We were interested to see the proportion of ectomycorrhizal fungi in the fungal community inhabiting decaying wood. Two DNA approaches were used: DGGE (denaturing gradient gel electrophoresis) fingerprinting combined with the sequencing of ITS1 rDNA and 454-pyrosequencing. Also fruitbodies of polypores species on the logs were investigated. We drilled saw-dust samples from four Norway spruce logs in the field. Sampling was performed at intervals of one and/or three meters in each log. Single as well as bulked samples were subjected to DNA analyses in order to estimate the resolution capacity of DGGE fingerprinting and pyrosequencing. Only a few ectomycorrhizal species were found in the logs. Highest fungal species diversity was observed through pyrosequencing approach. Resolution capacity of DGGE was far more limited compared to the pyrosequencing. Fruitbody data and mycelial data overlapped only partly. Our preliminary results show that the proportion of ectomycorrhizal fungi in the fungal community inhabiting decaying wood is minor. Pyrosequencing seems a promising tool for analyzing the fungal community in environmental samples.

47. PhyloChips for monitoring soil fungal communities

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In forest soils, communities of fungi are highly diverse, but little is known on their composition or dynamics. DNA sequencing of rDNA ITS is now routinely used to identify mycorrhizal fungi and providing novel insights into the processes shaping ECM communities. With recent improvements in genotyping tools and dedicated DNA databases, high-throughput molecular diagnostic tools, such as 454 pyrosequencing and DNA microarrays, are developed for identification of ECM taxa. In this study, we have identified and monitored ~100 ECM species, belonging to 21 fungal genera, in the Breuil-Morvan forest using a ribosomal DNA ITS array (PhyloChip). This array has been validated by ectomycorrhiza morphotyping and ITS sequencing. We have also designed a Nimblegen ITS oligoarray for the comprehensive identification of fungal species. We targeted the variation in the rDNA ITS to capture the range of diversity present in GenBank fungal ITS sequences (i.e., 10,300 species). Using this custom PhyloChip, we detected and monitored soil fungal populations collected under different hardwood and conifer species. The effects of natural sequence diversity and potential cross-hybridization in complex environmental samples will be discussed. Such genotyping tools will enable an understanding of environmental and anthropogenic impacts on fungal communities.

48. Effect of water availability on functional diversity of ectomycorrhizal fungal communities in two contrasted ecosystems, a pine forest of Gascogne Landes and a Mediterranean oak forest

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In temperate ecosystems, the symbiotic association of soil fungi and tree roots result in the formation of a dual root-fungus organ, the ectomycorrhiza (ECM). This interface between plant roots and soil crucially affects plant mineral nutrition. Previous studies showed that ectomycorrhizal fungal species have variable abilities to hydrolyse a wide range of soil organic molecules. In this study we investigated in two contrasted forest ecosystems (i) the distribution of fungal functions among ECM species and (ii) using field experiments, its response to induced changes in water availability. A preliminary molecular analysis showed high ECM species richness in both ecosystems that were not significantly affected by either an increase (Oak) or a decrease (Pine) of water availability in the experimental plots. Other similarities between ECM communities in Oak and Pine forests included (i) two very high hydrolytic activities (phosphatase and chitinase) and (ii) five low activities (glucuronidase, xyloxidase, cellobiohydrolase and beta-glucosidase). Interestingly, phosphatase activity in the Oak forest was significantly higher in summer than in autumn and increased when water availability was experimentally reduced. Consistently with the previous results, phosphatase activity in the Pine forest decreased markedly in irrigated plots. These patterns, which were observed for the dominant ribotypes in the two ECM communities, are discussed in relation to our knowledge about phosphorus availability and mobility in forest soils.

