Ectomycorrhizal Community Structure and Soil Characteristics of Mature Lodgepole Pine (*Pinus contorta*) and Adjacent Stands of Old Growth Mixed Conifer in Yellowstone National Park, Wyoming, USA

Running Head: ECM Community Structure in Yellowstone

Robert B. Douglas
Mendocino Redwood Company
32600 Holquist Lane/P.O. Box 489, Fort Bragg, CA 95437

V. Thomas Parker
Department of Biology, San Francisco State University
1600 Holloway Avenue, San Francisco, CA 94132

Kenneth W. Cullings
NASA-Ames Research Center, MS 239-4
Moffett Field, CA 94035-1000

Corresponding Author: Robert B. Douglas, Mendocino Redwood Company, 32600 Holquist Lane, Fort Bragg, CA 95437, rdouglas@mendoco.com, 707-962-2908

Abstract: Forest development patterns following disturbance are known to influence the physical and chemical attributes of soils at different points in time. Changes in soil resources are thought to have a corresponding effect on ectomycorrhizal (ECM) community structure. We used molecular methods to compare below-ground ECM species richness, composition, and abundance between adjacent stands of homogenous lodgepole pine and old growth mixed conifer in Yellowstone National Park (YNP). In each stand-type we collected soil cores to both identify mycorrhizae and assess soil chemistry. Although no statistical difference was observed in the mean number of ECM root tips per core between stand types, the total number of species identified (85 versus 35) and the mean number of species per core (8.8 ± 0.6 versus 2.5 ± 0.3) were significantly higher in lodgepole pine. Differences between the actual and estimated species richness levels indicated that these forest types support a high number of ECM species and that undersampling was severe. Species compositions were widely disparate between stands where only four species were shared out of a total of 116. Soil analysis also revealed that mixed conifer was significantly lower in pH, but higher in organic matter, potassium, phosphorus, and ammonium when compared to lodgepole pine stands. Species richness per core was correlated with these chemical data, however, analysis of covariance indicated that stand type was the only statistically significant factor in the observed difference in species richness. Our data suggest that ECM fungal richness increases as homogenous lodgepole pine stands grow and mature, but declines after Engelmann spruce and subalpine fir colonize. Despite difficulties linking species composition with soil chemistry, there are a variety of physical and chemical factors that could be influencing ECM community structure. Future field experiments are necessary to test some of the mechanisms potentially operating within this system.

Key Words: ectomycorrhizae, soil chemistry, community structure, ITS-RFLP, Yellowstone

INTRODUCTION

A prevalent view in fungal ecology holds that soil resources and fertility influence the ectomycorrhizal (ECM) plant-fungal relationship, as well as fungal colonization levels, species richness and composition patterns (Alvarez et al. 1979; Gehring and Whitham 1994; Bruns 1995; Gehring et al. 1998). Early models proposed that during forest development increasing plant litter and woody debris, along with changing host-carbohydrate supply, substantially alter the amount and quality of nutrients available to the ECM community (Dighton and Mason 1985; Last et al. 1987).

Previous studies examining ECM community structure have focused on a variety of forest systems, but relatively few have documented below-ground distribution patterns along successional gradients within natural systems where disturbance regimes have remained intact (Visser 1995; Jonsson et al. 1999b). Stand-replacing wildfire is a common disturbance in many forest ecosystems that may cause substantial nutrient loss through volatilization, change soil porosity and chemistry, and significantly reduce soil microbial biomass, particularly root symbionts such as ECM fungi (Neary et al. 1999; Stendell et al. 1999). Consequently, post-fire communities along with their associated ectomycorrhizae face environmental conditions quite unlike the pre-fire environment, and it may take years or centuries for these areas to recover to their previous state.

Currently, little is known about how temporal changes in soil factors accompanying vegetative successions affect ECM fungal diversity and composition in naturally regenerating forests. Although fire tends to cause rather abrupt and extreme environmental changes, forest succession, in contrast, tends to impart subtle changes to the community gradually through time with the slow accumulation of plant litter and woody debris and coincident changes in temperature and moisture regimes. A majority of below-ground studies have addressed ECM community structure within

specific forest habitats and/or host-specificity patterns in mixed forest communities (Gardes and Bruns 1996; Horton and Bruns 1998; Jonsson et al. 1999b; Taylor and Bruns 1999; Byrd et al. 2000; Cullings et al 2000) but only a few have provided information on soil nutrient status in relation to the fungal community (Gerhing et al. 1998; Horton et al. 1999; Grogan et al. 2000; Cullings and Makhija 2001).

In this study, we assessed below-ground ECM community structure and soil chemistry at two points of a natural successional gradient in Yellowstone National Park where periodic stand-replacing wildfire is common. Here we examined ECM community structure and its possible relationship with soil nutrients. Specifically, we compared ECM community patterns and soil characteristics between adjacent stands of mature lodgepole pine (just at a point where late-successional species begin to establish in the understory) and old-growth mixed conifer derived from the same rhyolitic parent material. Since a previous study examining host-specificity patterns in the mixed conifer stand demonstrated that many fungal species can associate with more than one species of plant host, possibly facilitating late-successional species (Cullings et al. 2000), we hypothesized that lodgepole pine and mixed conifer stands would have similar fungal compositions among system dominants.

MATERIAL AND METHODS

Study sites

The study area is located 17.9 kilometers west of Fishing Bridge along Grand Loop Road (UTM Zone 12, 4922400 m N, 540300 m E; elevation 2430 m) in Yellowstone National Park, Wyoming, and encompassed 4 hectares of variously aged coniferous forest (Figure 1). Individual

sampling sites were selected from two adjacent forest types representing distinct successional stages separated by 150-200 meters: 1) mature lodgepole pine stand (*Pinus contorta* Douglas ex Louden) regenerating from a fire approximately 135 years ago; and 2) a 250+ year-old mixed coniferous stand comprised of lodgepole pine, Engelmann spruce (*Picea engelmannii* Parry ex Engelmann), and subalpine fir (*Abies lasiocarpa* Nuttal ex Hooker). Secondary succession typically begins with lodgepole pines readily establishing in open sites following fire. This species forms monospecific stands for 80-150 years, after which shade-tolerant species such as Engelmann spruce and subalpine fir begin to colonize and later dominate (Despain 1990).

Soil type for the area is derived from rhyolitic and lake sediment parent materials and classified as Typic Cryochrepts with a coarse sandy loam (Rodman et al. 1996). Tree species composition and seasonal soil moisture patterns differed between forest types, while total basal area, mean canopy cover, and soil temperature regimes were similar (Table 1).

Soil Sampling

A total of 27 soil cores were collected from each forest type to assess below-ground ECM species richness, composition, and root tip abundance. A grid system was used to select core sites in two 50 x 50 m plots and one 30 x 80 m plot within the lodgepole pine stand in August 1998 and 1999, respectively. Using a random number generator, we randomly selected three sites within each plot to extract soil cores. At each site, three 8 x 24 cm soils were collected systematically: the first core was collected 25 cm due north of the randomly selected site while the subsequent two cores were taken at locations representing the vertices of an equilateral triangle. These soil cores were compared with a set of soil cores taken from an adjacent stand of mixed conifer in 1996.

An additional 27 soil cores were collected from each forest type in May 2000 immediately following snowmelt to assess chemical composition. A total of three soil cores, comprising the upper soil layer only (0-8 cm), were taken adjacent to sites where individual soil cores had been previously sampled for ectomycorrhizal fungi. These soil cores were pooled to ensure sufficient volume for chemical analyses (i.e., >300 g). Following field collection, soil was air-dried and passed through two sieve sizes, numbers 10 and 60. Samples were submitted to the DANR (Division of Agriculture and Natural Resources) Analytical Laboratory, University of California, Davis, and analyzed for pH, organic matter, total nitrogen, ammonium, nitrate, potassium, and phosphorus following standard procedures.

