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# Ectomycorrhizal sporophore distributions in a southeastern Appalachian mixed hardwood/conifer forest with thickets of *Rhododendron maximum*

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***Abstract:*** Sporophore abundance of putatively ectomycorrhizal fungi was compared in a mature mixed hardwood/conifer forest inside of (+) versus outside of (−) *Rhododendron maximum* thickets (RmT). Experimental blocks (1/4 ha) were established inside of (3) and outside of (3) RmT at the Coweeta Hydrologic Laboratory in Macon County, North Carolina, USA. Litter and organic layer substrates were removed, composited and redistributed among 90 2 × 2m plots within the blocks. Plots received either +RmT or −RmT litter, and either +RmT or −RmT organic layers, or were unmanipulated for controls. Sporophores of 67 putatively ectomycorrhizal species were collected from the blocks. Species diversity and overall community structure were similar inside of and outside of RmTs, and no grouping was detected by substrate type. Differences within the ectomycorrhizal fungus community were associated only weakly with environmental parameters, as indicated by ordination. In light of these results, recent observations of ectomycorrhizal suppression and strong shifts in the proportions of morphotypes on tree seedlings inside of RmT do not appear to be related to differences in sporophore distributions. The changes in seedling mycobiont dominance in relation to RmT and the influence this has on seedling health should be examined directly from root tips.

***Key Words:*** ericoid mycorrhiza, fungal community, indicator species, ordination

## INTRODUCTION

The important implications of spreading *Rhododendron maximum* L. thickets (RmT) in the southern Appalachian Mountains are well documented (see Walker et al 1999). Particularly, interest in this topic stems from severe suppression of tree seedling regeneration inside of RmT (e.g., Minkler 1941, Wahlenberg 1950, Phillips and Murdy 1985, Clinton et al 1994). A previous study found that total ectomycorrhizal colonization of hemlock (*Tsuga canadensis* L.) seedlings was reduced inside of RmT, and that this depression of mycorrhization was correlated with decreased productivity of the seedlings (Walker et al 1999). Furthermore, there was a shift in the proportion of ectomycorrhizal morphotypes toward *Cenococcum geophilum* Fr., a generalized, unspecialized, disturbance tolerant ectomycobiont in RmTs. These observations beg the following question: Is the ectomycorrhizal fungus community negatively affected by the presence of RmT?

The hypotheses addressed in this study were: (i) Is the assemblage of ectomycorrhizal fungi different when examined inside of versus outside of RmT? (ii) Are certain ectomycorrhizal fungus taxa indicators with regard to RmT? (iii) Are there differences in the ability to re-colonizing manipulated soil substrates from inside of versus outside of RmT among ectomycorrhizal fungus species? and (iv) Is there a strong relationship between sporophore distributions and abiotic environmental parameters?

*Sporophore sampling and ectomycorrhizal community analyses.*—Numerous studies have successfully used sporophore abundance (and or biomass) to assess ectomycorrhizal fungus community composition (e.g., Miller 1982a, Bills et al 1986, Villeneuve et al 1989, Nantel and Neumann 1992, Palmer et al 1994). The results produced by these studies appear to correspond well with plausible explanations. For example, Nantel and Neumann (1992) found that the strongest niche dimension of ectomycorrhizal communities was stand composition, but within the range of a stand, fungal assemblages differed in relation to edaphic characteristics. However, we know of no previous studies comparing sporophore assemblages in relation to an ericoid subcanopy shrub in the southeastern Appalachian Mountains.

The relationship between sporophore abundance (or biomass) and the amount of mycelium below ground is dependent upon species-specific differences in life history characteristics. Furthermore, sporophore production in basidiomycetes can be influenced strongly by environmental factors such as light (Miller 1967). However, an allometric relationship between sporophore and mycelial biomass does ap-

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pear to hold for some species (Laiho 1970, Newell 1984, Cotter and Bills 1985) and for even a complete community (Menge and Grand 1978). When a sporophore is produced, we know that part of the individual mycelial unit producing the sporophore is definitely present at that location. Sampling sporophore distributions therefore provides a valuable resource when evaluating ectomycorrhizal fungus communities.

It is known that sporophore abundance of a species is not tightly correlated with the abundance of mycorrhizae in a given area (Gardes and Bruns 1996, Dahlberg et al 1997). However, identifying ectomycorrhizae from root cores, like sampling sporophores, provides only a point estimate of the presence of an individual unit of mycelium below ground. In addition, the process of mycorrhization, like sporulation, is influenced by environmental and biotic factors. Finally, root colonization can be variable seasonally. For these reasons, mycorrhizae present in the root zone should still be compared with sporophore samples for the purpose of identifying the pool of fungi potentially available to seedlings. With this in mind, an additional study is being conducted to examine distributions of ectomycorrhizae from root samples in relation to RmT gradients.