49. Ectomycorrhizal fungal community associated to post-fire regenerated *Pinus pinaster* seedlings in Spain

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Recurring fire is a major disturbance in Mediterranean forest ecosystems. Mediterranean pines often show fire-adaptive traits like serotiny that allow to massive post-fire seed dispersal. However, the availability of ectomycorrhizal (EM) fungal inoculum in the soil is a main factor for subsequent seedling regeneration. In 2005, a major fire burned 15,000 ha of *P. pinaster* forest in Guadalajara-Spain. Our objective was to characterize the EM-fungal community associated to post-fire regenerated *P. pinaster* seedlings, and to check possible variations depending on fire intensity, site slope and season of sampling. Seedlings were highly mycorrhizal (>70%), and a total of 45 ITS-types (33% Ascomycetes; 60% Basidiomycetes) were described (almost all identified by sequencing). The community was dominated by Thelephoraceae, Russulaceae, and Pezizales. The relative frequency and abundance of fungi slightly varied with fire intensity, were more related to site slope, and significantly changed with season. The distribution of the EM fungi reflected different ecological preferences mostly conditioned by soil topology and seasonal changes.

50. Mycorrhizal plasticity in ectomycorrhizal fungi: the amazing shift from ectomycorrhizas to orchid mycorrhizas

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Most orchids associate with *Rhizoctonia*-like fungi that are also known as saprobic basidiomycetes. They colonize orchid roots and form intracellular hyphal pelotons when they proliferate in root cells; this is considered as the typical orchid mycorrhiza. Recently, investigations on some partly to fully heterotrophic orchid mycorrhizae revealed that they involve ectomycorrhizal fungi such as *Russula, Thelephora*, *Sebacina*, or even ascomycetes such as *Tuber*. These fungal species are known to be ectomycorrhizal on tree roots, and the same individual has been found both on orchid and on tree ectomycorrhizas surrounding the orchid. This demonstrates the ability of a fungal species, and even of an individual fungus, to form two different morphological types of mycorrhizas.

Three main differences can be underlined between orchid- and ecto-mycorrhizas formed by these fungi.

(i) The mycorrhizal morphology differs as the growth of the fungi occurs within or between the host cells, respectively. This raises many questions about the recognition process and suggests that the host plant critically contributes to the morphogenesis.

(ii) The physiology of the symbioses differs as the carbon flow is reversed in orchid mycorrhizae, raising questions on how carbon is transferred. Noteworthy, fungi are digested in orchid roots after some time, implying a higher turnover than in ectomycorrhizas.

(iii) The specificity differs, being often high in orchids, whereas ectomycorrhizas are often unspecific. Conversely, different fungi can be found on the same orchid root, whereas ectomycorrhizas are more exclusive.

We review ectomycorrhizal fungal species colonizing orchid roots, in temperate and tropical species, insisting on the symbiosis functioning and specificity in partly to fully heterotrophic orchids. For example, Russulaceae are associated to orchid roots all over the world, and illustrate the plasticity of association in a main ectomycorrhizal family.

51. *emerencia* – a web service for sequence based exploration of fungi

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emerencia (http://andromeda.botany.gu.se/emerencia.html) is a web service that automatically downloads all fungal ITS sequences from GenBank and then sorts out those that are not annotated with a species epithet (insufficiently identified sequence(s) – IIS) from those that have a full species name (fully identified sequence(s) – FIS). The IIS are then searched for their most similar counterpart among the FIS using BLAST, and the results are stored in a MySQL database. This information opens up new possibilities to explore the fungal ITS sequences. For example it is possible to search what sequences are associated with a particular study and what IIS are associated with any particular FIS. A new search function to search for IIS associated with a particular genus has also just been released. As the IIS can be examined in new contexts through these searches, it is also possible to put their metadata in new meaningful frameworks. In comparison with the FIS, the IIS are much more frequently annotated with geographic and ecological data and therefore hold great potential to contribute information in these regards. This presentation shows how such data, instead of just being left beckoning for analysis, can be used to synthesize information valuable for the advancement of mycology.

