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Morphological and Functional Diversity of Ectomycorrhizal Fungi on Roan Mountain (NC/TN)

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Abstract - A comparison of ectomycorrhizal morphotypes and hypogeous fungi (truffles and false-truffles) in northern hardwood and spruce-fir forests on Roan Mountain (NC/TN) was performed to increase our knowledge of the fungal communities in the Southern Appalachian high elevation forests. These forests are home to an endangered subspecies and mycophagist, the Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*), as well as the popular Christmas tree species and ectomycorrhizal host, Fraser fir (*Abies fraseri*). Ectomycorrhizal root tips were collected with soil cores and separated into morphotypes for quantification. Fruiting bodies of hypogeous fungi were sampled with a stratified random design, using exclosures to minimize mycophagy. *Elaphomyces muricatus* was the most commonly found hypogeous fruiting body, and *Cenococcum geophilum* the most commonly found ectomycorrhiza, in both forest types. *Elaphomyces muricatus* was most strongly associated with *A. fraseri*. There were more ectomycorrhizal morphotypes in the spruce-fir forest than in the northern hardwood forest. Functional groups of ectomycorrhizas were classified by exploration type. Historical land use on Roan Mountain is discussed in conjunction with the patterns found in this study, along with future concerns of the fragile Southern Appalachian spruce-fir ecosystem.

Introduction

High elevation spruce-fir forests in the Southern Appalachian Mountains have been classified as one of the most endangered ecosystems of North America (Noss and Peters 1995). The dominant organisms are red spruce (*Picea rubens* Sang.) and Fraser fir (*Abies fraseri* (Pursh) Poir.). Northern hardwood forests neighbor the spruce-fir forests, and are dominated by American beech (*Fagus grandifolia* Enrh.), yellow birch (*Betula alleghaniensis* Britt.), and maples (*Acer rubrum* L., *A. saccharum* L., and *A. pensylvanicum* L.). Red spruce, Fraser fir, American beech, and yellow birch form mutualistic associations with fungi on their roots called ectomycorrhizas. Ectomycorrhizas benefit the plant host with increased access to phosphorous, nitrogen, and water, whereas the fungal partner receives a ready source of carbon

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(Smith and Read 1997). The diversity of ectomycorrhizal (EM) fungi can be very high with just one plant species, such as Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) in the northwestern United States (Trappe 1977). However the diversity of EM fungi in high elevation spruce-fir and northern hardwood forests of the Southern Appalachians is still relatively unknown.

Bills et al. (1986) compared basidiomycete fruiting bodies (sporocarps) in red spruce and northern hardwood forests of West Virginia. However, within the past ten years, mycorrhizologists have determined that fruiting patterns differ among EM fungi, with some fruiting rarely, inconspicuously, or never (Gardes and Bruns 1996, and others . Gibson (1979) and Meier (1989) examined EM roots of Fraser fir and red spruce, respectively, but their emphasis was on total EM colonization and greenhouse experiments, rather than diversity of EM fungi in a natural ecosystem.

Loeb et al. (2000) examined the diversity of one group of EM fungi which form their sporocarps underground, hypogeous fungi (truffles and false-truffles). Hypogeous fungi have been documented in EM relationships and as food sources for some small mammals, such as the Carolina northern flying squirrel (CNFS) (*Glaucomys sabrinus coloratus* Handley), an endangered subspecies that has been found to consume at least six hypogeous genera (Weigl et al. 1999). Each of these studies contributed to the current picture of the EM fungal diversity present in the spruce-fir and northern hardwood forests of the Southern Appalachian Mountains.

To add another layer to this picture, we compared morphological types of EM root tips in spruce-fir and northern hardwood forests on Roan Mountain, a summit located on the border of North Carolina and Tennessee. This provides a measure of diversity that is not obtainable by sporocarp sampling alone. We also sampled for hypogeous sporocarps in the two forests to increase knowledge of hypogeous fungi, both as EM partners and as a food source for the CNFS.