Ectomycorrhizal Root Tip Sorting

Core samples were transferred to the lab on ice and refrigerated at 4 °C. Individual mycorrhizas were sorted within 10 days of collection to minimize DNA degradation. Beakers containing samples were filled with deionized water and left to soak overnight. Mycorrhizas were sorted using sieve trays to remove dirt and rock, then sequestered into epitubes based on color and branching pattern as seen with a dissecting scope (Aegerer 1987-1992). After sorting, all mycorrhizas were stored at –20 °C and later lyophilized for long-term storage.

ECM root tip abundance was calculated by counting the number of tips for individual morphotypes. In order to ensure accurate assessment of ECM species and their distribution, morphotypes were sampled for DNA extraction according to their abundance (Cullings et al. 2000). Tip numbers were consolidated for each fungal species following molecular identification and abundance data was tabulated for all soil cores.

DNA Extractions and PCR

DNA extractions of individual ECM root tip samples followed the CTAB miniprep method (Gardes and Bruns 1993). Amplifications of the variable internal transcribed spacer (ITS) region of ribosomal DNA were initially conducted on DNA extracts with the basidiomycete-specific primer combinations ITS1F and ITS4B. Samples failing to produce visible product with these primers were then screened with the fungal specific primer combination ITS1F and ITS4 to identify ascomycetes and other fungal taxa. A PCR Core Kit (Boehringer Mannheim) was used in all ITS amplifications. Cycling was conducted in a Perkin-Elmer 9600 thermocycler and consisted of 37 cycles with an initial denaturation step at 94 °C for 1 minute 25 seconds and 13 cycles at 94 °C for 35 seconds, 55 °C for 55 seconds, and 72 °C for 45 seconds. Parameters for the additional 13 and 11 cycling blocks were not changed, except that extension times were increased to 2 and 3 minutes, respectively. An additional 10-minute extension time followed the last cycle and the product was stored at 4 °C.

Molecular Identification

Successfully amplified ITS products were subjected to two restriction enzymes, *Hinf* I and *Alu* I. Restriction fragment length polymorphism (RFLP) patterns and DNA Molecular Weight Marker VIII (Boehringer Mannheim) were separated on 3.0% agarose mini-gels at 5V/cm, stained with ethidium bromide, and viewed with a Fisher Biotech Electrophoresis System. These patterns were compared with ITS-RFLP patterns from a sporocarp database comprised of 200 fungal specimens collected in the study area as well as other locations in YNP. Two restrictions enzymes are sufficient for identifying most fungal taxa within this system (Cullings et al. 2000; 2001).

Sequences corresponding to the 5.8S nuclear ribosomal RNA (rRNA) gene and mitochondrial large subunit (mlsu) rRNA gene were generated with ITS primers listed above and the basidiomycete-specific ML5/ML6 primers, respectively (Bruns et al. 1998; Cullings and Vogler 1998). Sequences of 5.8S rRNA gene were used to identify unknown fungal species amplified with ITS 1F/4 primers as either ascomycete or basisdiomycete, while mlsu rRNA sequences were used to identify unknown basidiomycetes to familyor sub-family. Cycle sequencing of double-stranded product was conducted using the fluorescent dideoxy-chain terminator with an ABI 377 automated sequencer. Recovered sequences were corrected for ambiguities in nucleotide identification and aligned using Sequencher 3.1.1. Sequences of 5.8S rRNA and mlsu rRNA regions were entered into their respective databases with PAUP 4.0 beta-version 8 (Swofford 2000).

Statistical analyses

We tested for mean differences in species richness, tip abundance, and soil chemistry per soil core between mixed conifer and lodgepole pine stands using the Student's t test, assuming unequal variances, and using the nonparametric alternative, Mann-Whitney U-test. All data were inspected for normality using the Kolomgorov-Smirnov test and for homogeneity of variance using the F-test. Data not conforming to parametric assumptions were log transformed.

We used analysis of covariance (ANCOVA) to examine both differences in species richness and ECM root tip abundance (at the soil core scale) and their possible relationships among soil parameters between stand types. Initially, we conducted a correlation analysis between soil parameters, ECM tip number, and ECM species richness for pooled soil core data. Significant correlates were included in a multiple regression model where only nonsignificant variables were

successively eliminated from the model. Once having a subset of significant covariates, we coded dummy variables to differentiate between stand types. All dependent variables were log transformed to ensure homoscedasticity and improve linearity. Statistical procedures were conducted with the program SPSS version 11.0 for Windows with alpha = 0.05 as the significance level for all tests. We also considered $0.05 < \text{alpha} < 0.10$ as representing a weak statistical trend.

To evaluate sampling effort in each stand we estimated species richness using the mark-recapture program CAPTURE, which recommends the most appropriate estimator based on species frequency distribution (Rexstad and Burnham 1991; Nichols et al. 1998). Several estimators included in the program were developed to adjust for behavioral and/or temporal responses of animals to trapping and are thus not relevant to ECM fungi. Therefore, we used the jackknife estimator associated with model M_h, which assumes heterogeneity in detection probabilities among species (Burnham and Overton 1979).

RESULTS

Fungal Distribution Patterns

We detected a total of 85 and 35 ECM fungal species representing a total of 5570 and 5933 root tips for lodgepole pine and mixed conifer stands, respectively (Table 2). Although there was no significant difference in mean number of root tips per core between stand types, the mean number of species per core was over three times higher in lodgepole pine than in mixed conifer (Table 2).

Estimated total species richness for lodgepole pine and mixed conifer stands were 135 and 67 species, respectively (Table 3). Both stands were primarily comprised of rare species (species found

in one core only). Differences between the actual and estimated richness can be attributed to the repeated detection of new species in each soil core. This indicated that for both stands 27 soil cores failed to accurately detect almost half the ECM species estimated to be present (Table 3). The models selected in CAPTURE also indicated that species distribution patterns were markedly different between stands (M_h in mixed conifer versus M_{tbh} in lodgepole pine; Table 3)

Only four fungal species were shared between stand types (*Inocybe*-25, *Tricholoma*-52, *Russula*-38, *Cortinarius*-11), most of which were infrequent to rare in lodgepole pine (Table 4). *Russula*-38 and *Cortinarius*-11 were the only two fungal species considered to be relatively frequent in mixed conifer that were also found in lodgepole pine (Table 4). Consistent with previous reports, we did not detect any ascomycetes in mixed conifer (Cullings et al 2000); but two species were found in lodgepole pine with one, *Cenococcum geophilum*, being a system dominant that comprised just over 8% of the total ECM root tips and was present in 17 soil cores.

Both stands differed in their composition of dominant fungal species (Figures 2a and 2b) and ranked abundance curves (Figure 3). Fungal species in mixed conifer typically exhibited lower core frequency and higher root tip numbers per core than species in lodgepole pine (Table 4). Five fungal species (*Cenoccocum*, Cantharelloid-1, unknown basidiomycete-1, Tricholomatoid-1, and *Cortinarius*-21) were found in 11 or more soil cores in lodgepole pine comprising 35.82% of the total ECM root tip number. In contrast, the three most abundant fungal species in mixed conifer (*Hygrophorous*-50, *Cortinarius*-11, and *Cortinarius*-65), representing 36.5 % of the total root tips, were found in five or less soil cores. On average, a single fungal species typically dominated individual soil cores in mixed conifer, whereas fungal species in lodgepole pine often comprised a smaller percentage of the total root tip abundance and were stratified among a greater number of cores.