## MATERIALS AND METHODS

The study was conducted at the Coweeta Hydrologic Lab, on a site that is dominated by mature northern red oak (*Quercus rubra* L.), which also includes eastern hemlock (*Tsuga canadensis* L.) and a variety of other taxa. All of the blocks for this study were located on a single hillside with similar aspect and slope. It is likely that roots of individual trees extend between the blocks; most of the tree species found on a particular block also occur in the forest surrounding all the other blocks. Therefore, the composition of the root zone in terms of canopy and subcanopy trees was treated as uniform across the blocks for this study.

*Plot layout and site preparation.*—Three ¼ ha blocks were randomly located inside of dense RmTs (+RmT) and three blocks were randomly positioned outside of RmTs (−RmT). Fifteen 2 × 2 m plots were systematically located within each block (90 plots total) and randomly assigned one of five treatments (3 plots/block × 6 blocks; 18 plots per treatment). The treatments applied were (i) unmanipulated controls, (ii) −RmT litter with −RmT organic layer (−L/−O), (iii) +RmT litter with +RmT organic layer (+L/+O), iv) +L/−O, and v) −L/+O. The litter (undecomposed leaf material on the soil surface) was removed from all +RmT plots other than controls, composited, and redistributed equally to all plots receiving the +L treatment. The −L, +O (organic layer: the layer between the litter and the mineral horizon) and −O substrates were manipulated in the same fashion. More details on site characteristics and the experimental design can be found in Walker et al (1999).

*Sporophore sampling.*—All sporophores of putative ectomycorrhizal fungi (Miller 1983 and 1982b, Bills 1986, Molina et al 1992) were collected from the substrate manipulation blocks throughout the fruiting season during 1996 and 1997. Collections were gathered once a week during all peak fruiting periods. During periods of sparse sporophore production, the plots were checked at least once every two weeks, and deteriorated, unidentifiable sporophores were rarely observed. All sporophores were identified in the field or dried and examined microscopically in the laboratory in cases when identification was ambiguous based solely on macromorphology. Voucher collections, color photos of fresh specimens, and detailed fresh descriptions were utilized for taxonomically difficult fungi.

*Analytical methods.*—The abundance of sporophores produced on a plot was estimated using the following categories: (i) solitary, 1–2 sporophores, (ii) low abundance, 3–5 sporophores, (iii) medium abundance, 6–10 sporophores, iv) abundant, >10 sporophores. For all analyses, the plots of the manipulation experiment were treated as sample units, and the abundance class for each species/plot pair was entered as a categorical variable (coded as 0 = absent 1 = solitary, 2 = low abundance, 3 = medium abundance, 4 = abundant). Abundance categories for all collection dates in the same year were summed prior to classifying the abundance. For example, a taxon that produced two sporophores one week and another three the next week on the same plot would be coded as medium abundance (category 1+ category 2 = category 3). Species that produced sporophores on an individual plot during both years of the study were placed in the higher abundance category plus one (e.g., 1996—category 2, 1997—category 2; coded as category 3).

Categories were employed for abundance because the types of analyses performed were designed for vegetation and depend on the abundance of individuals, not reproductive structures. Therefore use of absolute counts of sporophores for abundance would over-represent the importance of taxa that produce copious numbers of small sporophores. Because abundance was treated categorically, it is necessary to point out that the relative abundance values presented in TABLE II are not based on absolute numbers.

The groups used for all analyses were presence and absence of RmT, and the substrate treatments applied to the 2 × 2m plots. Substrate comparisons included all fungi collected on the plots. Control plots and substrate treatment plots were pooled for the inside versus outside of RmT comparison (TABLE I, FIGS. 3, 4) because there were no treatment effects, and scores for all fungi collected in the blocks (not necessarily on a 2 × 2m plot) were used for the multiresponse permutation procedures (MRPP) and indicator species analyses. Abundance was recorded similarly in a 2 × 2m area for sporophores within the blocks but not within a plot. The inside versus outside of RmT ordinations by reciprocal averaging (RA) and canonical correspondence analysis (CCA) only included those fungi collected from a plot.

MRPP (a nonparametric test that compares heterogeneity within predefined groups) was calculated using the Sorensen coefficient. The weighting factor applied to the items in each group was n/sum(n) where n is the number of items in the group. Mielke (1984) recommended this weighting for use with MRPP, and most recent applications of MRPP have followed suit (McCune and Medfford 1997). R values approaching 1 indicate groups with more similar samples, and negative R values are possible when groups are less similar than expected by chance. Statistical significance is based on a test of no difference between groups, and *P* values represent the chance of a more extreme *R* value originating randomly (based on a calculated mean within group homogeneity for all possible groupings of the data).

