



Belowground ectomycorrhizal community structure of mature lodgepole pine and mixed conifer stands in Yellowstone National Park

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Abstract

Forest development patterns following wildfire are known to influence the physical and chemical attributes of soils at different points in time, and are further thought to influence ectomycorrhizal (ECM) community structure. We used molecular methods to compare belowground ECM species richness, composition, and abundance between adjacent stands of homogenous lodgepole pine (established after a fire around 1867) and old growth mixed conifer (around 300 years old) 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 (81 versus 35) and the mean number of species per core (8.7 ± 0.5 versus 2.5 ± 0.3) were significantly higher in lodgepole pine. Species compositions were widely disparate between stands where only four of 112 species were shared. Soil analysis revealed that mixed conifer was significantly lower in mean pH, but higher in mean organic matter, potassium, phosphorus, and ammonium when compared to lodgepole pine. Although analysis of covariance did not statistically demonstrate that soil chemistry is driving ECM community structure with certainty, our data are, nonetheless, consistent with this hypothesis. Our data further suggest that fungal richness declines and composition shifts some time after Engelmann spruce and subalpine fir colonize mature lodgepole pine stands, and that time since last wildfire influences soil chemistry in this system. Moreover, because of data limitations, future field experiments will be necessary to determine if soil chemistry, as well as other biotic and abiotic factors not examined in this study, is a primary influence on ECM community structure at our study site in YNP.

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1. Introduction

A prevalent view in fungal ecology holds that soil resources and fertility influence the ectomycorrhizal

(ECM) plant–fungal relationship, fungal colonization levels, species richness and composition patterns (Alvarez et al., 1979; Dighton and Mason, 1985; Gehring and Whitham, 1994; Gehring et al., 1998; Conn and Dighton, 2000). 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 ECM fungi, and thus, affect overall ECM community structure (Dighton and Mason, 1985; Last et al., 1987).

Previous studies examining ECM community structure have focused on a variety of forest systems, but only a few have documented belowground 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 plant 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.

High ECM diversity, typical in most conifer dominated systems, is suspected to maintain belowground continuity in recovering plant communities by providing a pool of mycobionts capable of thriving under a variety of environmental conditions (Perry et al., 1989). The proximity of seedlings to sources of ECM inocula (e.g. forest edges, patches of surviving trees) is also thought to be a significant factor in the rate of plant establishment and succession following disturbance (Kranabetter, 1999; Kranabetter et al., 1999; Jonsson et al., 1999a).

Currently, little is known about how temporal changes in soil factors accompanying forest development and succession 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. Many belowground 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; Stendell et al., 1999; 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 (Gehring et al., 1998; Horton et al., 1999; Cullings and Makhija, 2002; Cullings et al., 2003).

In this study, we assessed belowground ECM community structure and soil chemistry at two points of a natural successional gradient in Yellowstone National Park (YNP) where periodic stand-replacing wildfire is common. Here, we examined ECM fungal species richness, abundance and composition, and their possible relationship to soil nutrients 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, both derived from the same soil parent material. 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 the establishment of late-successional species (Cullings et al., 2000). Based on this, and the close proximity of the stands to each other, we hypothesized that lodgepole pine and mixed conifer would have similar fungal richness and composition patterns among system dominants, as well as similar soil characteristics. Alternatively, however, if significant soil differences were found, we hypothesized shifts in ECM fungal community structure.

2. Methods

2.1. Study sites

The study area is located 17.9 km west of Fishing Bridge along Grand Loop Road (UTM Zone 12, 4,922,620 m N, 540,160 m E; elevation 2430 m) in Yellowstone National Park (YNP), Wyoming, and encompassed 4 ha of variously aged coniferous forest (Fig. 1). Individual sampling sites, separated