Materials and Methods

Study site

Roan Mountain is in the Southern Appalachian Mountains located on the border of North Carolina and Tennessee (36°12'N, 82°04'W), with forests in the Pisgah and Cherokee National Forests. The peak, at 1915 m, has many plant species that are more commonly found in more northern latitudes. These plants have disjunct populations at higher elevations of the Southern Appalachians (Brown 1941). Heath balds and spruce-fir forests dominate the plant community above 1700 m. Northern hardwood species, such as American beech, yellow

birch, and maples dominate at lower elevations. Soils on Roan Mountain are a gray-brown podsol (Brown 1941), with a parent material of granite and gneiss (USDA Soil Conservation Service 1953). Cooler temperatures, increased precipitation, and higher wind speed than the surrounding lower elevations characterize the climate of Roan Mountain. (Brown 1941).

Ectomycorrhizal root tips and hypogeous fungal fruiting bodies were sampled using stratified random sampling along elevation transects. We chose transects running along the slopes of the mountain according to telemetry data of CNFS (Weigl et al. 1999). Three transects were established in spruce-fir forests (SF) at approximately 1700 m, and three in northern hardwood forests (NF) at approximately 1550 m. Each transect was 100 m long. Five 1-m^2 plots in each transect were established using random numbers to generate one plot within each 20-meter segment.

EM root tip sampling

Eight-cm diameter soil cores were taken to a depth of 20 cm from the perimeter of plots established for sporocarp sampling. Five cores were taken from each forest type in November 1999 and February 2001. Each core was rinsed through a series of sieves (5.15 mm, 1.52 mm, and 0.5 mm) and the uppermost layer was examined for EM root tips. The lower layer (0.5 mm) was used for sclerotia sampling (see below). Ectomycorrhizal root tips were sorted using morphological characteristics described by Agerer (1987–1993). Ectomycorrhizas were quantified by recording the number of EM root tips per morphotype per core. In cases of thin or non-apparent mantles, sections were made to determine presence of a Hartig net at 1000X magnification.

Functional diversity was measured by classifying EM morphotypes into the five different exploration types given by Agerer (2001). The five exploration types used were: contact (no emanating hyphae or rhizomorphs), short distance (lots of emanating hyphae but no rhizomorphs), medium distance (sometimes emanating hyphae with rhizomorphs), long-distance (differentiated rhizomorphs up to decimeters long), and pick-a-back (this type "piggy-backs" on rhizomorphs of long distance exploration types) (Agerer 2001). The three subgroups under the medium distance exploration type given in Agerer (2001) (fringe type, mat type, and smooth type) were grouped together for statistical comparison.

Sporocarp sampling

In order to prevent consumption of sporocarps by mammals, plots were covered with aluminum window screening (North and Trappe 1994). Plots were covered for approximately three to four months.

Sampling occurred during the months of April through July 2000 and March through May 2001. Plots were excavated to a depth of 15 cm. A sieve (#4: 5.15 mm mesh size; Newark Wire Cloth Co., NJ) was used in the field to sort through clumped soil. Sporocarps were placed in waxed paper and labeled with date and location. Specimens were photographed and identified using Castellano et al. (1989) and Zhang and Mintner (1989). Sporocarps were then dried at 40 °C for at least 48 hours, and total biomass of sporocarps per plot was recorded. Specimens were deposited in the Appalachian State University Herbarium (BOON).

Sclerotia sampling

Sclerotia are masses of hyphae, often in a firm ball, that act as resting or overwintering structures. During the February 2001 EM root tip collection, each soil core was subsampled for *Cenococcum* sclerotia using methods developed by Trappe (1969). Soil was collected from the 0.5-mm soil sieve, placed in a resealable plastic bag, and four subsamples of 15 mL were randomly taken from each sample. Numbers of sclerotia were recorded for all soil cores from the 2001 sampling.