Cluster analysis is a method for grouping similar items based on two or more characteristics. Typically a distance measure between items is calculated by methods similar to those used in numerical taxonomy or phenetics. Cluster analyses were calculated with the aid of Numerical Taxonomy and Multivariate Analysis System version 1.8 (Rohlf 1994) using only fungi collected from a plot. The cluster analysis produced a dendrogram, which was defined by the unweighted pair-group method and arithmetic average (UPGMA), and employed the Bray-Curtis coefficient (Rohlf 1994). Cluster analyses generated by PC-ORD Multivariate Analysis of Ecological Data version 3.0 for windows (McCune and Medfford 1997) using Sorensen's distance and UPGMA or nearest neighbor joining gave similar results. Subsampling (with 500 repetitions) was used to generate the species to area curve.

Indicator species analysis was performed using Dufrene and Legendre's (1997) method, which is based both on the abundance and frequency of species in *a priori* groups. The indicator species analysis uses a Monte Carlo technique to test statistical significance based on repeated randomizations (1000 in this study) of the dataset, such that *P* values represent the probability of a higher maximum indicator value arising randomly. Relative abundance is the abundance of a certain taxon in proportion to the abundance of the taxon in all groups. The relative frequency is the percentage of sample units in each group containing a given taxon. The indicator value is a measure of both the relative abundance and reliability of occurrence of the taxon in the group, and ranges from 0–100 (100 representing perfect indication). The relative abundance is relative to the classes (four) used to record sporophore abundance in the field and therefore are not absolute numbers.

Ordination techniques including CCA (Ter Braak 1986) and RA are used to describe multidimensional data such as species composition on a reduced number of axes while retaining as much of the original information as possible. These are known as eigenvalue type analyses. For RA, the methods involve reciprocation between weighting rows (plots in this study) and columns (species in this study) to obtain a unique solution. Sample positions in RA ordination space are defined by Chi-squared distances. CCA employs the same analytical method as RA, except that CCA is constrained by multiple regression of the species—plot matrix against a second matrix containing environmental parameters. Similar samples are positioned close to one another in both RA and CCA ordination space, thus grouping is evident when the samples appear clustered on the ordination diagram.

The sporophore abundances for 44 putatively ectomycorrhizal species from 43 plots (20 −RmT, 23 +RmT) were used to conduct RA and CCA. CCA included five environmental parameters: soil pH, Ca, P, moisture, and weighted canopy openness. Plots with fewer than two ectomycobiont taxa were excluded from both RA and CCA analyses. Down-weighting rare species gave similar results. CCA axes were scaled to optimize representation of plots. The species-area curve, indicator species, MRPP, and CCA analyses were generated by PC-ORD Multivariate Analysis of Ecological Data version 3.0 for windows (McCune and Medfford 1997).

Soil cation concentrations and pH were determined by the Soil Testing Laboratory at Virginia Tech by means of inductively coupled plasma mass spectrometry. Canopy openness was determined by means of canopy hemispherical photographs taken during the maximum seedling growth period (July). The images were processed using FEW 4.0 (M. Ishizuka, pers comm) to derive weighted canopy openness, or the ratio of unobstructed sky to the whole hemisphere. Values for soil moisture were collected in July 1996 by Time Domain Reflectometry (Tektronix model 1502C TDR cable tester, Heerenveen, The Netherlands), at a depth of 0–15 cm, in the center of each plot. Methods for characterization of the environmental parameters are described completely in Nilsen et al (2001).

## RESULTS

*General assessment of the ectomycorrhizal fungus community.*—A total of 67 putatively ectomycorrhizal fungus taxa were collected from the blocks, of which 49 species were collected from a plot (TABLE I). All collections except two were identified to species, one of which (*Cortinarius* "sp. 1") possibly has not yet been described. The species–area curve (FIG. 1) appears to be increasing even at the maximum area sampled in this study. The ectomycorrhizal families Russulaceae (*Lactarius* 10 species, *Russula* 6 species), Boletaceae (13 species), and Amanitaceae (10 species) were dominant on the substrate manipulation blocks (TABLE I). Dominant ectomycorrhizal species in the plots in descending order based on percent frequency (percent of plots with the taxa present) were: *Russula silvicola* Shaffer (29% frequency), *Laccaria laccata* (Scop. ex Fr.) Berk. & Br. (28% frequency), *Cantharellus ignicolor* Peterson (19% frequency), *Lactarius speciosus* (Burl.) Sacc. (17% frequency), *Boletus affinis* Pk. (14% frequency), and *Clavulinopsis fusiformis* (Fr.) Cor. (13% frequency) (TABLE I). Note, however, that the substrate manipulation on these sites possibly affected the distribution of individual fungi on the plots (see following section).