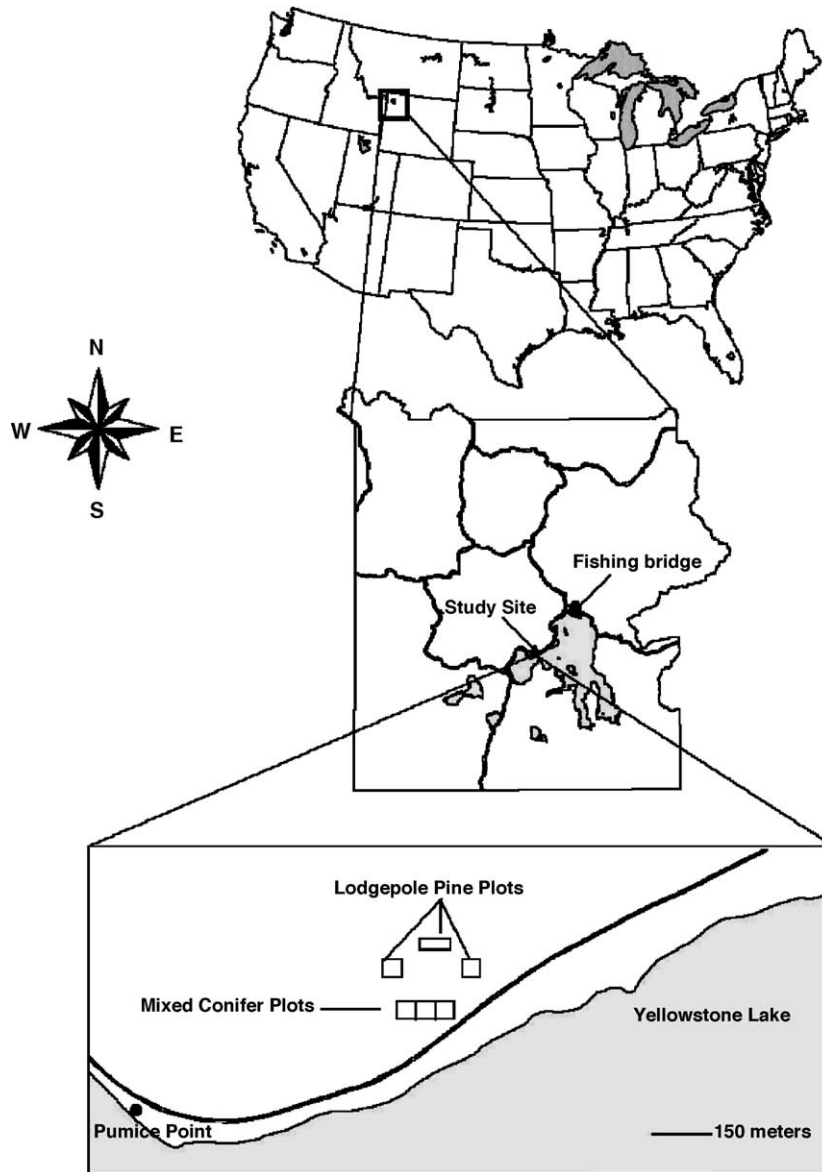


Fig. 1. Location map of Yellowstone National Park and study site.

by 75–200 m, were selected from two adjacent forest types representing distinct successional stages: (1) a 135-year-old lodgepole pine stand (*Pinus contorta* Douglas ex Louden); and (2) a 300-year-old mixed coniferous stand comprising lodgepole pine, Engelmann spruce (*Picea engelmannii* Parry ex Engelmann), and subalpine fir (*Abies lasiocarpa* Nuttall ex Hooker). Lodgepole pine stand-age was estimated by

counting tree ring data sampled from 99 trees surrounding soil core locations, and was consistent with previous research indicating the presence of a stand-replacing wildfire around 1867 (W. Romme, pers. comm). Tree size and frequency distributions indicated an old-growth-type structure for the mixed conifer stand, and the juxtaposition of both stand types were further corroborated by an existing

Table 1
Description of the forest stands

Stand characteristics	Lodgepole pine ^a	Mixed conifer ^a
Forest age (years)	135	300
Size of area (ha)	2	2
Canopy cover (%)	56.2 (0.83)	60.6 (1.6)
Total basal area (m ² /ha)	44.1	42.1
<i>P. contorta</i>	44.1	12.1
<i>P. engelmannii</i>	–	22.8
<i>A. lasiocarpa</i>	–	7.2
Total tree density (trees/ha)	1400.5	1782.5
<i>P. contorta</i>	1400.5	594.2
<i>P. engelmannii</i>	–	891.3
<i>A. lasiocarpa</i>	–	297.0

^a Values in parentheses are standard errors.

stand-age model for YNP (Tinker et al., 2003). Secondary succession typically begins with lodgepole pine readily establishing in open sites following fire, and depending on soil conditions, this species may form monospecific stands for 80–150 years, after which shade-tolerant species such as Engelmann

spruce and subalpine fir begin to colonize and later dominate (Romme, 1982; Despain, 1990).

Soils at the study site consist of glacial lacustrine sediments derived from rhyolite and are classified as sandy, mixed, superactive Typic Cryumbrepts and Cryochrepts (Rodman et al., 1996). Tree species composition and seasonal soil moisture patterns differed between forest types, while total basal area and mean canopy cover were similar (Table 1). Soil temperature was similar in the beginning of the growing season, but became slightly higher in the lodgepole pine stand as the growing season progressed (Table 2). Although understory vegetation was not quantitatively assessed, species composition and density differed between forest types. *Carex geyeri* was the dominant understory plant within the lodgepole pine sites, along with an occasional *Vaccinium scoparium* and *Fragaria vesca*. Bare ground and large woody debris in the form of treefalls were also more apparent. In contrast, mixed conifer sites contained a dense understory of *V. scoparium*, interspersed with *F. vesca*, *Chimaphila umbellata*, and

Table 2
ECM community and soil characteristics in lodgepole pine and mixed conifer

Characteristic	Lodgepole pine ^a			Mixed conifer ^a		
ECM community						
Total ECM root tips	5570			5933		
Mean root tips per core	206.3 (19.4)			219.7 (42.0)		
Mean species per core	8.67 (0.53) ^b			2.48 (0.27)		
Total species detected	81			35		
Estimated richness ^c	142 (18.3)			67 (13.0)		
95% C.I. ^c	116–189			51–104		
Model ^c	M _{tbh}			M _h		
Soil parameters (0–8 cm depth)						
pH	4.02 (0.04) ^b			3.71 (0.03)		
Organic matter (%)	6.74 (0.83) ^b			9.74 (0.86)		
Total nitrogen (%)	0.24 (0.02)			0.22 (0.01)		
NO ₃ ⁻ -N (μg/g)	0.70 (0.16)			0.81 (0.24)		
NH ₄ ⁺ -N (μg/g)	13.6 (2.1) ^b			19.9 (1.3)		
X-K ⁺ (μg/g)	176.3 (12.7) ^b			238.5 (11.7)		
Bray-P (μg/g)	47.2 (2.7) ^b			79.8 (6.7)		
	May	July	Sept.	May	July	Sept.
Mean soil moisture (%)	19.0 (1.7) ^b	4.8 (0.8) ^b	1.0 (0.3) ^b	27.1 (1.7)	13.5 (1.2)	4.3 (1.0)
Mean soil temperature (°C)	4.5 (0.1)	15.8 (0.5) ^b	12.0 (0.5) ^b	4.0 (0.2)	14.3 (0.3)	9.7 (0.9)

^a Values in parentheses are standard errors.