Data analysis

Ectomycorrhizal root tips were counted for each morphotype in each soil core. Effect of year or core location on total EM root tip abundance was analyzed using the PROC GLM procedure in SAS. Shannon-Weiner diversity indices were calculated for each soil core and averaged for each forest type (Krebs 1999). For morphotypes that appeared in more than one soil core, root tip abundance data were transformed using log(x + 1). A multivariate analysis of variance (MANOVA) was performed to determine any effects of location, year, or location*year on EM morphotype community composition. The exploration types were also used to compare the two forest types, using the two-way factorial MANOVA.

Associations between sporocarp and occurrence of tree species in plots were determined using the following association formula based on presence/absence data:

$$\text{Association} = \sqrt{\frac{(ad - bc)}{(a + b)(c + d)(a + c)(b + d)}}$$

Where a = number of plots with both species present, b = number of plots with species one present, c = number of plots with species two present, and d = number of plots with neither species present (Krebs 1999).

Univariate and multivariate analyses were performed using SAS Software, version 8 (SAS Institute, Cary, NC). A one way analysis of variance (ANOVA) tested year and plot location effects on sporocarp biomass and sporocarp number. A one-way ANOVA tested effect of location on number of sclerotia per soil core.

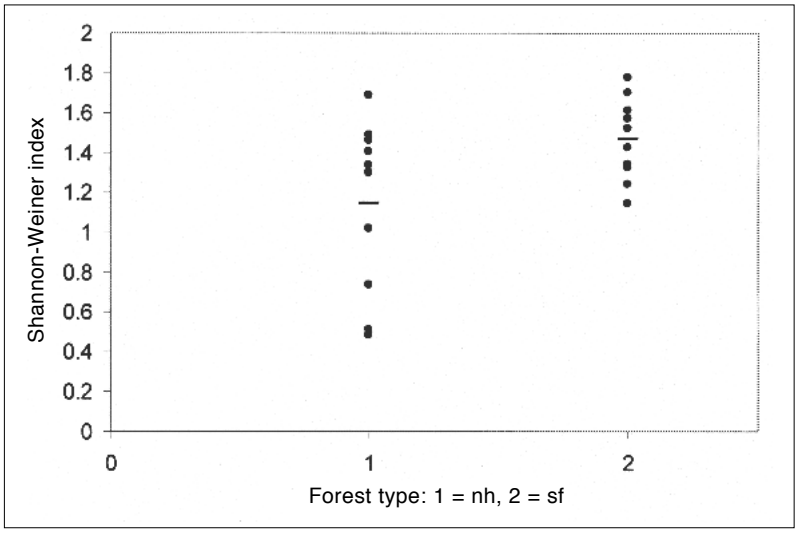

Figure 1. Shannon-Weiner diversity indices for each soil core in the two forest types (spruce-fir[sf] and northern hardwood [nh]). Horizontal lines indicate the mean for each forest type.

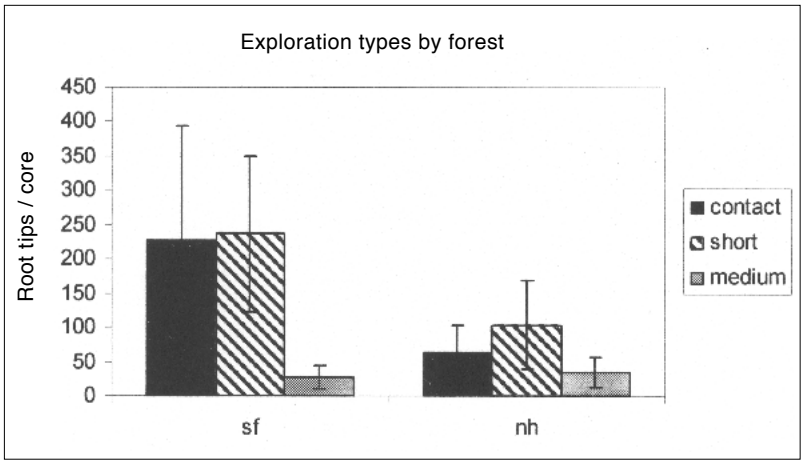

Figure 2. Functional diversity of EM in spruce-fir (sf) and northern hardwood (nh) forests. Contact, short, and medium describe exploration types as given by Agerer (2001). Columns represent average of 10 cores per forest, error bars represent +/- one standard deviation.