The percent total of ECM root tip distributions according familial groupings was also very different between stand types (Figure 4). Identified root tips collected in lodgepole pine were distributed in eight taxonomic categories (7 families+Ascomycetes), six of which had similar tip distribution percentages. In contrast, root tips collected in mixed conifer were distributed among six families (Gomphidiaceae not shown), with the Cortinariaceae disproportionately representing greater than 50% of the total root tips. Also, all families represented in mixed conifer were represented in lodgepole pine, except for the Gomphidiaceae (*Chroogomphus*); however, the Thelephoraceae, Cantharellaceae, and Ascomycota were not represented in mixed conifer.

Soil Chemistry

Mean values of five soil parameters were significantly different between stand types ($p < 0.01$; Table 5). On average, mixed conifer was significantly lower in pH, but significantly higher in percent organic matter, ammonium, potassium, and phosphorus than lodgepole pine. Average total nitrogen and nitrate levels were similar between stands.

Correlation analysis illustrated several moderate to low, but significant associations between soil chemical variables. Both pH and percent organic matter were significantly correlated with one another, and both were significantly correlated in different ways with total nitrogen, phosphorus, potassium, and ammonium (Table 6). While species richness per core was positively correlated with pH and negatively correlated with organic matter, phosphorus, potassium, and ammonium, it was neither correlated with total nitrogen nor nitrate. ECM root tip abundance was not correlated with any of the soil parameters or species richness. Analysis of covariance indicated that stand type was the only significant factor in predicting species richness on a per core basis (Table 7). Two of seven

chemical covariates (pH, ammonium) were tested in the best regression model, however neither covariate was shown to be a significant predictor of species richness after stand type was differentiated in the model.

DISCUSSION

In this study we found evidence suggesting both a significant decrease in below ground ECM species richness and an extreme change in fungal composition during forest development as mature, homogenous stands of lodgepole pine make a transition to mixed species stands with the colonization of shade-tolerant Engelmann spruce and subalpine fir. Our soil chemistry data further indicated that during this transition there are significant increases in organic matter, phosphorus, potassium, and ammonium, and a significant decrease in pH as mixed conifer stands establish and mature. However, other abiotic or biotic factors associated with each stand type must also be considered when explaining our results.

Species richness and composition patterns

Species richness patterns were consistent with previous below ground investigations that found high species richness in homogenous stands of lodgepole pine (Stoll 1998; Byrd et al. 2000) and comparatively lower richness in mixed conifer (Cullings et al. 2000, 2001). These data also correspond with the pattern and predictions discussed in several studies describing increased fungal richness during forest development followed by a decrease in richness as canopy closure and soil moisture increase (Dighton and Mason 1985; Last et al. 1987).

Both stands were in close proximity to each other but only shared four species in common out of a total of 116. This result contrasted with several studies showing higher percentages of species overlap in homogenous pine (Visser 1995; Stoll 1998; Byrd et al. 2000) and in mixed conifer/hardwood stands (Bills et al. 1986; Jonsson et al. 1999a) of different ages. This result ran counter to our initial hypothesis that both stands would share a subset of fungal species, primarily among system dominants. Proximity to forest edges or even single surviving trees following disturbance has been known to influence the availability of fungal innocula for colonizing tree species (Kranabetter 1999; Kranabetter et al. 1999) and may also be responsible for maintaining fungal composition over time (Jonsson et al. 1999a). However, in our study area proximity to sources of fungal innocula does not appear to play an obvious role in ECM community structure patterns between adjacent forest stands.

Despite the lack of species overlap, most ECM basidiomycete families were shared between stands. The dominance of the Cortinariaceae in mixed conifer was extreme relative to the distribution of the other families. The prevalence of the Cortinariaceae has not only been documented in undisturbed stands of mixed conifer (Cullings et al. 2000, 2001) but has also been documented in progressively older stands comprised of a single plant-host (Visser 1995; Byrd et al. 2000). High species richness exhibited by this family is not easily generalized to a specific forest type and/or age; however, in Yellowstone, as well as other boreal forests, the prevalence of this family is common (Visser 1995; Jonsson et al. 1999b). In contrast, Wurzburger et al. (2001) noted the absence of the Cortinariaceae from coastal mixed coniferous forest in California, but found them to be dominant in nearby highly acidic pygmy forests. In YNP others have noted the predominance of the Cortinariaceae in highly acidic thermal soils, suggesting that in addition to high taxonomic diversity, certain species within the family might be adapted to specific environments and/or soil

conditions (Cullings and Makhija 2001). Our results suggest that higher soil pH within mixed conifer at our study site may provide a more suitable environment for many species within this family. However, this interpretation might be viewed with some reservation since differentiating members of the Cortinariaceae by the RFLP method make it likely that we have underestimated their presence in lodgepole pine, where close to 25% percent of the basidiomycetes were not identified to family (Karen et al. 1997)

The apparent absence of ascomycetes in mixed conifer, especially *Cenococcum geophilum*, is peculiar. *Cenococcum geophilum* is described as an abundant, cosmopolitan species that typically lacks sexual structures but can readily reproduce via sclerotia and/or mycelia; this species can be found in numerous habitat types and seral stages (Visser 1995; Smith and Read 1997; Jonsson et al. 1999b), and in some cases, nearly every soil sample (Jonsson et al. 2000). Here in YNP, *C. geophilum* has been reported in 8 year-old lodgepole pine stands following fire and clear-cutting, as well as in mature lodgepole pine stands similar to our study (Stoll 1998; Byrd et al. 2000). Furthermore, several other research projects in mixed conifer within YNP have also failed to detect *C. geophilum*, suggesting that over time this species may be reduced in areas where disturbance has been absent for some time (Cullings et al. 2000, 2001). During forest development, ascomycetes have been known to progressively decline in the fungal community, possibility indicating that their life-cycles respond to specific physical and chemical cues associated with specific disturbance events, especially fire (Wicklow 1988).

Soil chemistry

Correlations between chemical data and community patterns suggest that higher ECM richness occurs in areas having lower soil fertility and lower organic matter content. Circumstantially, our data also support the hypothesis that soil fertility influences fungal species composition, though we were unable to statistically test this because there was very little species overlap and a majority of species were unique to each stand type. Several studies have documented different ECM species compositions and higher colonization levels in soils having both lower soil fertility and lower organic matter content, but none found species richness to be correlated with soil nutrient status (Alvarez et al. 1979; Gerhing and Whitham 1994, 1995; Gehring et al. 1998). Interestingly, we found no difference in colonization levels between stands as inferred by ECM root tip abundance.

While we demonstrated that both lodgepole pine and mixed conifer do indeed differ in soil chemistry, our overall statistical model failed to identify any specific nutrient or soil factor as driving richness patterns with certainty. Soil factors such as pH and ammonium are known to influence ECM community structure, but neither covariate was included in the final model as being a significant predictor of species richness (Baar 1996; Cullings and Makhija 2001). However, ammonium was the only covariate that represented a possible trend at $P=0.09$, well above our predetermined alpha level. Thus, it is possible that small differences in ammonium concentration are having a disproportionately large effect on the fungal community even though this phenomenon was not conclusively demonstrated.