^b Significantly different ($P < 0.05$) from mixed conifer.

^c Estimations made with program CAPTURE (Rexstad and Burnham, 1991) using jackknife-estimator associated with M_h (Otis et al., 1978) for both stands. Although CAPTURE selected M_{tbh} as the best fitting model for the lodgepole pine stand, it does not have an estimator for this model. As a result, we used the estimator associated with model M_h because of its robustness to deviations in model assumptions.

several graminoid species. Dense mats of lichens and mosses were common and large woody debris was found in various stages of decomposition.

2.2. Soil sampling

A total of 27 soil cores were collected from each forest type to assess belowground ECM species richness, composition, and root tip abundance. A grid system was used to select core sites in two 50 m × 50 m plots and one 30 m × 80 m plot within the lodgepole pine stand during August 1998 and 1999, respectively (Fig. 1). We randomly selected three sites within each plot to extract soil cores. At each site, three 8 cm × 24 cm soil cores 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 cores were compared to another 27 cores collected in the same manner as above from three plots in a 50 m × 150 m area of adjacent mixed conifer in 1996 (Fig. 1).

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 mineral layer (0–8 cm depth), 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 a 2-mm mesh screen. Samples were submitted to the DANR (Division of Agriculture and Natural Resources) Analytical Laboratory, University of California at Davis, Davis, CA, USA, and analyzed for pH, organic matter, total nitrogen, ammonium, nitrate, potassium, and phosphorus following standard procedures (Cullings and Makhija, 2002; Cullings et al., 2003).

2.3. Ectomycorrhizal root tip sorting

Core samples were transferred to the laboratory on ice and refrigerated at 4 °C. Individual mycorrhizas were hand-sorted within 10 days into epitubes based on color and branching pattern as seen with a dissecting scope (Agerer, 1987–1997). 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. Following molecular identification (replicate number based on abundance, Cullings et al., 2000), tip numbers were consolidated for each fungal species and abundance data were tabulated for all soil cores.

2.4. 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 the nuclear ribosomal RNA gene repeat (rRNA) 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 followed the cycling parameters in Cullings et al. (2000).

2.5. Molecular identification

Successfully amplified ITS products were subjected to two restriction enzymes, *Hinf* I and *Alu* I, which are sufficient for identifying most fungal taxa within this system (Cullings et al., 2000, 2001). Restriction fragment length polymorphism (RFLP) patterns were compared with ITS-RFLP patterns from a sporocarp database comprising 200 fungal specimens collected in the study area as well as other locations in YNP.

Sequences corresponding to the 5.8S nuclear rRNA gene and mitochondrial large subunit (mtLSU) 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 basidiomycete, while mtLSU rRNA sequences were used to identify unknown basidiomycetes to family or 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 nuclear rRNA and mtLSU rRNA genes were entered into their respective databases with PAUP 4.0 beta-version 8 (Swofford, 2000).

Additional confirmation of known and unknown RFLP-species was further corroborated by searching for similar ITS sequences in GenBank using the alignment tool BLAST (Wurzbarger et al., 2001). Recent publications have warned of misleading sequence information contained within GenBank, and hence, an increased potential for misidentifying unknown species using BLAST searches (Vilgalys, 2003; Bruns and Shefferson, 2004). We tested this method using BLAST with ITS sequences from seven known species and they were all correctly identified to genus (see Appendix A).

2.6. 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 both the Student's *t*-test, assuming unequal variances, and the non-parametric Mann–Whitney *U*-test. All data were inspected for normality and homogeneity of variance using the Kolmogorov–Smirnov test and *F*-test, respectively. Data not conforming to parametric assumptions were log-transformed.

We used analysis of covariance (ANCOVA) to examine both species richness and ECM root tip abundance (at the soil core scale) and their possible relationships with soil chemistry 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 backwards elimination multiple regression model where only non-significant variables were successively eliminated. Once having a subset of significant covariates, dummy variables were coded 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.

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). We used the jackknife estimator associated with model M_{th} , which assumes heterogeneity in detection probabilities among species, to estimate richness in both stands (Otis et al., 1978; Burnham and Overton, 1979). This model is considered robust to deviations in model assumptions and is preferable to count data, which assume that all species have an equal probability of detection (Nichols et al., 1998).

3. Results

3.1. Fungal distribution patterns

We detected a total of 81 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 (Table 2).

Only four fungal species were shared between stand types (*Inocybe*-25, *Tricholoma*-52, *Russula*-38, *Cortinarius*-11), most of which were infrequent–rare in lodgepole pine (Table 3). *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 3). Consistent with previous reports, we did not detect any ascomycetes in mixed conifer (Cullings et al., 2000). Two species of ascomycetes were found in lodgepole pine including a system dominant, *Cenococcum geophilum*, which comprised just over 11% of the total ECM root tips and was present in 19 soil cores (70%).