TABLE I. Indicator species analysis for ectomycorrhizal sporophores inside versus outside of *Rhododendron maximum* thickets

| | Rel. Abun.[a] | | Rel. Freq.[b] | | Indicator[c] | | |
|---|---|---|---|---|---|---|---|
| | −RmT[d] | +RmT[e] | −RmT | +RmT | −RmT | +RmT | *P*[f] |
| *Amanita brunnescens* | 20 | 80 | 2 | 8 | 0 | 7 | 0.382 |
| *Amanita caesarea* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Amanita cinnereoconia* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Amanita citrina* var. *lavendula* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Amanita flavoconia* | 33 | 67 | 2 | 4 | 1 | 3 | 0.999 |
| *Amanita gemmata* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Amanita onusta* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Amanita pantherina* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Amanita rubescens* | 0 | 100 | 0 | 8 | 0 | 8 | 0.124 |
| *Amanita virosa* | 18 | 82 | 4 | 10 | 1 | 9 | 0.271 |
| *Austroboletus betula* | 14 | 86 | 4 | 17 | 1 | **14** | 0.042 |
| *Austroboletus gracilus* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Boletellus chrysenteroides* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Boletus affinis* | 56 | 44 | 13 | 15 | 7 | 6 | 0.999 |
| *Boletus bicolor var. bicolor* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Boletus griseus* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Boletus ornatipes* | 33 | 67 | 2 | 2 | 1 | 1 | 0.999 |
| *Boletus pallidus* | 50 | 50 | 6 | 6 | 1 | 3 | 0.999 |
| *Boletus subtomentosus* | 50 | 50 | 2 | 2 | 1 | 1 | 0.999 |
| *Camarophyllus borealis* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Camarophyllus pratensis* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Cantharellus ignicolor* | 29 | 71 | 15 | 23 | 4 | 16 | 0.148 |
| *Clavaria vermicularis* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Clavariadelphus pistillaris* | 100 | 0 | 6 | 0 | 6 | 0 | 0.270 |
| *Clavariadelphus truncatus* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Clavicorona pyxidata* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Clavulinopsis fusiformis* | 61 | 39 | 15 | 10 | 9 | 4 | 0.468 |
| *Coltricia cinnamomea* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Cortinarius alboviolaceus* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Cortinarius bolaris* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Cortinarius collinitus* | 25 | 75 | 2 | 6 | 1 | 5 | 0.606 |
| *Cortinarius iodes* | 0 | 100 | 0 | 4 | 0 | 4 | 0.516 |
| *Cortinarius* sp. 1 | 100 | 0 | 4 | 0 | 4 | 0 | 0.499 |
| *Craterellus cornucopiodes* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Elaphomyces* sp. 1 | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Entoloma grayanum* var. *grayanum* | 67 | 33 | 4 | 2 | 3 | 1 | 0.999 |
| *Gomphus flocossus* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Hydnellum ferrugineum* | 50 | 50 | 2 | 2 | 1 | 1 | 0.999 |
| *Hygrophorus eburneus* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Inocybe cf. fastigiata* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Inocybe mixtilis* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Laccaria laccata* | 61 | 39 | 33 | 23 | 20 | 9 | 0.239 |
| *Lactarius allardii* | 0 | 100 | 0 | 4 | 0 | 4 | 0.498 |
| *Lactarius camphoratus* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |
| *Lactarius chrysorheus* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Lactarius gerardii* | 56 | 44 | 10 | 4 | 6 | 2 | 0.447 |
| *Lactarius griseus* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Lactarius helvus* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Lactarius piperatus* var. *glaucescens* | 25 | 75 | 6 | 6 | 2 | 5 | 0.661 |
| *Lactarius piperatus* var. *piperatus* | 42 | 58 | 4 | 8 | 2 | 5 | 0.657 |
| *Lactarius speciosus* | 87 | 13 | 27 | 6 | **24** | 1 | 0.004 |
| *Lactarius volemus* | 40 | 60 | 8 | 10 | 3 | 6 | 0.757 |
| *Lactarius zonarius* | 50 | 50 | 2 | 2 | 1 | 1 | 0.999 |
| *Leccinum rubropunctum* | 71 | 29 | 4 | 4 | 3 | 1 | 0.853 |

