Ectomycorrhizal fungal communities of black spruce differ between wetland and upland forests

Susan J. Robertson, Linda E. Tackaberry, Keith N. Egger, and Hugues B. Massicotte

Abstract: Ectomycorrhizal (ECM) communities of black spruce (*Picea mariana* (Mill.) BSP) seedlings were characterized from three habitats spanning a moisture gradient in central British Columbia: black spruce dominated wetlands, black spruce – tamarack wetlands, and black spruce – lodgepole pine uplands. Morphological and molecular (PCR-RFLP) analyses indicated a diverse community of root-associated ECM fungi consisting of 33 morphotypes and 65 genotypes. ECM abundance varied significantly between habitats for six morphotypes. Although many occurred in all three habitats, some occurred in only one or two, and some genotypes had distributions that suggested habitat specificity across the moisture gradient. Intraspecific variation (defined as genotype variation within morphotypes) ranged from one to seven genotypes, depending on ECM morphotype. Both morphological and molecular analyses showed that ECM diversity was greater in upland than in wetland habitats and greater in black spruce – tamarack wetlands than in black spruce dominated wetlands ($\alpha \leq 0.05$). Morphological assessment captured contributions to diversity by both abundant and less abundant ECM morphotypes, whereas molecular analysis revealed patterns of genetic variation and habitat distribution at a finer resolution. The study presents the first comprehensive information on black spruce ECM and suggests that ECM community composition and richness varies across the moisture gradient in response to soil heterogeneity and alternate hosts (tamarack and lodgepole pine).

Résumé : Les communautés ectomycorhiziennes (ECM) des semis d'épinette noire (*Picea mariana* (Mill.) BSP) ont été caractérisées dans trois habitats couvrant un gradient d'humidité dans le centre de la Colombie-Britannique : les terres humides dominées par l'épinette noire, les terres humides dominées par l'épinette noire et le mélèze et les hautes terres dominées par l'épinette noire et le pin lodgepole. Des analyses morphologiques et moléculaires (PCR-RFLP) ont révélé qu'il existait une communauté diversifiée de champignons ECM associés aux racines comprenant 33 morphotypes et 65 génotypes. L'abondance de six morphotypes d'ECM variait significativement selon l'habitat. Bien que plusieurs morphotypes aient été présents dans les trois habitats, certains étaient présents dans seulement un ou deux habitats et la distribution de certains génotypes suggérait qu'ils étaient spécifiques à un des habitats couvrant le gradient d'humidité. La variation intraspécifique (définie comme la variation du génotype dans un morphotype) allait de un à sept génotypes dépendamment du morphotype. Tant les analyses morphologiques que moléculaires ont montré que la diversité des ECM était la plus grande sur les hautes terres comparativement aux terres humides et plus grande sur les terres humides dominées par l'épinette noire et le mélèze que celles dominées par l'épinette noire ($\alpha \leq 0{,}05$). L'évaluation morphologique a permis de détecter la contribution à la diversité tant des morphotypes d'ECM abondants que moins abondants alors que l'analyse moléculaire a révélé les patrons de variation génétique et de distribution des habitats avec une meilleure résolution. Cette étude présente les premières informations détaillées sur les ECM de l'épinette noire et indique que la composition et la richesse de la communauté d'ECM varient en fonction du gradient d'humidité en réponse à l'hétérogénéité du sol et aux hôtes alternes (mélèze et pin lodgepole).

[Traduit par la Rédaction]

Introduction

The distribution of tree species and forest ecosystems across boreal landscapes is primarily determined by climate (Pitelka et al. 1997; Chapin et al. 2004). Current climate change models predict that changes in temperature (generally warming, with greater increases in winter than in summer and in minimum versus maximum diurnal temperatures) and precipitation (regional increases or decreases) will result in a spatial redistribution of major forest types and potential loss of habitat for some important boreal species (Hamann and Wang 2005). For example, increased precipitation in flat, maritime-influenced areas may lead to creation of peatlands and favour wetland species over other forest species (Chapin et al. 2004). Under conditions of continued warming and adequate drainage, forests may migrate northward if soil moisture does not limit tree growth. In areas influenced by more continental climates, drier soils may lead to replacement of certain forest species with steppe communities (Chapin et al. 2004). The response of plant communities to

Received 11 May 2005. Resubmitted 16 December 2005. Accepted 23 December 2005. Published on the NRC Research Press Web site at http://cjfr.nrc.ca on 1 April 2006.