Both stands differed in their composition of dominant fungal species (Fig. 2a and b). Dominant fungal species in mixed conifer typically exhibited lower core frequency and higher root tip numbers per

Table 3

Thirty-five ECM fungal taxa detected in lodgepole pine and mixed conifer illustrating core frequency and relative root tip abundance

Lodgepole pine			Mixed conifer		
Fungal species	Core frequency	ECM tip abundance (%)	Fungal species	Core frequency	ECM tip abundance (%)
<i>Piloderma</i> -1 ^a	21	7.16	<i>Russula</i> -38	8	5.31
<i>Cenococcum geophilum</i> ^b	19	11.02	<i>Hygrophorus</i> -50	5	14.17
B-1	14	11.44	<i>Cortinarius</i> -11	5	11.53
<i>Cortinarius</i> -69 ^b	14	6.68	<i>Cortinarius</i> -10	5	7.99
<i>Tricholoma</i> -55 ^b	11	7.97	<i>Suillus tomentosus</i>	5	4.38
Thelephoroid-1	11	4.74	<i>Cortinarius</i> -65	2	10.75
<i>Suillus</i> -1 ^b	10	2.24	<i>Russula</i> -43	3	3.03
<i>Thelephora</i> -2 ^a	9	5.24	<i>Cortinarius</i> -6	2	5.28
<i>Russula</i> -2 ^a	6	2.21	<i>Russula</i> -39	2	2.95
B-5	5	4.17	B-80	2	2.92
B-10	5	0.68	<i>Suillus</i> -2	2	2.36
Thelephoroid-3	4	2.15	<i>Tricholoma</i> -52	2	1.55
B-11	4	0.36	<i>Hebeloma</i> -15	2	1.30
B-9	4	0.16	<i>Cortinarius</i> -17	1	4.21
<i>Phialophora</i> -1 ^b	3	1.74	<i>Inocybe</i> -11	1	4.05
<i>Russula</i> -38 ^b	3	1.42	<i>Russula</i> -37	1	2.19
F-8	3	1.22	<i>Cortinarius</i> -20	1	2.11
<i>Inocybe</i> -25	3	0.65	<i>Cortinarius</i> -109	1	1.69
BF-2	3	0.47	B-81	1	1.52
B-6	3	0.25	<i>Hygrophorus</i> -49	1	1.26
B-16	2	3.48	<i>Inocybe</i> -25	1	1.26
Cortinarioid-2 ^c	2	2.37	<i>Cortinarius</i> -15	1	1.18
Tricholomatoid-2	2	1.92	<i>Inocybe</i> -15	1	1.18
<i>Hygrophorus</i> -87	2	1.08	<i>Cortinarius</i> -NM	1	1.01
B-14	2	0.70	<i>Russula</i> -11	1	0.96
B-13	2	0.52	<i>Lactarius</i> -42	1	0.84
<i>Cortinarius</i> -11	2	0.23	<i>Tricholoma</i> -54	1	0.76
<i>Cortinarius</i> -23	2	0.18	<i>Cortinarius</i> -16	1	0.67
Cortinarioid-1 ^c	2	0.11	<i>Cortinarius</i> -14-96	1	0.51
<i>Gymnomyces</i> -1 ^a	1	2.68	B-83	1	0.51
<i>Inocybe</i> -77	1	1.44	B-82	1	0.34
<i>Tricholoma</i> -52	1	1.10	<i>Cortinarius</i> -207	1	0.13
<i>Cortinarius</i> -19 ^b	1	0.50	<i>Cortinarius</i> -14	1	0.05
<i>Tricholoma</i> -51	1	0.11	B-84	1	0.03
<i>Gautieria</i> -1 ^a	1	0.05	<i>Chroogomphus</i> -35	1	0.02
46 more RFLP taxa	1 or 2	11.58			

The -oid suffix indicates mtLSU placement with family or higher order grouping; prefix 'B' indicates amplification with basidiomycete specific primers (ITS 1F/4B); prefix 'BF' indicates 5.8S placement with basidiomycetes; and prefix 'F' indicates amplification with fungal specific primers (ITS 1F/4).

^a Identified to genus using BLAST search for partial and complete ITS sequences (>95% sequence match).

^b RFLP match to fruiting body RFLP pattern and confirmed using BLAST search for partial and complete ITS sequences.

^c Blast search for partial or complete ITS supports designation (≥91% sequence match).

core than species in lodgepole pine (Fig. 2b). Four fungal species (*C. geophilum*, B-1, *Piloderma*-1, and *Cortinarius*-69), comprising 36.3% of the total ECM root tip number, were found in 14 or more soil cores in lodgepole pine. In contrast, the three most abundant fungal species in mixed conifer (*Hygrophorus*-50, *Cortinarius*-11, and *Cortinarius*-65), representing

36.5% of the total root tips, were found in only 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 but were stratified among a greater number of cores.