Although total nitrogen content was similar between stands, the observed differences in ammonium concentrations suggest that ammonification rates, which typically increase as both temperature and moisture increases, could be different in mixed conifer and lodgepole pine stands. We propose that during forest development the increasing deposition of organic matter over time,

coupled with the colonization of gaps by subalpine fir and Engelmann spruce, increase tree density and soil moisture retention within the stand. The increased soil moisture results in higher nitrogen mineralization, which subsequently influences fungal composition and reduces fungal species richness. Thus, it is conceivable that nutrient concentration, particularly ammonium, is a mere by-product of the physical changes occurring to the soil during vegetative succession

Increased ammonium levels in soil are thought to cause either the plant-host or the fungus to shift carbon allocation away from the root system or vegetative mycelium, respectively, towards amino acid synthesis (Wallender 1995; Hampp et al. 1999). Recent laboratory studies have demonstrated that fungal species vary in their ability to deal with increased exogenous ammonium, some showing different respiration rates, extramatrical mycelial biomass, and ammonium transfer rates to the plant host (Bidartondo et al. 2001). However, it is unclear if increased ammonium concentration affects the number of roots colonized by ECM fungi. Presumably, since this mechanism affects carbon investment in fungal vegetative and reproductive structures, we would expect to see not only lower fungal species richness, but also lower numbers of root tips in areas with higher ammonium levels. Because we observed differences in species richness and a similar abundance of roots tips in each stand, it remains inconclusive if these patterns are due to different ammonium concentrations.

Higher mean concentration of organic matter, lower mean pH values, and the more apparent presences of large, decaying woody debris in the mixed conifer stand suggests that phenolic compounds may be more prevalent here. The apparent higher nutrient surplus in mixed conifer could be a result of phenolic compounds forming colloidal structures in the humus layer that bind potassium, ammonium, and various forms of phosphates, thus increasing soil nutrient retention (Hättenschwiler and Vitousek 2000). Because soils in lodgepole pine stands likely have lower

phenolic concentrations, they also have lower capacity for retaining soil nutrients and therefore have both higher nutrient availability and soil leaching capacity. These conditions, coupled with lower mineralization rates and the intense competition for and rapid assimilation of available nutrients by mycorrhizae and other soil organisms, result in lower observed nutrient surpluses. Furthermore, mixed conifer stands may be more environmentally stable because they provide a continual source of nutrients that can only be accessed by fungal species possessing lignases and phenol oxidases (Dighton and Mason 1985; Bending and Read 1995a, b).

Disturbance patterns

Although we inferred a peak and subsequent decrease in below ground ECM richness along two points of a natural successional gradient, the suggested age-related changes in fungal richness contrasts with previous studies that observed a similar community pattern within considerably younger and homogenous forests outside this region (Dighton and Mason 1985; Last et al. 1987). Despite this temporal difference, our results are the first to suggest a general pattern of fungal richness from a below-ground perspective consistent with the Dighton and Mason (1985) model (henceforth referred to as Dighton and Mason model) that follows a timescale particular to the lodgepole pine-mixed conifer forest system in YNP. While the Dighton and Mason model is primarily based on observations from homogenous plantation and oldfield systems over a relatively short time period (6 to27 years), their predictions of higher organic matter content and higher soil moisture are universal among forest succession patterns. Our data are consistent with these factors as potential influences on ECM community structure in mixed conifer; however, to empirically demonstrate this requires a more rigorous experimental approach that must also differentiate the

possible effects of changing plant-host composition. We suggest that in addition to disturbance type, growing season length and climate, both of which influence forest growth and soil development patterns, might explain some of the temporal discrepancies between our data and the Dighton and Mason model.

Stand-replacing wildfire and fire interval patterns not only have considerable influence on vegetation patterns in YNP, they also appear to be closely linked with ECM community structure. Previous research in YNP, including our study, indicates that ECM species richness progressively increases after homogenous stands of lodgepole pine establish and mature (Stoll 1998; Byrd et al. 2000). Visser (1995), working in a wildfire-driven system similar to lodgepole pine, noted that ECM species richness progressively increased in chronosequences of jack pine (Pinus banksiana) then leveled off in mature stands that were 122 years-old. In contrast to our results, Jonsson et al. (1999b) found no significant differences in below-ground ECM species richness between chronosequences of recently burned and late-successional stands of Scots pine (Pinus sylvestris). They attributed this pattern to the low intensity surfaces fires that typify Scots pine forests in Sweden which fail to completely combust both the soil organic layer and the ectomycorrhizae below ground. Based on the severity of disturbance, one might intuitively expect lodgepole pine to have lower richness than mixed conifer since it was more recently affected by fire. However, data from YNP suggests that ECM fungi recover rapidly within the first eight years following wildfire (Stoll 1998). Therefore, stand-replacing wildfire in YNP may actually increase fungal richness in the post-fire pine community by combusting phenolic compounds that inhibit ECM fungi and/or leaving activated charcoal behind in the soil, which may have a detoxifying effect for over a century (Wardle et al. 1998).

Plant host composition

Initial hypotheses suggested that many fungal species have high specificity for specific plant hosts and that with increasing plant richness there would also be a concomitant rise in ECM fungal richness. Our data suggest just the opposite: as plant species richness increases ECM composition changes and richness decreases. Lodgepole pine is a primary colonizer that grows fast in open and moderately xeric conditions, whereas Engelmann spruce and subalpine fir grow and survive in areas with relatively higher soil moisture and shade (Stohlgren and Bachand 1997). It is possible that the quantity and/or quality of carbon resources available to ECM fungi are different between lodgepole pine and mixed conifer stands. Although this is speculative and remains to be tested, the increased prevalence of shade-tolerant species might limit the amount of carbon available to the soil because these tree species have slower growth rates; hence, the predominance of Engelmann spruce and subalpine fir in mixed conifer stands may only support fungal species capable of thriving under conditions of lower respiration and plant carbohydrate availability. Additionally, many lodgepole pine trees living in the mixed conifer stand are the oldest and largest tree species within the stand and are reaching the end of their lifespan and may have limited carbohydrates to invest in ECM fungi.

Sampling and community variation

Most below-ground ECM studies encounter high variation in species frequency and abundance which can be problematic when trying to infer relationships between fungi and environmental factors (see Horton and Bruns 2001). We encountered a few dominant species in each stand, a high number of rare species, and again relatively little overlap in composition. The estimated

level of total species richness was very different from what was observed in each stand respectively, indicating that we undersampled the community. Therefore, any differences in community composition that we have attributed to environmental factors could also be due to sampling error. Also, since we collected soil cores for both ECM identification and chemical analyses in different years, seasonal effects could have introduced more variation than we were able to account for, thus obfuscating any real relationships prevalent in the community. Similarly, we cannot discount the possibility of spurious relationships being generated in this way as well. In any case, caution should be exercised when attributing cause to any of the community patterns we have described above.

CONCLUSION

In summary, we found evidence that total below-ground ECM species richness declines along a natural successional gradient from homogenous stands of mature lodgepole pine to old growth mixed conifer. Evidence suggests that species richness increases in homogenous stands of lodepole pine, but dramatically declines after colonization and establishment of shade-tolerant species. We found marked differences in soil chemistry between stand types and several significant correlations between the community and soil data; however, the soil variables as sampled in this study did not have a significant relationship with species richness in the final model. Moreover, the broad category of stand-type was significant in the model, possibly representing unmeasured environmental factors that are influencing the ECM community. Species richness patterns were consistent with a generalized version of the Dighton and Mason (1985) model that follows a timeframe specific to the lodgepole pine/mixed conifer system in YNP. Future research of ECM communities in Yellowstone should address seasonal and annual variation in nutrient dynamics, including phenolic compounds, plant

carbohydrate supply, and other chemical variables not examined in this study, and how these might be related to the ECM community during succession. Together, this would enhance our understanding of vegetation dynamics in this important temperate ecosystem and would provide land managers with information on ECM fungi adapted to specific environments that could enhance forest regeneration and growth.

ACKNOWLEDGEMENTS

This project was funded by National Science Foundation grants DEB-9420141 and DEB-9726404 awarded to V.T. Parker and Ken Cullings. We thank Yellowstone National Park for their assistance and cooperation in all phases of this project, Ann Rodman for soil survey information, J.R. Blair and Dennis Desjardin for fungal identifications, and Ed Connor for advice on statistics. We are deeply indebted to Jerry Douglas for assisting with fieldwork and travel duties, Heather Brown-Douglas for additional financial and logistical support, as well as Frank Cipriano and the Conservation Genetics laboratory at San Francisco State University where all molecular work was conducted.