TABLE I. Continued

| | Rel. Abun.[a] | | Rel. Freq.[b] | | Indicator[c] | | |
|---|---|---|---|---|---|---|---|
| | −RmT[d] | +RmT[e] | −RmT | +RmT | −RmT | +RmT | *P*[f] |
| *Phellodon melaleucus* | 100 | 0 | 4 | 0 | 4 | 0 | 0.499 |
| *Phylloporus rhodoxanthus* | 67 | 33 | 2 | 2 | 1 | 1 | 0.999 |
| *Pulveroboletus ravanelii* | 25 | 75 | 2 | 4 | 1 | 3 | 0.729 |
| *Rozites caperata* | 50 | 50 | 2 | 2 | 1 | 1 | 0.999 |
| *Russula aeruginea* | 78 | 22 | 8 | 4 | 6 | 1 | 0.409 |
| *Russula incarnaticeps* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Russula krombholzii* | 100 | 0 | 13 | 0 | **13** | 0 | 0.023 |
| *Russula rosea* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Russula silvicola* | 33 | 67 | 23 | 35 | 7 | 24 | 0.113 |
| *Russula variata* | 80 | 20 | 10 | 4 | 8 | 1 | 0.215 |
| *Strobilomyces floccopus* | 33 | 67 | 2 | 4 | 1 | 3 | 0.999 |
| *Tricholoma davisiae* | 0 | 100 | 0 | 2 | 0 | 2 | 0.999 |
| *Tricholoma sejunctum* | 100 | 0 | 2 | 0 | 2 | 0 | 0.999 |

[a] Relative abundance, percent (based on four abundance catogories) of sporophores of the taxa produced in the group.
[b] Relative frequency, percent of plots on which the taxa occurred in the group.
[c] Indicator values, a combination of the relative abundance and relative frequency.
[d] Blocks in forest without *Rhododendron maximum* thickets (RmT).
[e] Blocks inside RmT.
[f] Bold numbers are the maximum (significant) indicator values. *P* represents the probability of a higher maximum indicator value arrising randomly. Because no grouping was detected based on treatment type, substrate manipulation and control plots are combined.

*Ectomycorrhizal fungi in response to substrate treatments.*—The ectomycorrhizal fungi collected on the plots did not show a pattern reflecting substrate treatment type in the cluster analysis. Treatment types appeared to be randomly scattered on the dendrogram termini. Because there was no resolution of substrate treatments or block type (+RmT versus −RmT) in the cluster analysis, the dendrogram is not presented. Within group homogeneity for MRPP using substrate treatments as groups was low ($R = 0.026$, $P \ll 0.01$).

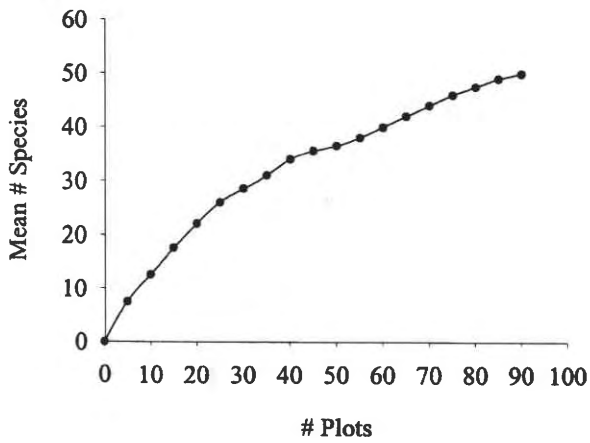


FIG. 1. Species versus area curve—average number of species versus number of 2 × 2 m plots for all ectomycorrhizal fungi at the substrate manipulation site, based on subsampling with 500 repititions.

Ordination by RA did not reveal treatment level grouping (FIG. 2). Fungi that were significantly indicative of treatment type are presented in TABLE II.

*Ectomycorrhizal fungi in response to RmT.*—As was the case for the substrate treatments, block type (+RmT

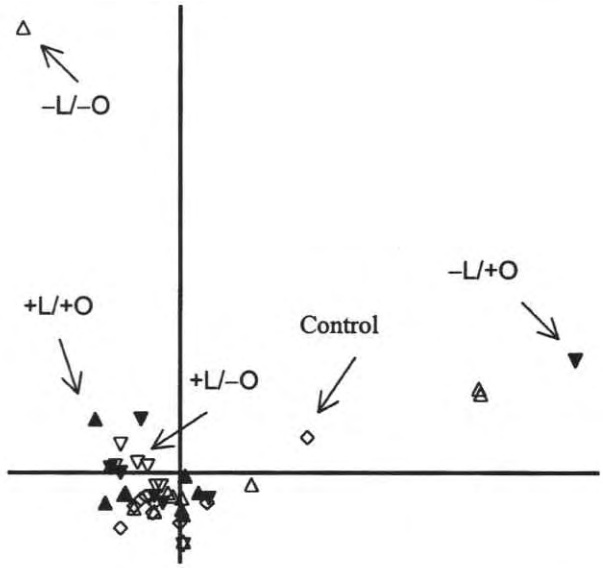


FIG. 2. RA ordination diagram of 43 2 × 2m plots based on sporophore abundance of 44 putatively ectomycorrhizal fungus taxa grouped by substrate treatment type. Substrate treatment abbreviations are as for TABLE II.