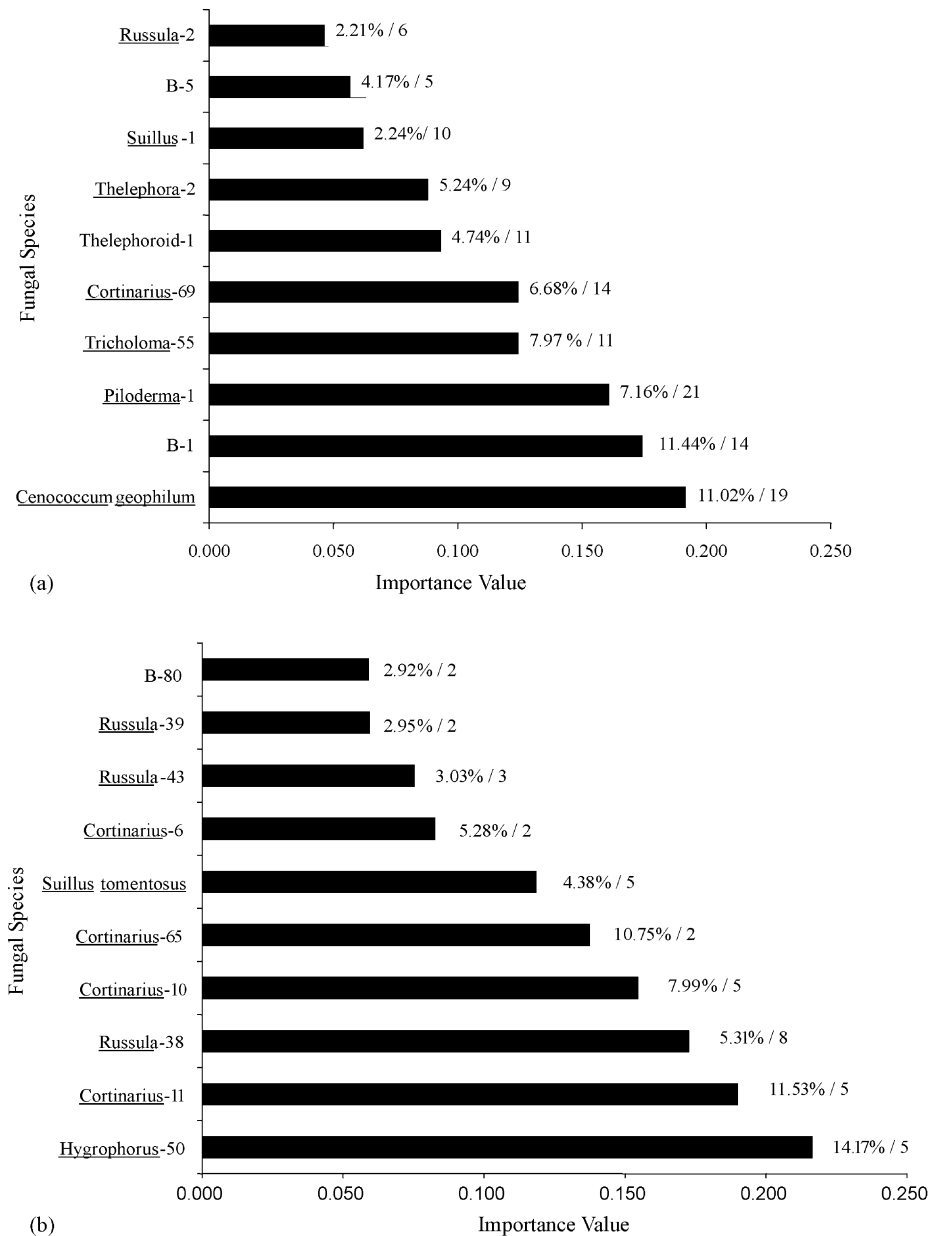


Fig. 2. (a and b) Ranked species distributions according to importance values based on the sum of relative ECM root tip abundance and relative core frequencies for the top 10 fungi in (a) mature lodgepole pine and (b) mixed conifer. Numbers at the end of each bar correspond to the percent of total root tips and core frequency for each species. Species identified to family or sub-family are followed by the -oid suffix. Abbreviation for unidentified taxa: B, basidiomycete (primers 1F/4B, mtLSU, or 5.8S placement).

Estimated total species richness for lodgepole pine and mixed conifer stands were 142 and 67 species, respectively (Table 2). Both stands were primarily comprised of rare species (species found in one or two

soil cores only), but nearly three times as many fungal species were detected in three or more soil cores in lodgepole pine than mixed conifer (Table 3). Differences between the actual and estimated richness can

be attributed to the accumulation of new species in each soil core with no leveling-off as more samples were analyzed. 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 2), which was probably due to the higher detection frequencies of several fungal species in lodgepole pine (Fig. 2a; Table 3).

The ranking of importance values based on fungal family and other inclusive groupings was also very different between stand types (Fig. 3). Identified root tips collected in lodgepole pine were distributed in nine taxonomic categories (eight families + ascomycetes), five of which had similar importance value rankings. However, there were a significant number of unidentified basidiomycetes (39 species representing 27.5% of the total root tips) and unidentified fungi (18 species representing 7.5% of the total root tips) that could potentially change these rankings in lodgepole

pine. Root tips collected in mixed conifer were distributed among six families, with the Cortinariaceae disproportionately representing greater than 50% of the total root tips. All families found in mixed conifer were also found in lodgepole pine, except for the Gomphidiaceae (*Chroogomphus*); members of the Thelephoraceae, Gautieraceae, Atheliaceae, and Ascomycota were found only in lodgepole pine.