LITERATURE CITED

Agerer, R. 1987-1997. *Colour Atlas of Ectomycorrhizae*. 1st-8th Edition. Einhorn-Verlag, Germany.

Alvarez, I. F., D. L. Rowney, and F. W. Cobb, Jr. 1979. Mycorrhizae and growth of white fir seedlings in mineral soil with and without organic layers in a California forest. Canadian Journal of Forest Research 9:311-315.

Baar, J. 1996. The ectomycorrhizal flora of primary and secondary stands of *Pinus sylvestris* in relation to soil conditions and ectomycorrhizal succession. Journal of Vegetation Science 7:497-504.

Bending, G. D., and D. J. Read. 1995a. The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. Foraging behaviour and translocation of nutrients from exploited litter. New Phytologist 130:401-409.

Bending, G. D., and D. J. Read. 1995b. The structure and function of the vegetative mycelium of ectomycorrhizal plants. VI. Activities of nutrient mobilizing enzymes in birch litter colonized by Paxillus involutus (Fr.) Fr. New Phytologist 130:411-417.

Bidartondo, M. I., Ek, H., Wallander, H., and B. Soderstrom. 2001. Do nutrient additions alter carbon sink strength of ectomycorrhizal fungi. New Phytologist 151: 543-550.

Bills, G. F., G. I. Holtzman, and O. K. Miller, Jr. 1986. Comparison of ectomycorrhizal--basidiomycete communities in red spruce vresus northern hardwood forests in West Virginia. Canadian Journal of Botany 64:760-768.

Bruns, T. D. 1995. Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant and Soil 170:63-73.

Bruns, T. D., and M. Gardes. 1993. Molecular tools fo rthe identification of ectomycorrhizal fungi-taxon-specific oligonucleotide probes for suilloid fungi. Molecular Ecology 2:233-242.

Bruns, T. D., T. M. Szaro, M. Gardes, K. W. Cullings, J. Pan, D. L. Taylor, T. R. Horton, A. M. Kretzer, and M. Garbelotto. 1998. A sequence database for the identification of ectomycorrhizal basidiomycetes by sequence analysis. Molecular Ecology 7:257-272.

Burnham, K. P., and W. S. Overton. 1979. Robust estimation of population size when capture probabilities vary among animals. Ecology 60:927-936.

Byrd, K. B., V. T. Parker, D. R. Vogler, and K. W. Cullings. 2000. The influence of clear-cutting on ectomycorrhizal fungus diversity in a lodgepole pine (Pinus contorta) stand, Yellowstone National Park, Wyoming, and Gallatin National Forest Montana. Canadian Journal of Botany 78:149-156.

Cullings, K. W., and D. R. Vogler. 1998. A 5.8S nuclear ribosomal RNA gene sequence database: applications to ecology and evolution. Molecular Ecology 7:919-923.

Cullings, K. W., D. R. Vogler, V. T. Parker, and S. K. Finley. 2000. Ectomycorrhizal specificity patterns in a mixed Pinus contorta/Picea engelmannii forest in Yellowstone National Park. Applied and Environmental Microbiology 66:4988-4991.

Cullings, K. W., D. R. Vogler, V. T. Parker, and S. Makhija. 2001. Defoliation effects on the ectomycorrhizal community of a mixed *Pinus contorta/Picea engelmannii* stand in Yellowstone Park. Oecologia 127: 533-539.

Cullings, K. W. and S. Makhija. 2001. Ectomycorrhizal fungal associates of Pinus contorta in soils associated with a hot spring I Norris Geyser Basin, Yellowstone National Park, Wyoming. Applied and Environmental Microbiology 67:

Despain, D. 1990. *Yellowstone vegetation: Consequences of environment and history in a natural setting.* Roberts Rinehart Publishers, Santa Barbara, CA.

Dighton, J. and. P. A. Mason. 1985. Mycorrhizal dynamics during forest tree development. Pages 117-139 *in* D. Moore, Casselton, L.A., Wood, D.A., and J.C. Frankland, editor. Developmental Ecology of Higher Fungi. Cambridge University Press.

Gardes, M., and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113-118.

Gardes, M., and T. D. Bruns. 1996. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. Canadian Journal of Botany 74:1572-1583.

Gehring, C. A., T. C. Theimer, T. G. Whitham, and P. Keim. 1998. Ectomycorrhizal fungal community structure of pinyon pine growing in two environmetal extremes. Ecology 79:1562-1572.

Gehring, C. A. and T. G. Whitham. 1994. Comparisons of ectomycorrhizae on pinyon pines (Pinus edulis; Pinaceae) across extremes of soil type and herbivory. American Journal of Botany 81: 1509-1516.

Grogan, P., Baar, J., and T. D. Bruns. 2000a. Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. Journal of Ecology 88: 1051-1062.

Hampp, R., J. Wiese, S. Mikolajewski, and U. Nehls. 1999. Biochemical and molecular aspects of C/N interaction in ectomycorrhizal plants: an update. Plant and Soil 215:103-113.

Hattenschwiler, S., and P. M. Vitousek. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends in Ecology and Evolution 15:238-243.

Horton, T. R., and T. D. Bruns. 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (Pseudotsuga menziesii) and bishop pine (Pinus muricata). New Phytologist 139:331-339.

Horton, T. R., and T. D. Bruns. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular Ecology 10:1855-1871.

Horton, T. R., T. D. Bruns, and V. T. Parker. 1999. Ectocmycorrhizal fungi associated with Arctostaphylos contribute to Pseudotsuga menziesii establishment. Canadian Journal of Botany 77:93-102.

Jonsson, L., A. Dahlberg, M.-C. Nilsson, O. Karen, and O. Zackrisson. 1999a. Continuity of ectomycorrhizal fungi in self-regenerating boreal Pinus sylvestris forests studied by comparing mycobiont diversity on seedlings and mature trees. New Phytologist 142:151-162.

Jonsson, L., A. Dahlberg, M-C. Nilsson, O. Zackrisson, and O. Karen. 1999b. Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. Molecular Ecology 8:205-215.

Jonsson, L., A. Dahlberg, and T-E. Brandrud. 2000. Spatiotemporal distribution of na ectomycorrhizal community in an oligotrophic Swedish Picea abies forest subjected to experimental nitrogen addition: above- and below-ground views. Forest Ecology and Management 132:143-156.

Karen, O., N. Hogberg, A. Dahlberg, L. Jonsson, and J.-E. Nylund. 1997. Inter- and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. New Phytologist 136:313-325.

Kranabetter, J. M. 1999. The effect of refuge trees on a paper birch ectomycorrhiza community. Canadian Journal of Botany 77:1523-1528.

Kranabetter, J. M., S. Hayden, and E. F. Wright. 1999. A comparison of ectomycorrhiza communities from three species planted on forest gap edges. Canadian Journal of Botany 77:1193-1198.

Last, F. T., Dighton, J., and Mason, P.A. 1987. Succession of sheathing mycorrhizal fungi. Trends in Ecology and Evolution 2:157-161.

Neary, D. G., Klopatek, C. C., DeBano, L. F., and P. F. Ffolliott. 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecology and Management 122: 51-71.

Newton, A. C. and J. Haigh. 1998.Diversity of ectomycorrhizal fungi in the UK: a test of the species-area relationship and the role of host preference. New Phytologist 138: 619-627.

Nichols, J. D., T. Bouliner, J. E. Hines, K. H. Pollock, and J. R. Sauer. 1998. Inference methods for spatial variation in species richness and community composition when not all species are detected. Conservation Biology 12:1390-1398.