52. Ectomycorrhizal fungal diversity: targeting root tips or extra-radical mycelium? A case study

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Molecular techniques applied to ectomycorrhizal (EM) fungal communities have shown poor overlap in species richness and evenness recorded from the analyses of sporocarps and root tips. Here, we have investigated and contrasted the EM fungal diversity recorded from sampling root tips and cloning the extra-radical mycelium. We generated 2297 fungal internal transcribed spacer sequences from 1150 EM root tips and 1147 bacterial clones containing fungal DNA, obtained from the organic and mineral soil horizons within the rhizosphere of a poplar plantation. Considering basidiomycetes identified as EM fungal operational taxonomic units (OTUs), 40 EM OTUs were identified from the 1720 EM basidiomycetes sequences retrieved. The root tip analysis provided 28 EM OTUs whereas soil cloning found 22 EM OTUs. The most common EM fungal OTUs recovered overlapped between both data sets, whereas rare OTUs remained specific to one data set. EM communities recovered from sampling root tips and cloning the extra-radical mycelium differed significantly from each other, while rarefaction curves were fully saturated. Targeting EM root tips and extra-radical mycelium by direct sequencing and soil-cloning were complementary approaches as the combination of the two data sets provided an increase of 22.7% in EM fungal diversity.

53. Plant genetics alter ectomycorrhizal community composition, which feeds back to influence plant performance

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Understanding the genetic components of community structure and how community feedbacks may affect the performance of foundation species that can 'drive' whole ecosystems are key issues in ecology and evolution. Genetic influences on below-ground microbial communities may be particularly important because of their large impacts on a wide variety of ecosystem processes. Because of their ability to influence plant growth and productivity ectomycorrhizal fungi (EMF) represent a model community to examine possible feedbacks between community and plant phenotypes. We used field and greenhouse experiments to observe the EMF communities of pinyon pine (*Pinus edulis*) that show a genetic resistance or susceptibility to chronic herbivory. We found that *P. edulis* EMF communities were more strongly influenced by variation in plant traits associated with resistance and susceptibility to herbivory than by herbivory itself; indicating the potential importance of plant genetics in determining EMF communities. This importance of genetics was further supported by the finding that seedlings grown from different genotypes supported different EMF communities, and that total seedling biomass was strongly correlated with EMF community composition. Our findings demonstrate potential feedbacks between plant and EMF community phenotypes that could explain important links between plant genes and below-ground microbes involved in ecosystem functioning.

54. Ectomycorrhizal fungal symbionts of *Pisonia grandis* and mycorrhizal status of *Pisonia sechellarum* (Nyctaginaceae)

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Nyctaginaceae is considered to be a nonmycorrhizal family, with a few species known to form ectomycorrhiza (ECM). *Pisonia grandis* forms distinct type of ECM with certain fungal species that belong to the Thelephoraceae. There is no information on the mycorrhizal status of *Pisonia sechellarum*. Roots of *P. grandis* and *P. sechellarum* were collected from small islands of Cousin and Silhouette, respectively. Fungal species were indentified from the roots by anatomotyping and rDNA sequencing. In addition, fungal fruit-bodies were collected from the sampling area and sequenced. Three distantly related ECM fungal species belonging to Thelephoraceae were identified from roots of *P. grandis.* In addition, fruit-bodies of two new *Tomentella* species were found from Cousin and described as new species. Comparison of sequences from ECM and fruit-bodies showed that species that were found as fruit-bodies also formed ECM on the roots of *P. grandis*. None of these symbionts were previously detected from different ecosystems in the neighbouring islands of Praslin and Mahé. In contrast to *P. grandis, P. sechellarum* was detected as an arbuscular mycorrhizal (AM) plant. In addition to abundant root endophytes, three AM fungal species were revealed from the roots. There was no evidence for ECM formation in *P. sechellarum.*

55. Molecular identification of mycorrhizal fungi of dipterocarp seedlings in Indonesian rubber agroforests

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Little is known about ectomycorrhiza (EcM) communities in disturbed ecosystems in the tropics after, for example, change from natural forests to other types of land use such as rubber agroforests (RAF). In the presence study, mycorrhizal colonization of Dipterocarpaceae from two different experiments were assessed. In the first experiment, the EcM inoculum potential in the soil from seven land use types was studied, based on colonization of nursery-grown plants of *Shorea lamellata* and *Shorea selanica*. In the second, root tips of same plant species for analysis were harvested in-situ in RAF with different land use histories. Sequencing of ribosomal DNA (rDNA) was applied to identify EcM fungi on root tips. The first experiment yielded three genera of EcM fungi: *Tomentella, Laccaria* and an unidentified member of the Sclerodermataceae. In addition, an endophytic *Curvularia* was obtained. The field experiment yielded five basidiomycetous genera of EcM fungi: *Tomentella, Pisolithus, Clavulina, Sebacina* and *Sistotrema*. The EcM fungal community in dipterocarp seedlings comprises numerous species. EcM propagules persists in the soil after forests change RAF. Isolation from mycorrhizal roots in experiment 1 only yielded Ascomycetous fungi, probably mostly endophytic species, and there was no overlap with the results of direct sequencing.