3.2. Soil chemistry

Mean values of five soil parameters were significantly different between stand types ($P < 0.01$; Table 2). 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 low, but significant associations between soil chemical vari-

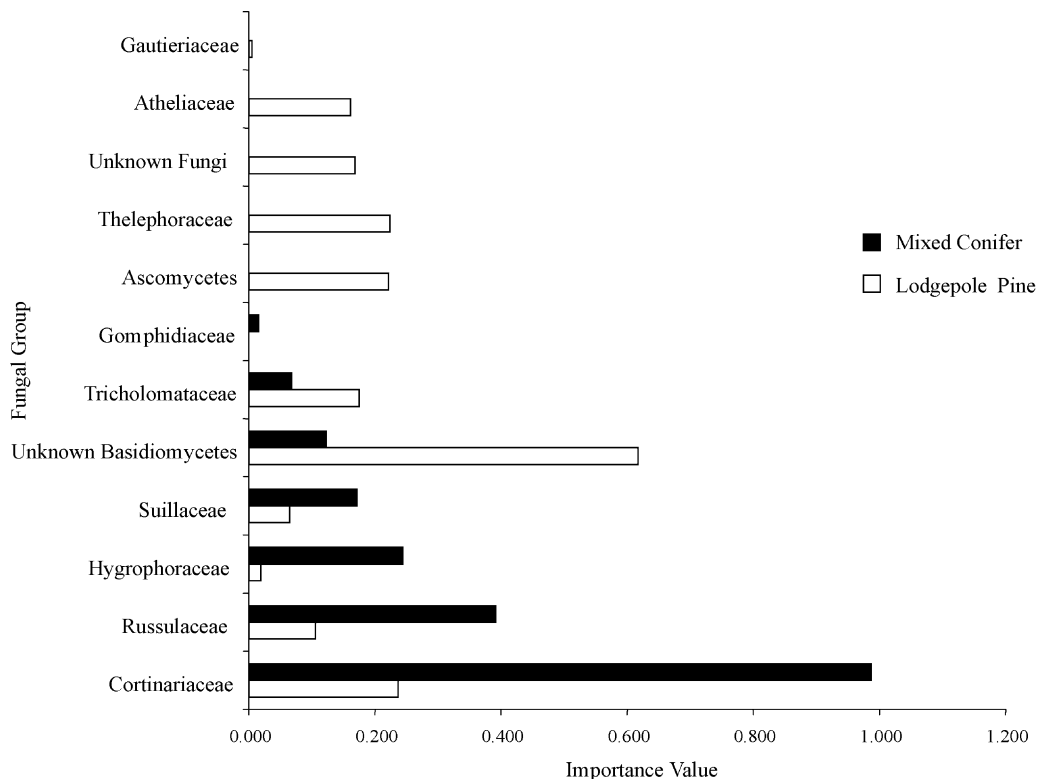


Fig. 3. Comparison of importance values according to familial grouping, fungus type, and unknown fungal species in mature lodgepole pine and mixed conifer.

ables and species richness per core for the pooled stand data (lodgepole + mixed conifer) only. Here, species richness was negatively correlated with ammonium, potassium, phosphorus, and organic matter, and positively correlated with pH (data not shown). Two of seven chemical covariates (pH, ammonium) were tested in the best regression model and neither covariate was significantly related to species richness after stand type was differentiated in the ANCOVA model. Stand type was the only factor explaining differences in mean species richness per core.

4. Discussion

Plant-host species, plant-host age, as well as soil nutrients and organic matter content may influence ECM species distribution patterns (Alvarez et al., 1979; Dighton and Mason, 1985; Bills et al., 1986; Gehring et al., 1998). Areas with high nutrient availability, particularly nitrogen, often exhibit lower ECM sporocarp and root tip abundance, and sometimes lower belowground species richness when compared to nutrient-poor areas (Lilleskov et al., 2001, 2002; Peter et al., 2001; Avis et al., 2003). Also, studies conducted in extreme environments (e.g. polluted, thermal, or desert areas) or areas with different plant hosts have found widely different fungal species compositions in comparison to less extreme reference stands (Gehring et al., 1998; Lilleskov et al., 2001, 2002; Cullings and Makhija, 2002), further reinforcing the idea that ECM species have diverse physiological tolerances enabling them to survive in many habitat types and conditions. In Yellowstone, wildfire and succession interact to create a mosaic of vegetative types and soil conditions that may select for different species of ECM fungi.

4.1. Species richness and composition patterns

Contrary to our initial hypothesis we found neither significant fungal species overlap, nor similar levels of species richness between lodgepole pine and mixed conifer stands. Although between-stand soil core distances ranged between 75 and 200 m, only four of 112 species were shared amongst both stands. Byrd et al. (2000) observed a similar disparity in species

overlap (10 of 106 species) among young and mature lodgepole pine stands, which in contrast to our study, were separated by 45 km. Others have noted higher percentages of both above- and belowground fungal species overlap in different aged homogenous pine (Visser, 1995; Stoll, 1998) and in mixed conifer/hardwood stands (Bills et al., 1986; Jonsson et al., 1999b), but information on proximity of sampling sites was often lacking. While proximity to fungal inocula is hypothesized to be an important factor in maintaining fungal composition over time (Jonsson et al., 1999a), we found no evidence to support this given our sampling design. If, however, this phenomenon occurs at a very small spatial scale, then it is possible that our between-stand sampling distances may have been too large to detect it (Lilleskov et al., 2004).

Species richness patterns were consistent with previous belowground investigations in Yellowstone that found high richness in homogenous stands of mature lodgepole pine (Stoll, 1998; Byrd et al., 2000) and comparatively lower richness in mixed conifer (Cullings et al., 2000, 2001). This suggests that as lodgepole pine transitions to a mixed conifer state, ECM species richness declines and composition shifts. Dighton and Mason (1985) and Last et al. (1987) proposed a model of fungal community structure predicting similar patterns, but in a totally different system following shorter time scales. They hypothesized that in addition to host-carbohydrate availability, soil organic matter and nutrient availability are important in shaping fungal distribution patterns as forests age. Interestingly, we found differences in community structure and soil chemistry between two points along a successional gradient.