Rexstad, E. and K.Burnham. 1991. User's guide for interactive program CAPTURE. Abundance estimation of closed animal populations. Colorado State University, Fort Collins.

Rodman, A., H. F. Shovic, and D. Thoma. 1996. Soils of Yellowstone National Park. Publication YCR-NRSR-96-2. Yellowstone Center for Resources, Yellowstone National Park, Wyoming.

Smith, S. E., and D. J. Read. 1997. Mycorrhizal Symbiosis, Second Edition edition. Acadamic Press.

Stendell, E. R., Horton, T. R., and T. D. Bruns. 1999. Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada ponderosa pine forest. Mycological Research 103: 1353-1359.

Stohlgren, T. J. and R. R. Bachand. 1997. Lodgepole pine (*Pinus contorta*) ecotones in Rocky Mountain National Park, Colorado, USA. Ecology 78: 632-641.

Stoll, W. W. 1998. Ectomycorrhizal fungal community composition and structure in young lodgepole pine forests. Master's Thesis. San Francisco State University.

Swofford, D. L. 2000. Phylogenetic Analysis Using Parsimony. Version 4. Sinauer Associates, Sunderland, Massachusetts, USA.

Taylor, D. L., and T. D. Bruns. 1999. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Molecular Ecology 8:1837-1850.

Visser, S. 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytologist 129:389-401.

Wallender, H. 1995. A new hypothesis to explain allocation of dry matter between mycorrhizal fungi and pine seedlings in relation to nutrient supply. Plant and Soil 168-169: 243-248.

Wardle, D. A., O. Zackrisson, and M.-C. Nilsson. 1998. The charcoal effect in Boreal forests: mechanisms and ecological consequences. Oecologia 115:419-426.

Wicklow, D. T. 1988. Parallels in the development of post-fire fungal and herb communities. Proceedings of the Royal Society of Edinburgh 94B: 87-95,

Wuzburger, N., Bidartondo, M. I., and C. S. Bledsoe. 2001. Characterization of Pinus ectomycorrhizas from mixed conifer and pygmy forests using morphotyping and molecular methods. Canadian Journal of Botany 79: 1211-1216.

Table 1: Description of the forest stands

Stand Characteristics	Mixed Conifer			Pure Lodgepole		
Forest Age (yrs)	~250-300			~135		
Size of Area (ha)	1			2		
Canopy Cover (%)	60.6 (1.6)			56.2 (0.83)		
Total Basal Area (m^2/ha)	42.1			44.1		
Pinus contorta	12.1			44.1		
Picea engelmannii	22.8			-		
Abies albicaulis	7.2			-		
Total Tree Density (trees/ha)	1782.5			1400.5		
Pinus contorta	594.2			1400.5		
Picea engelmannii	891.3			-		
Abies albicaulis	297.0			-		
	May	July	Sept.	May	July	Sept.
Seasonal Soil Moisture (%)*	27.1 (1.7)	13.5 (1.2)	4.3 (1.0)	19.0 (1.7)	4.0 (0.6)	1.0 (0.3)
Seasonal Soil Temperature (C)*	4.0 (0.2)	14.3 (0.3)	9.7 (0.9)	4.5 (0.1)	16.3 (0.4)	12.0 (0.5)

* Data collected during growing season in 2000

Table 2: Ectomycorrhizal Root Tip and Species Distribution (SE)

	Mixed Conifer	Lodgepole Pine	p-value
Total ECM Tips[1]	5933	5570	-
Mean Tip #/Core	219.7 (42.0)	206.3 (19.4)	0.77*
Mean # Species/Core	2.48 (0.27)	8.81 (0.56)	<0.01*

[1]Based on the total of Polymerase Chain Reaction (PCR) amplied tips.

* Student's t test assuming unequal variances

Table 4: Top 35 ECM fungal taxa detected in lodgepole pine and mixed conifer illustrating core frequency and relative root tip abundance

Fungal Species Mixed Conifer	Core Frequency	% ECM Tip Abundance	Fungal Species Lodgepole Pine	Core Frequency	% ECM Tip Abundance
Russula-38	8	5.31	BF-1	21	7.14
Hygrophorous-50	5	14.17	*Cenococcum geophilum*	17	8.68
Cortinarius-11	5	11.53	Cantharelloid-1	14	8.61
Cortinarius-10	5	7.99	Tricholomatoid-1	14	6.65
Suillus tomentosus	5	4.38	Thellephoroid-1	11	4.74
Cortinarius-65	2	10.75	*Suillus*-1	10	2.37
Russula-43	3	3.03	Thellephoroid-2	8	5.09
Cortinarius-6	2	5.28	Tricholomatoid-55	8	2.76
Russula-39	2	2.95	Russuloid-2	6	2.21
B-80	2	2.92	*Cortinarius*-23	5	5.20
Suillus-2	2	2.36	B-5	5	4.16
Tricholoma-52	2	1.55	B-10	5	0.68
Hebeloma-15	2	1.30	*Russula* 38	4	4.25
Cortinarius-17	1	4.21	Thellephoroid-3	4	2.15
Inocybe-11	1	4.05	B-11	4	0.36
Russula-37	1	2.19	B-9	4	0.16
Cortinarius-20	1	2.11	F-8	3	1.22
Cortinarius-109	1	1.69	*Inocybe*-25	3	0.65
B-81	1	1.52	BF-2	3	0.47
Hygrophorous-49	1	1.26	B-6	3	0.25
Inocybe-25	1	1.26	B-16	2	3.50
Cortinarius-15	1	1.18	Cortinaroid-2	2	2.37
Inocybe-15	1	1.18	Tricholomatoid-2	2	1.92
Cortinarius-NM	1	1.01	AF-5	2	1.65
Russula-11	1	0.96	F-3	2	1.40
Lactarius-42	1	0.84	*Hygrophorous*-87	2	1.08
Tricholoma-54	1	0.76	F-6	2	0.97
Cortinarius-16	1	0.67	*Cortniarius*-11	2	0.23
Cortinarius-14-96	1	0.51	*Cortinarius*-23b	2	0.18
B-83	1	0.51	Cortinaroid-1	2	0.11
B-82	1	0.34	Russuloid-3	1	2.69
Cortinarius-207	1	0.13	*Inocybe*-77	1	1.44
Cortinarius-14	1	0.05	*Tricholoma*-52	1	1.10
B-84	1	0.03	*Cortinarius*-19	1	0.50
Chroogomphus-35	1	0.02	*Tricholoma*-51	1	0.11
			50 more RFLP Taxa	-	12.96

Table 3: Observed and Estimated ECM Fungal Species Richness for Lodgepole and Mixed Conifer Stands. *Estimations were calculated assuming Model M_h in CAPTURE (Rexstad and Burnham 1991)

Stand Type	Species Detected	Estimated Richness	Model Selected	SE	95% CI
Mixed Conifer	35	67	M_h	13.07	51-104
Lodgepole	85	137*	M_{tbh}	16.92*	114-182*

Table 5: Soil parameters shown with mean values (SE)

Parameter	Mixed Conifer	Pure Lodgepole	p-value
pH	3.71 (0.03)	4.02 (0.04)	<0.01
% Organic Matter	9.74 (0.86)	6.74 (0.83)	<0.01
% Total Nitrogen	0.22 (0.007)	0.24 (0.02)	0.70
$N\text{-}NO_3^-$ ppm	0.81 (0.24)	0.7 (0.16)	0.66
$N\text{-}NH_4^+$ ppm	19.9 (1.3)	13.6 (2.1)	<0.01
$X\text{-}K^+$ ppm	238.5 (11.7)	176.3 (12.7)	<0.01
Bray-P ppm	79.8 (6.7)	47.2 (2.7)	<0.01

Note: Soil data based on n=27 soil samples for each stand type. P-values are based on Student's t-test assuming unequal variances

Table 6: Correlation analysis (Pearson's r) of soil chemical variables to ECM species richness and tip abundance for pooled stand data.