TABLE II. Indicator values by substrate treatment for ectomycorrhizal sporophores on 90 2 × 2 m plots (only taxa with significant indicator values are shown)

| | −L/−O[a] | +L/+O[b] | +L/−O[c] | −L/+O[d] | Control | P[e] |
|---|---|---|---|---|---|---|
| *Amanita flavoconia* | 0 | 0 | **17** | 0 | 0 | <0.05 |
| *Austroboletus betula* | 1 | 0 | 0 | 0 | **26** | <0.01 |
| *Hydnellum ferrugineum* | 0 | 0 | 0 | 0 | **15** | <0.06 |
| *Russula variata* | 0 | 2 | **18** | 0 | 0 | <0.06 |
| *Strobilomyces floccopus* | 0 | 0 | **17** | 0 | 0 | <0.04 |

[a] −RmT (forest without *Rhododendron maximum* thickets) litter with −RmT organic layer (−L/−O).
[b] +RmT litter with +RmT organic layer (+L/+O).
[c] +RmT litter with −RmT organic layer (+L/−O).
[d] −RmT litter with +RmT organic layer (−L/+O).
[e] Statistical significance as for Table I. Bold numbers are the maximum (significant) indicator values.

versus −RmT) did not define any groups of plots in the cluster analysis. The cluster analysis dendrogram lacked any resolution for block type, and plots from inside of RmT and outside of RmT were interspersed throughout the entire tree. MRPP did not detect grouping at the block type level either, with $R = 0.006$ ($P < 0.02$; probability of a more extreme $R$ arising randomly). Individual fungal taxa that were significant indicators of block type were *Austroboletus betula* (Schw.) E. Horak, *Lactarius speciosus*, and *Russula krombholzii* Shaffer (TABLE I). Other species that showed potential as indicators were *Amanita brunnescens* Atk., *Amanita rubescens* (Pers. per Fr.) S.F. Gray, *Cantharellus ignicolor*, *Russula silvicola*, and *Russula variata* Banning & Pk. (TABLE I). Twenty-five species of putatively ectomycorrhizal fungi were collected only from +RmT blocks. However, of those 22 species, 19 species were collected only once. There were 17 species of putatively ectomycorrhizal fungi on only −RmT blocks; of those species, 14 were collected only once (TABLE I).

Plots from in versus out of RmT were not obviously grouped by RA (FIG. 3). The absence of any detectable clumping in the RA ordination indicates that the sporophore community was similar inside versus outside of RmT. Ordination by RA (FIG. 3), which is not constrained by environmental parameters, lacked the slight shift in + versus −RmT plots evident in CCA (FIG. 4).

Canonical correspondence axes 1, 2, and 3 (not

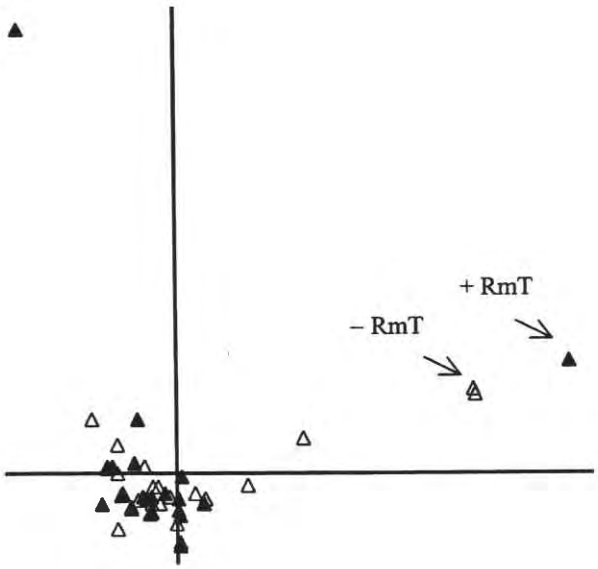


FIG. 3. RA ordination diagram of plots inside (23, +RmT) versus outside (20, −RmT) of *Rhododendron maximum* thickets based on sporophore abundance of 44 putatively ectomycorrhizal fungus taxa.

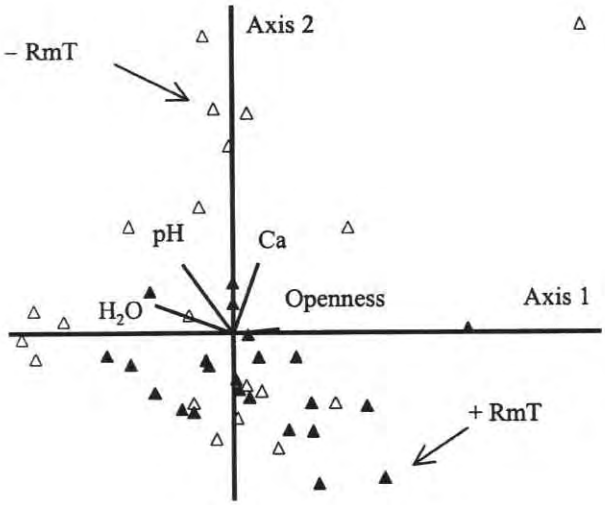


FIG. 4. CCA ordination diagram of plots inside (23, +RmT) versus outside (20, −RmT) of *Rhododendron maximum* thickets. Axis 1 and 2 explain 4.5% and 3.8% respectively of the variation in sporophore abundance of 44 putatively ectomycorrhizal species. Vectors depict the strength and direction of correlation with environmental parameters with $r^2 > 0.2$, Openness: weighted canopy openness, pH: soil pH, $H_2O$: soil moisture, Ca: soil Calcium.