While most ECM basidiomycete families were shared between lodgepole pine and mixed conifer (Cortinariaceae, Russulaceae, Tricholomataceae, Hygrophoraceae, and Suillaceae), within-family species distributions, as noted by importance values, were typically different between stands. The dominance of the Cortinariaceae in mixed conifer was extreme relative to the distribution of the other families, and its prevalence has been previously documented in undisturbed stands of mixed conifer (Cullings et al., 2000, 2001) and mature stands comprised of a single plant-host (Visser, 1995; Byrd et al., 2000). High species richness exhibited by the Cortinariaceae is not

easily generalized to forest age or a specific forest type; however, in YNP as well as other boreal forests, the predominance of this family is common (Visser, 1995; Jonsson et al., 1999b). Certain species within this family appear to be adapted to acidic soils (Wurzburger et al., 2001; Cullings and Makhija, 2002), which may explain their dominance in mixed conifer.

Horton and Bruns (2001) noted that many below-ground studies have found members of the Russulaceae and Thelephoraceae to be dominant in coniferous ecosystems worldwide. We documented the presence of both families in lodgepole pine, but only the presence of the Russulaceae in mixed conifer. Because 35% of the root tips in lodgepole pine were unidentified fungi, it is unknown whether the Thelephoraceae and Russulaceae are the most dominant fungal families at our study site. However, of the species we were able to identify, no single taxonomic group dominated the lodgepole pine stand. In fact, fungal species were more evenly distributed in most of the families compared to mixed conifer. Although the Russulaceae and Thelephoraceae are indeed a significant component in some forest stands in YNP, no clear patterns have emerged as to their dominance here (Byrd et al., 2000; Cullings et al., 2000, 2001).

The apparent absence of ascomycetes in mixed conifer, especially *C. geophilum*, is surprising. *C. geophilum* is an abundant, cosmopolitan species lacking sexual structures that readily reproduces via sclerotia and/or mycelia. It is found in numerous habitat types and seral stages, and in some instances, nearly every soil sample (Visser, 1995; Dahlberg et al., 1997; Smith and Read, 1997; Jonsson et al., 1999b, 2000; Horton and Bruns, 2001). 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; Cullings et al., 2003). However, a previous study in YNP failed to detect *C. geophilum* and other ascomycetes in old growth mixed conifer stands, suggesting that over time these species may be reduced in areas where disturbance has been absent for some time (Cullings et al., 2000). The dominance of *Cenococcum* in lodgepole pine and its apparent absence from our mixed conifer site is so striking that it merits further investigation.

4.2. Soil chemistry

Although we were unable to demonstrate a significant statistical relationship between ECM community structure and soil factors, we found that lodgepole pine not only contained a different suite of fungal species, but also had relatively lower nutrient concentrations and higher fungal species richness than mixed conifer. Several ECM studies in natural environments have associated soils containing lower organic matter and lower fertility with supporting different ECM species compositions and higher colonization levels, but not higher species richness (Alvarez et al., 1979; Gehring and Whitham, 1994; Gehring et al., 1998).

Coniferous forests in the Northern hemisphere are typically nitrogen-limited and research has shown a link between high soil-nitrogen levels and declines in above- and belowground ECM species richness, colonization levels, as well as changes in community composition (Lilleskov et al., 2001, 2002; Peter et al., 2001, Avis et al., 2003). We found substantial differences in fungal species richness and composition, but no strong correlations between community and nitrogen data. Although mean total nitrogen and nitrate levels were similar between stands, mean ammonium levels were statistically different, but still very small compared to the differences seen in other studies (Lilleskov et al., 2001, 2002). It is unknown whether these small differences in ammonium levels are biologically significant, and thus capable of influencing plant-fungal relationships (Wallender, 1995; Hamppe et al., 1999; Bidartondo et al., 2001).

The higher levels of fine organic matter and soil nutrients, as well as the presence of more decaying large woody debris and a well-developed layer of understory plants in the mixed conifer stand suggests that time since last wildfire is an important factor in determining not only plant community composition, but also soil moisture retention and fertility in this system. We propose that during forest development, the colonization of gaps by subalpine fir and Engelmann spruce, coupled with the increasing deposition of organic matter over time, increase tree density and soil moisture retention, which, in turn, creates an environment more conducive for nutrient mineralization (Singer and Munns, 1991).

Although we found higher mean concentration of extractable nutrients in mixed conifer, these results do not necessarily mean that nutrient availability is higher here. We assume that plant species composition and stand age influences the quality and quantity of litter input in each stand, thus contributing to differences in soil chemistry and mineralization rates (Conn and Dighton, 2000). Quantification of mineralization rates via litter bags or resin balls will be necessary to determine more accurately the total amount nutrients available to the fungal and plant communities in each stand throughout the growing season.

4.3. Sampling, community variation, and data limitations

Many belowground ECM studies encounter high variation in species frequency and abundance, which can be problematic when comparing species richness and composition between stands (see Horton and Bruns, 2001). Although we encountered several dominant species in each stand, both communities still had a high number of rare species and relatively little overlap in composition. The estimated level of total species richness was much higher than what was observed in each stand, respectively, indicating that undersampling was a problem. The use of species richness estimators addressed this issue, but given that our sample size represented a miniscule part of the community, it is entirely possible that we failed to accurately characterize species composition, and hence, underestimated the number of species shared between stands.