	OM	Total-N	Bray-P	X-K^+	NH_4^+	NO_3^-	ECM Tips	Species Richness
pH	-0.573**	-0.308*	-0.467**	-0.362**	-0.404**	-0.103	-0.058	0.556**
OM		0.517**	0.487**	0.514**	0.458**	-0.034	0.113	-0.340*
Total-N			0.054	0.042	0.465**	-0.041	0.185	0.088
Bray-P				0.528**	0.404**	0.002	0.235	-0.326*
X-K^+					0.522**	0.144	0.006	-0.362**
NH_4^+						0.083	0.194	-0.404**
NO_3^-							-0.083	-0.103

* $p < 0.05$
** $p < 0.01$

Table 7: Results of analysis of covariance relating species richness per core to soil characteristics and stand type

Source	df	Type III ss	*F*	*P*	R^2
Model	3	2.949	41.077	0.000	0.711
Stand Type	1	1.376	57.491	0.000	
Intercept	1	0.052	2.187	0.145	
pH	1	0.004	0.183	0.670	
NH_4^+	1	0.069	2.895	0.095	
Error	50	1.197			

Figure 1: Location Map of Yellowstone National Park and Study Site

Figures 2a-2b: Ranked species distributions according to importance values based on the sum of relative ECM root tip abundance and relative core frequencies for the top 26 fungi in (a) mixed conifer and (b) mature lodgepole pine. Species identified to family or sub-family are followed by the *-oid* suffix. Abbreviations for unidentified taxa: B-basidiomycete (primers 1F/4B, mtLSU, or 5.8S placement); A-ascomycete (5.8S placement); F-fungal (1F/4).

Figure 3: Rank abundance curve for all detected species in mixed conifer and mature lodgepole pine stands depicted on a logarithmic scale.

Figure 4: Percent total ECM root tip abundance according to familial grouping, fungus type, and percent unknown in mixed conifer and mature lodgepole pine.

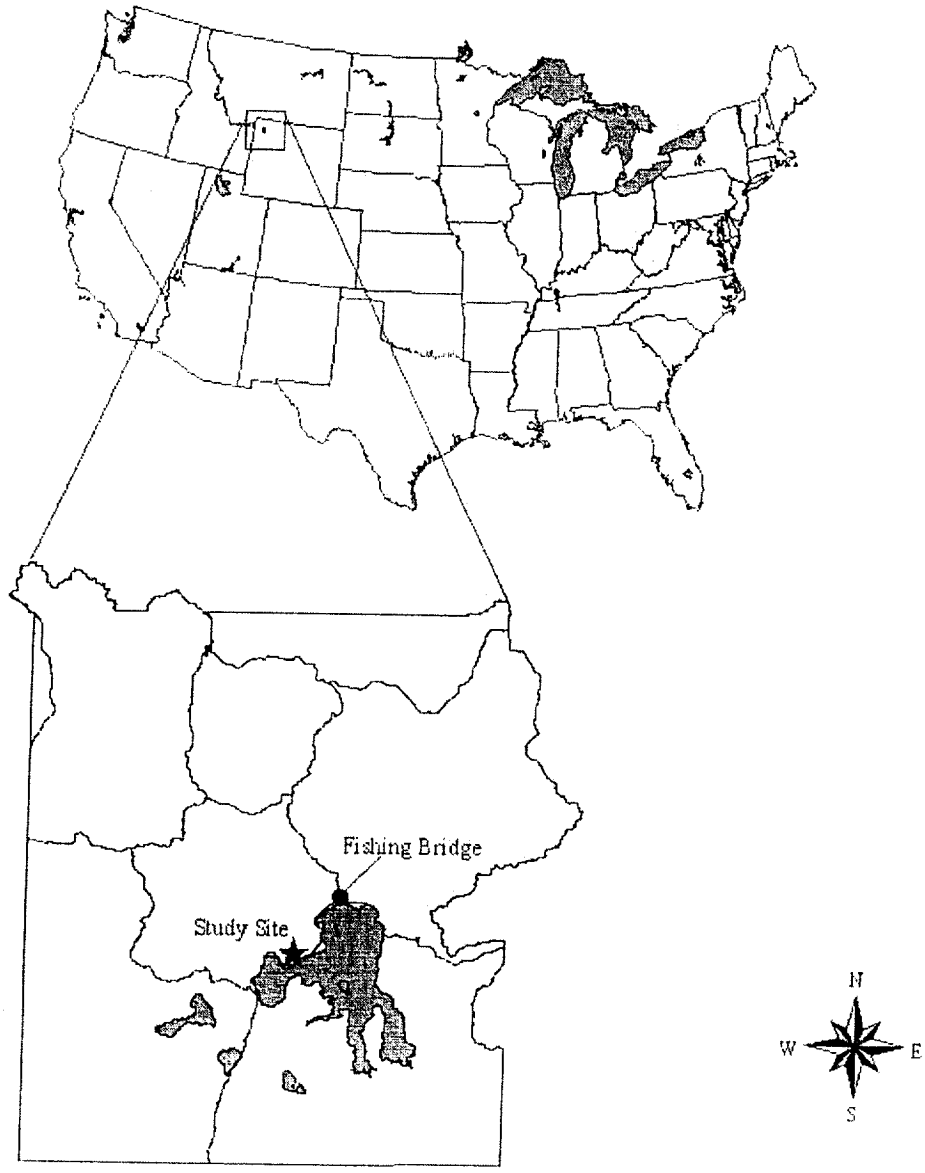
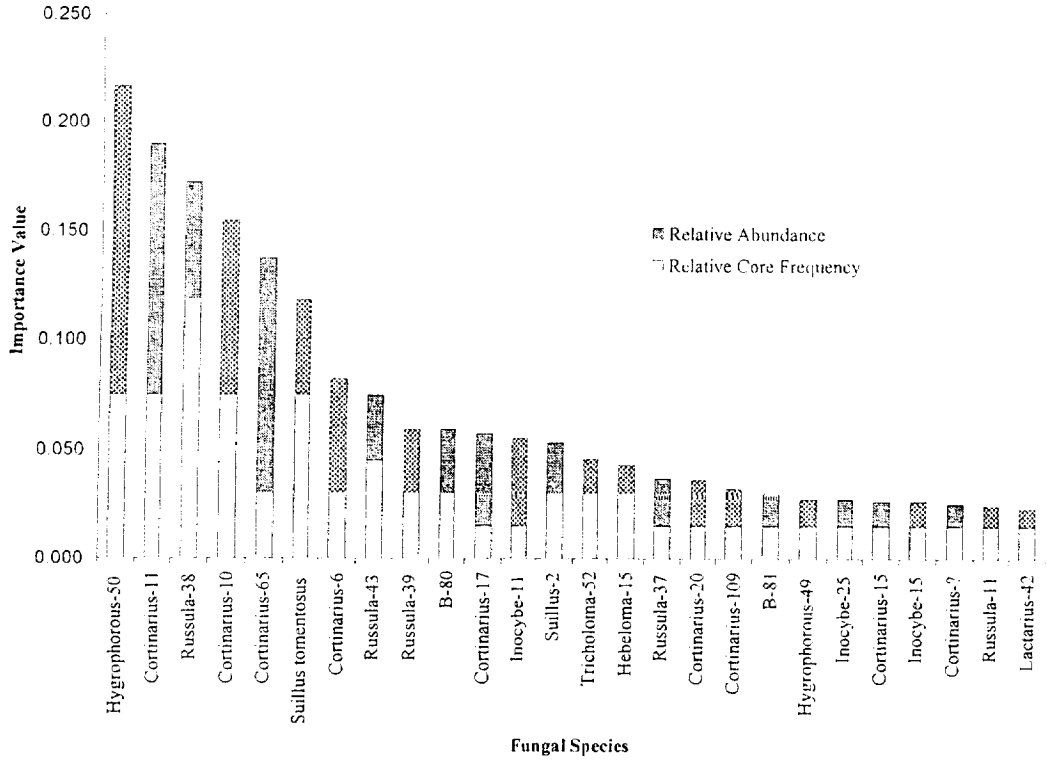
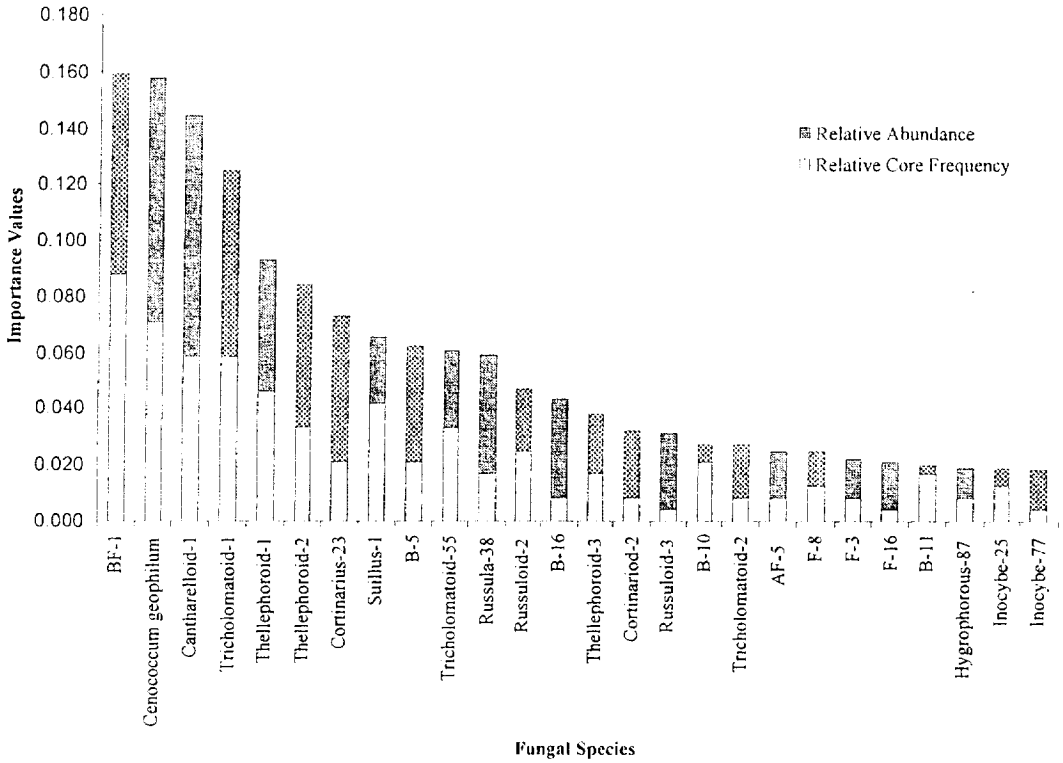