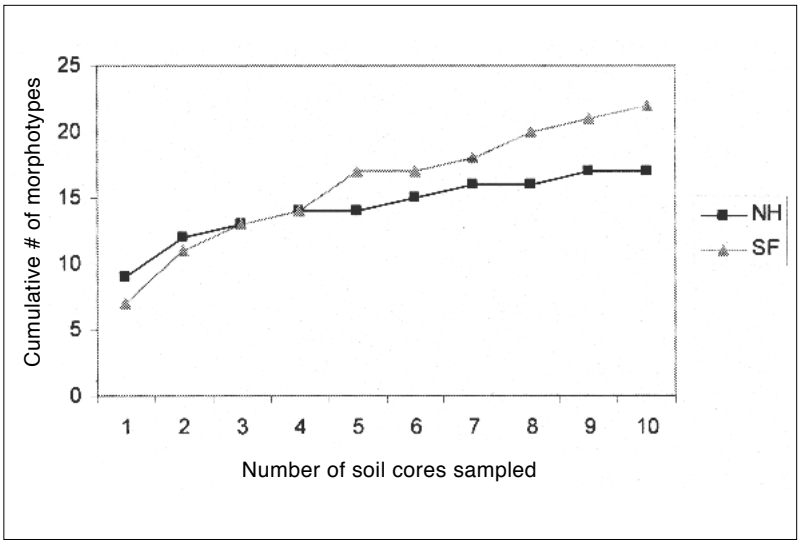

Figure 3. Morphotype – effort curve. This curve represents the cumulative morphotypes found in each forest type as sample size increased.

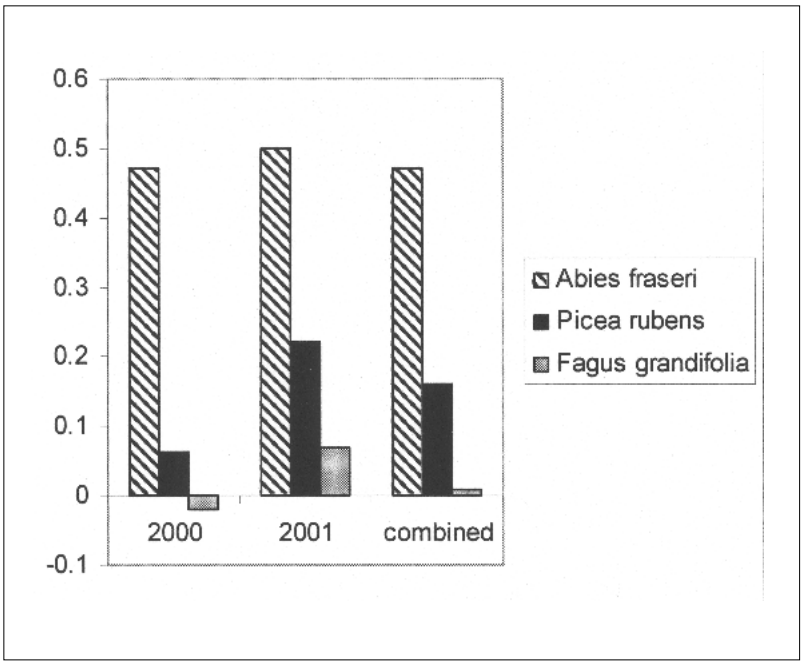

Figure 4. Association values for dominant tree species and *E. muricatus*. Values between -0.1 and 0.1 represent neutral associations, values outside of this range represent positive or negative associations.

Results

The EM community

A total of 27 EM morphotypes were described, with 5 unique EM morphotypes found in northern hardwood forests, 10 unique morphotypes found in spruce fir forests, and 12 found in both forest types. Low species evenness was found, with one to two morphotypes comprising most of the root tips and the other morphotypes appearing rarely. The average Shannon-Weiner diversity index for northern hardwood samples was 1.14 (s.d. = 0.41) and for spruce-fir samples it was 1.47 (s.d. = 0.19) (Fig. 1). There was no significant difference between forest type or year sampled for total EM root tips. Forest type explained a significant amount of variance in the abundance of morphotypes found in more than one core (Wilk's Lambda df = 8, F = 7.9, P = 0.0019). Year sampled had no effect.