Because soil cores were collected for both ECM community and chemical analyses in different years, seasonal variation could have limited our ability to discern any relationship between them. Soil chemistry was also assessed at only a single point in time (late spring), which may not reflect soil conditions or nutrient levels throughout the growing season or year, let alone the total amount of nutrients released over time. Alternatively, soil moisture, rather than soil fertility, must also be considered a potential factor that may select for certain species of ECM fungi (Cullings et al., 2003); unfortunately, we did not measure this parameter in such a way that it could easily be linked to the ECM community data.

Previous research has shown that multiple-host fungi are common in several mixed species stands including mixed conifer stands in YNP (Horton and Bruns, 1998; Cullings et al., 2000). However, if the environmental context of the plant–fungal mutualism is different in each stand, then this may limit the breadth of fungal species able to colonize specific plant hosts or individuals of a host species. Thus, plant species commonly sharing fungal species within a stand may not share them with the same plant species in another stand. In this study, we did not investigate the potential effects of plant-host composition on fungal community structure, but since plants are the primary source of carbon for ECM fungi their possible influence on the community patterns cannot be discounted here. Plant-carbohydrate availability varies with plant species, age, nutritional status, etc. (Grayston et al., 1996), and is hypothesized to constrain ECM species composition to those with compatible carbon budgets (Dighton and Mason, 1985; Cullings et al., 2001). In future, studies measuring rhizosphere carbohydrate availability in each stand may address the effects of plant-host composition on ECM fungal community structure.

Overall, statistical analysis showed little support for linking ECM community structure with soil chemistry, and the biological significance of the observed soil chemical differences needs further investigation. However, we recognize that our design may have been more appropriate for assessing ECM community structure and soil chemistry, rather than testing the relationship between the two. Testing for potential causal mechanisms will require a rigorous experimental design that controls for multiple sources of temporal and spatial variation in soil factors and the ECM fungal community, as well as the potential influence of plant-host composition.

5. Conclusion

In summary, our data were consistent with a general model of fungal succession advanced in the literature (Dighton and Mason, 1985; Last et al., 1987) as well as the hypothesis that soil fertility influences richness and composition but not coloni-

zation levels. We found that fungal species richness was higher and composition was different in mature, homogenous lodgepole pine compared to old growth mixed conifer, suggesting that ECM richness declines and composition shifts some time after the establishment of shade-tolerant species. However, because several sources of variation were not controlled for here, it is unclear what factors are responsible for the observed fungal distribution patterns. Although several previous studies in YNP add support to our view that ECM community patterns observed in both stands are real, it remains to be seen if these patterns represent functional changes in the way ECM fungi cycle nutrients in this system. Future research on ECM communities in Yellowstone should address seasonal variation in nutrient availability, focusing on mineralization rates, phenolic compounds, as well as potential plant-host influences on soil chemistry and carbohydrate supply, and how these might be related to the ECM community during succession. This would enhance our understanding of vegetation dynamics

and ecosystem function and would provide land managers with information on ECM fungi adapted to specific environments that could enhance forest regeneration and growth.

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Appendix A

GenBank Accession Numbers for fungal taxa identified or confirmed by DNA sequencing of internal transcribed spacer (ITS) regions and/or mitochondrial large subunit (mtLSU) rRNA gene

Species	GenBank Accession Numbers		
	ITS (primers ITS-1F/ITS-4)	mtLSU rRNA gene (primers ML5/ML6)	ITS sequence similarity using BLAST searches ^a
<i>Piloderma</i> -1	AY822743	–	99% (577/580 base pairs (bp)) AY010281
<i>C. geophilum</i> ^b	AY822735	–	98% (939/948 bp) AY299214
<i>Cortinarius</i> -69 ^b	AY822739	–	96% (658/683 bp) AF335446
<i>Tricholoma</i> -55 ^b	AY822748	AY822756	97% (739/759 bp) AB078341
<i>Suillus</i> -1 ^b	AY822745	–	99% (667/673 bp) STU74614
<i>Thelephora</i> -2	AY822747	–	98% (677/685 bp) TTU83486
<i>Russula</i> -2	AY822744	AY822754	98% (591/598 bp) AY061720
<i>Phialophora</i> -1 ^b	AY822741	–	98% (824/834 bp) PFI534704
<i>Russula</i> -38 ^b	AY822742	AY822752	99% (599/604 bp) AF495466
BF-2	AY822734	–	–
Cortinarioid-2	AY822736	AY822749	91% (622/683 bp) AY174831
Cortinarioid-1	AY822740	AY822751	94% (279/296 bp) AF539731
<i>Gymnomyces</i> -1	AY822746	AY822753	96% (672/699 bp) AY239317
<i>Cortinarius</i> -19 ^b	AY822737	AY822750	97% (691/710 bp) AY174785
<i>Gautieria</i> -1	AY822738	–	97% (801/820 bp) AF377093
Thelephoroid-1	–	AY822757	–
Tricholomatoid-2	–	AY822758	–
Thelephoroid-3	–	AY822755	–

^a Percent pairwise similarity with identified sequences (Accession Numbers included) contained in GenBank.

^b Initially identified to genus by ITS-RFLP pattern.

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