Figure 1 : Location map for Yellowstone National Park and study site.

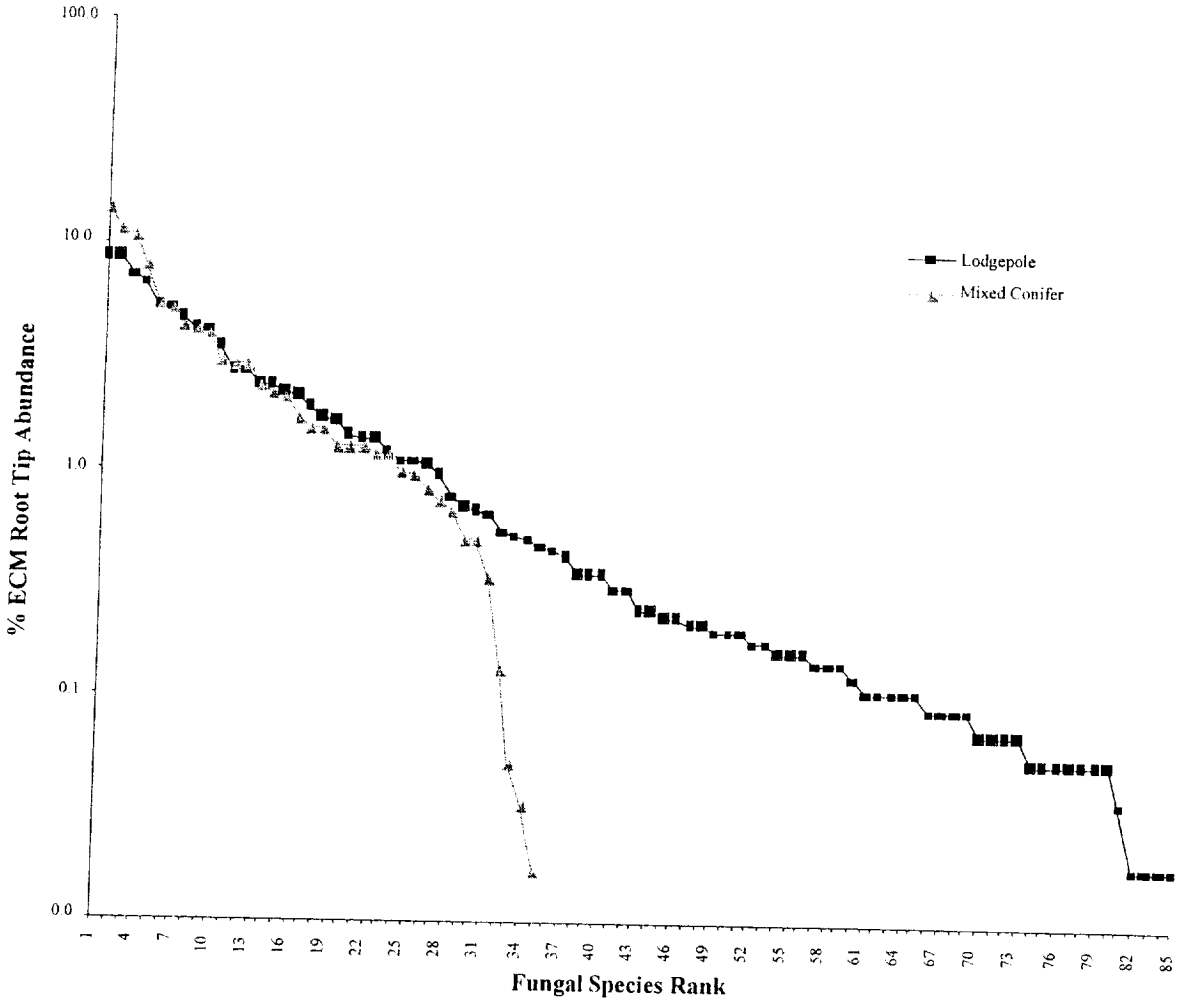
Ranked Fungal Taxa According to Importance Values Based on Relative Core Frequency and Relati Abundance in Mixed Conifer



Ranked Fungal Taxa According to Importance Values Based on Relative Abundance and Relative Co Frequencies in Lodgepole Pine



Rank Abundance for ECM Fungal Species in Lodgepole Pine and Mixed Conifer



Percent Total of ECM Root Tip Abundance in Lodgepole Pine and Mixed Conifer Stands According to Inclusive Groupings