Based on exploration types, there was no significant difference by year or by forest type (Fig. 2). New morphotypes versus number of soil cores examined revealed unique morphotypes were still being found in new spruce-fir soil cores, whereas additional northern hardwood soil cores appeared to be reaching an asymptote after 6 soil cores (Fig. 3).

No morphotype's genetic pattern (restriction fragment length polymorphism or RFLP, determined using methods from Gardes and Bruns (1993) matched *Elaphomyces granulatus* Fr. or *E. muricatus* Fr., *Alpova* sp., or *Scleroderma* sp. genetic patterns (C.E. Bird ,unpubl. data). Two morphotypes were similar to the hypogeous genera *Tuber* and *Rhizopogon*. The *Tuber*-like root tips had cystidia visible (although similar cystidia are found in *Russula* ectomycorrhizas) giving the root tip a spiny appearance. The *Rhizopogon*-like root tip grew in a tight coralloid pattern, without the tough covering typical of many *Rhizopogon* ectomycorrhizas. These tips were not a dominant component of the soil cores examined.

The most common and abundant morphotype in both forests is identical to the morphotype produced by *Cenococcum geophilum* Fr.. This distinctive EM fungus was identified by its shiny black mantle and copious amounts of emanating black hyphae (Agerer and Gronbach 1988). Microscopic verification was made by confirming the star-like arrangement of hyphae in the mantle.

Sporocarp diversity

Two species of hypogeous fungi, *Elaphomyces granulatus* and *E. muricatus*, were found fruiting in red spruce/Fraser fir (SF) plots. *Elaphomyces muricatus* was the only species found in northern hardwood (NH) plots. Between April and June of 2000, three SF plots and two NH plots had *E. muricatus* sporocarps. Between March and May

of 2001, three of 15 SF plots and three of 15 NH plots had *E. muricatus* sporocarps. There was no significant effect of sampling year or forest type on sporocarp biomass or numbers of sporocarps.

Elaphomyces muricatus fruiting was most strongly associated with *A. fraseri*, slightly positive to neutrally associated with *P. rubens*, and neutrally associated with *F. grandifolia* (Fig. 4). An absolute value of 1.0 indicates a strong association, with the sign indicating positive or negative associations. Absolute values less that 0.1 indicate a neutral association.

All sclerotia found in soil samples were consistent with those produced by *Cenococcum* species. *Cenococcum* sclerotia were more abundant in samples from spruce-fir forests than northern hardwood forests ($df = 38$, $F = 7.10$, $p = 0.01$). Mean sclerotia abundance was 1.27 per mL soil in northern hardwood and 1.93 per mL soil in spruce-fir.

Discussion

This study found more similarity between coniferous forest- and mixed hardwood forest-EM morphotypes than previous studies that had compared EM fungi based on sporocarp abundance (Bills et al. 1986). Also, the trend towards more EM morphotypes in spruce-fir forests contrasts with higher diversity of epigeous (above-ground fruiting) basidiomycete species found in the hardwood plots of the Bills et al. (1986) study. The diversity estimates in this study are conservative, as morphotyping alone allows for lumping of taxa together. The differences and similarities between the two forests may become more apparent when morphotypes can be further identified using DNA sequencing.

Based on morphotype data, the spruce-fir ecosystem has more EM morphotypes than the northern hardwood. However, some of the symbiotic fungi in the spruce-fir forests are present in adjacent northern hardwood forests, suggesting there may be some buffering between these two forest types. This has important implications for restoration of red spruce and Fraser fir. Increased threats to these forests need to be monitored, and the specificity of fungi in these two forests needs to be further assessed.