shown by convention) captured 4.5%, 3.8%, and 2.9%, respectively, of the variation in abundance data from 44 putatively ectomycorrhizal taxa (FIG. 4). Plots from in versus out of RmT were not obviously grouped by CCA. The limited variation explained by CCA was associated with environmental parameters. Axis scores derived from species versus scores that are linear combinations of the environmental variables were highly correlated (Pearson correlation coefficients for axis 1 = 0.943, axis 2 = 0.919, and axis 3 = 0.858). Standardized canonical coefficients versus axis 1 were $R = 0.37$ for openness, $R = -0.32$ for pH, $R = -0.33$ for $H_2O$, $R = -0.30$ for P, and $R = 0.42$ for Ca. Standardized canonical coefficients versus axis 2 were $R = 0.07$ for openness, $R = 0.42$ for pH, $R = 0.03$ for $H_2O$, $R = 0.10$ for P, and $R = 0.45$ for Ca.

## DISCUSSION

*Ectomycorrhizal diversity at the substrate manipulation site.*—As mentioned in the introduction, species richness is likely underreported in this study because hypogeous fungi and mycorrhizae were not sampled. In addition, the species-area curve indicates that species richness was probably underestimated due to the limited area sampled. The species-area curve continues to increase at the maximum area sampled (90 plots) (FIG. 1). Bills et al (1986) suggested 100 contiguous 2 × 2 m plots as a guideline for subsampling a limited area, and Nantel and Neumann (1992) followed suit. However, the species–area curve continued to climb even at this size (Bills et al 1986). For this study, plots were separated by walkways to isolate the effects of the substrate treatments.

Ectomycorrhizal communities vary in relation to stand age (Marks and Foster 1967, Mason et al 1982, Miller 1983, Dighton et al 1986), the host tree composition of a stand (e.g., Villeneuve 1989, Bills et al 1996), and the edaphic characteristics within the range of a particular assemblage (Nantel and Neumann 1992). Throughout the range of habitats where RmTs are present at Coweeta, we have recorded approximately 250 species of ectomycorrhizal fungi. The results of this study were derived solely from fungi fruiting at the substrate manipulation sites and therefore are not necessarily indicative of other ectomycorrhizal communities where RmTs occur.

*Ectomycorrhizal fungi in response to treatments.*—Treatment effects on the distribution of ectomycorrhizal fungi were species specific (TABLE II). However, treatment effects did not relate to overall ectomycorrhizal community structure (FIG. 2). There were few differences in mycorrhizal colonization levels by treatment type (Walker et al 1999), so the lack of grouping of ectomycorrhizal sporophores by treatment type is not incongruent. The homogenization of the substrates was a fairly severe disturbance to the mycelium in the substrates, which meant that the fungi fruiting within the plots had to re-grow from severed hyphal fragments or other propagules if they originated in the substrates. In addition, there is no way to estimate how frequently fungi fruiting in the plots originated from the underlying mineral horizon (which was left intact in all treatments), or how frequently they originated from undisturbed mycelium in the walkways between the plots. Nonetheless, the ability of ectomycorrhizal fungi to re-colonize the substrates from +RmT versus −RmT blocks was not generally different.

Several species of ectomycorrhizal fungi, including *Amanita flavoconia* Atk., *Russula variata*, and *Strobilomyces floccopus* (Vahl. ex Fr.) Karst, were indicative of the +L/−O substrate combination (TABLE II). The reasons for this are not clear. Other fungi such as *Austroboletus betula* and *Hydnellum ferrugineum* (Fr. : Fr.) Karst were most abundant on control plots (TABLE II). This observation may indicate lack of tolerance to disturbance.

The difference between the number of species on the plots versus the total number on the blocks (18 species) could easily be explained by increased sample area. This also tends to indicate that diversity of ectomycorrhizal fungi on the plots was not severely depressed by the disturbance to the plots during the substrate manipulation. Brundrett and Abbott (1995) found that some bait plants (*Mirbelia dilatata* R. but not *Eucalyptus calophylla* Lindley) formed somewhat fewer mycorrhizae in treatments where hyphal networks in soil cores were disrupted by breaking up the core. However, they also reported highly variable mycorrhizal colonization levels related to the amount of organic matter and hyphae in the soil.