56. Ectomycorrhizal fungal ecology in Australian Nothofagus forests: host specificity, seedling establishment and associated microfungi

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Ectomycorrhizal (EcM) symbiosis is a widespread plant nutrition strategy in Australia, especially in semiarid regions. This study focused on ecosystems including *Nothofagus cunninghamii* as a (sub)dominant component. We addressed the diversity and community structure of EcM fungi (EcMF), their host specificity and role in seedling establishment in three primary forests in Tasmania and Victoria states. EcMF and associated microfungi 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.

Both Tasmanian sites hosted a highly diverse community of EcMF dominated by the cortinarius, tomentella-thelephora, clavulina, russula-lactarius and laccaria lineages. Individual species had a strong host preference, a likely factor contributing to the high richness. Decaying wood was an important substrate for *Nothofagus* regeneration that was probably facilitated by EcM symbiosis. EcMF were species-poor in decayed wood, comprising a few dominant species of several fungal lineages that were taxonomically unrelated to taxa in boreal ecosystems. EcM-associated ascomycetous microfungi were highly diverse, but did not exhibit any preference for EcMF or plant hosts. These fungi, particularly from Helotiales and Sordariales comprised common soil fungi, root endophytes, ericoid mycorrhizal fungi and mycopathogens.

57. The effects of litter quality and quantity on the interaction between ectomycorrhizal fungi and native tree seedlings, modulated by the soil environment

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Soluble litter extracts have been shown to modify mycorrhizal fungal activity. Here we specifically investigate what the role of leaf litter quality and quantity have on naturally growing Pedunculate Oak seedlings growth, survival and ectomycorrhizal development and how these factors affect or are affected by soil properties. To study the effects of litter quality on native plants we used the litter of the most problematic invader tree species in Central Europe, the Locust Tree (*Robinia pseudoacacia* L.). We applied the following treatments: (i) complete litter withdraw, (ii) the litter quantity doubled, (iii) original litter layer replaced by Locust Tree (*Robinia pseudoacacia* L.) litter (iv) litter layer disturbed (as control for disturbance) and (v) control. Twelve seedlings were randomly chosen (600), measured and marked from each plot (50). At the end of the experiments survived seedlings were dig out and measured the followings: length of stem, length of roots, number and size of leaves, mycorrhizal level on the root system (on 200 root tips per seedlings), and the proportion of mycorrhizal morphotypes. During the study soil samples were taken three times from each plots.

We have showed that leaf litters quality and quantity have significant effect on the ECM community. The mycorrhizal level of the seedlings root tips was significantly lower in the locust tree litter plots, but increased in the litter removed plots, compared to the control. Mycorrhizal level tended to decrease in the doubled litter plots. Our results clearly show that the litter of the locust trees can disturb the delicate relation between the native oak seedlings and their mycorrhizas and therefore might suppresses the growth of native trees. This can enhance the spread of this aggressively invasive species in ecosystem consisted of native species.

58. Below-ground monitoring of stipitate hydnoid fungi

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Sporocarps of stipitate hydnoids often grow at specific sites characterised by exposed mineral soil free from organic material, on sandy, gravely or shingle substrates. At these sites sporocarps of several species co-occur, and irregularly re-occur over a number of years. At some sites sporocarp production has ceased in recent years. We hypothesised that the gregarious fruiting of stipitate hydnoid fungi is regulated by site conditions, and/or the below-ground structure of the stipitate hydnoid community and/or interactions with other ECM fungi. In addition, we tested the hypothesis that mycelium of stipitate hydnoid fungi persists below-ground at sites where sporocarps have been previously, but are no longer, recorded.