Morphological classification revealed a greater diversity of EM fungi in the spruce-fir than northern hardwood forests. However, there was no difference between forest types when functional diversity of ectomycorrhizas was compared. Although there are some different species in the two forests, it appears that most of the fungi operate through direct contact with the substrate or over very short distances (< 2 mm) (Agerer 2001). This suggests that even though the tree hosts and litter composition are very different in these two forest types, the EM fungi

form similar structures to fulfill their role in the ecosystem. Also, none of the morphotypes found in this study formed the longer range exploration types of hyphae or the parasitizing type such as *Gomphidius* (Agerer 2001). The reasons for the lack of these exploration types of EM fungi are unknown.

In this study, the most frequent and abundant EM fungus at the root tip level was *Cenococcum geophilum*, a common ectomycorrhizal fungus. No sexual stage (fruiting body) of this fungus has ever been observed; presence of *C. geophilum* can only be observed through soil core samples. *Cenococcum* forms a tough mantle that is resistant to decomposition; hence more tips may have been recorded than were actually alive (T. Horton, Syracuse, NY, pers. comm.). Further examinations should be certain the root tip is turgid, indicating it is an active root tip.

This study found the same hypogeous genus as Loeb et al. (2000) on Roan Mountain (NC/TN) although a different species was more common (*E. muricatus*). The strongest association value was with Fraser fir, suggesting there may be some positive interaction between Fraser fir and *E. muricatus*. Trees which have been found in proximity to *E. muricatus* include *Abies amabilis* Forbes, *Alnus* spp. , *Betula* spp., *Castanea sativa* Mill., *Fagus sylvatica* L., *Juniperus communis* L, *Picea abies* Kurst., and *Pinus sylvestris* L (Trappe 1971). Loeb et al. (2000) found importance values of red spruce correlated with presence of *E. granulatus*. The specificity of these two *Elaphomyces* species needs to be further examined.

The abundance of *Elaphomyces* sporocarps found in this study may be due to the fact that *Elaphomyces* species form decay resistant outer layers, as compared with species which were not found in this study, such as *Rhizopogon* and *Tuber* species. More ephemeral hypogeous species may be encountered with more intensive repeated sampling (every two weeks for example).

The occurrence of *Elaphomyces muricatus* in both forest types suggests that this is a food source for the CNFS available both within and outside of the spruce-fir forest. Weigl et al. (1999) found that 40% of CNFS fecal samples contained *Elaphomyces* spores, and others have found *Elaphomyces* spores in squirrel feces, especially in the spring when other hypogeous genera are less abundant (Mitchell 2001). North et al. (1997) found that *E. granulatus* and *E. muricatus* had much lower palatability than *Truncocolumella citrina* or *Rhizopogon subcaerulescens*. Cork and Kenagy (1989) found that golden-mantled squirrels (another mycophagist) did not obtain sufficient nutrition to grow and reproduce from a diet of *Elaphomyces granulatus* alone. Both *Elaphomyces* species are, however, very common and persistent in the soil, which may account for their use as a food source by the squirrels.

The low diversity of hypogeous fungi found in both forests may be explained by past disturbance, from both humans and insects. Byrd et al. (2000) found that EM species composition changed when *Pinus contorta* stands were clear-cut. Roan Mountain was intensely logged during the late-1920s through mid-1930s, removing all spruce and fir trees larger than six inches in diameter (Brown 1941). Hardwoods were logged less intensely, with only larger trees removed (Brown 1941). Balsam woolly adelgid infestations on Roan Mountain were detected in 1962, followed by a large wave of mortality; some recovery has occurred since that time (Smith and Nicholas 1999). This disturbance history may explain why two separate studies on Roan Mountain have failed to find a large diversity of hypogeous fungi. Many hypogeous species, such as some *Rhizopogon* sp., are host-specific (Molina et al. 1992). EM fungi specific to red spruce or Fraser fir may have become less abundant, whereas more generalist species such as *Elaphomyces* spp. and *C. geophilum* have become more abundant. In order to test this hypothesis, spruce-fir and northern hardwood forests that were not logged in the earlier part of this century should be surveyed and their hypogeous and EM communities compared.

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