*Ectomycorrhizal fungi inside versus outside of* Rhododendron maximum *thickets.*—Because the $R$ and $P$ values for MRPP ($R = 0.006$; $P < 0.02$, probability of a more extreme $R$ arising randomly) are so low in this study, it is highly unlikely that a difference in ectomycorrhizal fungus communities sampled inside versus outside of RmT would be documented through additional sporophore sampling. Lack of grouping in versus out of RmT (FIG. 3) could potentially be related to any combination of the following factors: (i) confounding effects from disturbance of the plots by treatment application, (ii) confounding effects from the treatments themselves, (iii) the limited area sampled, (iv) the variable nature of the effect of RmT on individual ectomycobiont species, (v)

the failure to detect potentially dominant ectomycorrhizal fungi which fruit rarely or never above ground, and (vi) strong relationships between sporophore distributions and abiotic environmental parameters that are uncorrelated with RmTs. However, with the absence of detectable grouping at the treatment level (FIG. 2), including unmanipulated controls, the first two points seem unlikely.

As mentioned above, strong relationships between abiotic environmental parameters and species composition could uncouple or mask any relationship between sporophore distributions and RmTs. Sporophore distributions were not explained by either abiotic factors or RmTs, however. CCA, which uses environmental variables to constrain the ordination of plots and taxa, captured only a small portion (11.2%) of the variation in sporophore composition in this study (FIG. 4). This result indicates that abiotic factors did not strongly confound our analyses of the influence of RmT on sporophore distribution, implying that sporophore distributions were indeed similar inside of and outside of RmT (FIG. 3). It should be noted that in community analyses such as this, high levels of variation are frequently encountered because of the multitude of potentially important factors affecting fungal distribution and sporulation. Therefore, although the level of species variation captured by CCA was rather low, relationships between sporophore distributions and soil pH, Ca, P, moisture, and weighted canopy openness should probably be considered in future studies (FIG. 4).

It is known that the total percent mycorrhizal colonization of some tree seedling taxa is lower, while the level of colonization by *Cenococcum geophilum* is higher, in RmT at the study site (Walker et al 1999). If the ectomycorrhizal community is not different in and out of RmT, then the failure of the seedlings to form as many mycorrhizae in RmT is probably due to factors affecting the process of mycorrhization, and not due to a dearth of potential fungal associates. The seedlings are most likely cut off from potential ectomycorrhizal inoculum by the presence of the dense rhododendron root mats in the thickets. The rhododendron root mat, which is symbiotic with ericoid mycorrhizal fungi, occupies the organic layer and may spatially separate the seedlings from potential ectomycorrhizal colonization sources deeper in the soil profile. In addition, competition for nutrients in the mycorrhizosphere may or may not be important. A less parsimonious possibility is that the effect of RmT on total colonization may be due to inhibition of only that portion of the ectomycorrhizal community compatible with the two species of seedlings previously assayed, i.e., hemlock and red oak (Walker et al 1999). To date, we have no evidence to distinguish among these hypotheses. A study designed to resolve this issue by testing for differences among ectomycobiont communities on seedling roots across undisturbed transects with varying ericaceous shrub densities (including RmT) is underway.

Although there was no apparent change in overall community composition, indicator values suggest that individual ectomycorrhizal fungi (*Austroboletus betula, Lactarius speciosus,* and *Russula krombholzii*) were correlated more strongly with either forest type (+RmT or −RmT) (TABLE I). However, because of the limited area sampled in this study, additional observations of these taxa will be necessary if they are to be informative for indicator analyses.

*Summary and conclusions.*—We have found that: (i) In general, the diversity and community structure of ectomycorrhizal fungi is not apparently different inside versus outside of RmT, (ii) Individual ectomycorrhizal fungus taxa are potentially distributed differentially with regard to RmT, (iii) The portion of the ectomycorrhizal fungus community capable of recolonizing manipulated soil substrates is not different for substrates from inside versus outside of RmT, and (iv) The observed relationships between sporophore distributions and abiotic environmental parameters is weak. The differences in mycorrhizal colonization found previously on seedlings within versus outside of RmT (Walker et al 1999) were not reflected in the distribution of ectomycorrhizal fungus sporophores. Because the mycorrhizal colonization of seedlings is depressed, but sporophore dominance and diversity are similar inside versus outside RmTs, it is thought that RmTs affect the process of seedling mycorrhization. Factors such as the availability of light and the relationship between light quantity and mycorrhizal colonization should be the focus of further investigations of the inhibition of seedlings by RmT. Most importantly, the types of mycorrhizae formed by seedling root systems should be directly compared across environmental gradients associated with changes in the density of ericoid shrubs.

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