The below-ground composition of the stipitate hydnoid community, and the total ECM fungal community, was studied around two fruiting sites using species-specific probes and T-RFLP profiling. Species-specific probes were also used to check for the persistence of DNA and RNA of four species at former fruiting sites.

There was no evidence that below-ground interactions among hydnoids, or between hydnoids and other ECM fungi regulated hydnoid mycelial community composition. It is more likely that edaphic factors determine fruiting sites. The DNA and RNA of target species was detected at sites up to 4 years after the last recorded fruiting, suggesting that metabolically active mycelium persists between fruiting events.

59. Multi-scale structure of populations of the ectomycorrhizal basidiomycete *Laccaria amethystina* in Europe

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Laccaria amethystina is an ectomycorrhizal basidiomycete, unspecifically associated with tree species in temperate forests of Eurasia, coniferous or deciduous. The population genetics of this model species has been investigated at local scales in Europe (Fiore-Donno and Martin, 2001; Roy *et al.*, 2008) and Japan (Wadud *et al.*, 2006), showing a high local genetic diversity, but no differentiation between populations at the French country scale (500 km). We thus studied the structure of *L. amethystina* populations at the European continental scale. First analyses, based on microsatellite markers developed thanks to *L. bicolor*'s genome availability (Martin & Selosse, 2008), confirmed a strong local structure of the populations, but revealed a panmictic European population, with little differentiation from Spain to Poland. A multi-locus phylogeographic approach of this species in Eurasia, using coding loci, shows unexpectedly reduced genetic variation among and between European populations. Interestingly, we observed a strong divergence with Japanese samples that could be an evidence for a speciation at Eurasian level, instead of only one *L. amethystina* species.

60. Quantifying biomass and respiration of ectomycorrhizal fungal mycelium in pure culture – Does diversity matter?

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The extramatrical mycelium of mycorrhizal fungi is an important sink of host carbon and makes a substantial contribution to soil respiration. In nature, ectomycorrhizal fungi often have different functional traits and exist in complex multi-species assemblages. This raises the possibility that ectomycorrhizal diversity may have an important role in forest ecosystem functioning. We investigated the relationship between species diversity and substrate quality on biomass production by ectomycorrhizal fungi in pure culture. A diversity gradient was created ranging from 1 to 8 species, with all species being represented in monoculture. Three substrates with C:N ratios of 10:1, 20:1 and 40:1 (where C was held constant) were used in order to determine effects of substrate quality and temporal niche complementarity. Biomass was found to increase with increasing diversity, although in the multiple combinations one species tended to produce greater biomass consistently, and usually this was either *Paxillus involutus* or *Suillus bovinus*. Overall biomass decreased as the C:N ratio increased, although monoculture species varied in biomass between different C:N ratios. Further measurements are underway to quantify CO_2 efflux from the communities. We conclude that although biomass did increase in the treatments with greater species richness, this may be due to the presence of certain high biomass-producing species.

61. Symbionts of *Epipactis helleborine* from heavy metal rich and nonpolluted areas in Poland – molecular studies

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Epipactis helleborine is an orchid easily surviving in heavy metal polluted areas such as post-flotation wastes. According to previous research (Jurkiewicz et al. 2001, *Protoplasma* 218:117–124) mycorrhizal fungi play an important role in detoxification of heavy metals and act as filters within the roots. During the present study we aimed at identifying those symbionts. Orchid roots were collected from Zn-Pb wastes in southern Poland and molecular studies have been carried out. PCR products of fungal ITS region were sequenced and symbionts were assigned to three genera: *Helvella*, *Tuber* and *Wilcoxina*. All of those fungi belong to the same order *Pezizales* and are also known to form ectomycorrhizas with trees shearing the habitats with orchid. *Pinus silvestris* and *Betula verrucosa* could be the trees to share the symbionts with orchids on the wastes and preliminary studies of their ectomycorrhizas showed that this is the case. Interestingly, the identity of *E. helleborine* symbionts from industrial wastes did not differ from natural habitats that were also studied.

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