S.J. Robertson,[1] L.E. Tackaberry, K.N. Egger, and H.B. Massicotte. Ecosystem Science and Management Program, College of Science and Management, University of Northern British Columbia, 3333 University Way, Prince George, BC V2N 4Z9, Canada.

[1]Corresponding author (e-mail: robertss@unbc.ca).

 doi:10.1139/X06-001

temperature and precipitation changes is related to complex interactions between soil biota, water table depth, nutrient availability, root distribution, and soil and root respiration (Wurzburger et al. 2004). A better understanding of the ecological, economic, and social consequences of plant migration may be gained through studies directed below ground, as the functioning of terrestrial ecosystems and their ability to adapt to environmental changes appears to depend on soil communities (Dahlberg 2001; Hart et al. 2001; Prosser 2002).

Ectomycorrhizas (ECMs) are essential to ecosystem functions including biogeochemical cycling, maintaining soil structure and forest food webs, and buffering plants against environmental stress (Brundrett 1991; Colpaert and van Tichelen 1994). The structure (species composition) and diversity (species richness and abundance) of ECM fungal communities vary across landscapes according to host specificity and edaphic factors (Molina et al. 1992; Allen et al. 2003). For example, Gehring et al. (1998) reported a change in pinyon pine (*Pinus edulis* Engelm.) ECM community composition across a soil moisture gradient, but no significant difference in species richness. The ECM community composition of white spruce (*Picea glauca* (Moench) Voss), Norway spruce (*Picea abies* (L.) Karst.), and European beech (*Fagus sylvatica* L.) also varied across nitrogen (N) deposition gradients, accompanied by a decline in ECM species richness with increasing soil N availability (Taylor et al. 2000; Lilleskov et al. 2002). Very few studies have attempted to relate ECM distribution to environmental gradients independent of host plant (O'Dell et al. 1999).

Black spruce (*Picea mariana* (Mill.) BSP), a dominant species of the Canadian boreal forest, exhibits broad tolerance to soil moisture levels. It typically grows in *Sphagnum*-dominated wetlands characterized by cold, poorly aerated peat soils with low nutrient availability, either in pure stands or mixed with tamarack (*Larix laricina* (Du Roi) K. Koch) (MacKenzie and Moran 2004). Along peatland gradients in Wisconsin, larch was found to dominate the wetter, more minerotrophic (fed by mineral-rich ground water) sites, whereas black spruce dominated drier, more ombrotrophic (fed by very nutrient-poor precipitation) sites (Montague and Givnish 1995). Black spruce is well adapted to water-saturated soil conditions, establishing on hummocks of woody debris and producing shallow roots that spread laterally (Lieffers and Rothwell 1987; Roy et al. 1999). Despite low dissolved oxygen and reducing conditions in wetland soils that may inhibit ECM formation by limiting fungal metabolism (Walker 1987), many woody plant species (including black spruce) in northern wetlands have been found to be ectomycorrhizal (Thormann et al. 1999; Wurzburger et al. 2004).

In adjacent upland forests, black spruce associates with lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia*), as well as with other tree, shrub, and herb species that generally indicate low to medium productivity plant communities developed over nutrient-poor, water-deficient soils (Krestov et al. 2000). Drier upland soils often exhibit a root-restricting clay horizon near the surface that perches the water table during wet periods of the year, leading to seasonal cycles of moisture surplus and deficit (Krestov et al. 2000). Upland trees often appear more robust than their wetland counterparts; however, improved edaphic conditions do not seem to result in ecotypic variation in black spruce morphological features or water relations (Parker et al. 1983; Zine El Abidine et al. 1994). Results from greenhouse and field experiments with Norway spruce (*Picea abies* (L.) Karst.) suggest that survival in seasonally dry soils may be due to an increased branching density of fine roots in response to water stress, allowing for uptake of water from dry soil via ECM (Feil et al. 1988). The presence of ECM on naturally regenerating and outplanted black spruce has been confirmed in upland habitats (Malloch and Malloch 1981; McAfee and Fortin 1989), but the ECM communities associated with this host species have not been previously described.

The objective of this study was to describe the ECM fungal communities associating with naturally regenerating black spruce seedlings from forest sites demarcating wet and dry habitats in central British Columbia. Sites included black spruce dominated wetlands, black spruce – tamarack wetlands, and black spruce – lodgepole pine uplands, which represent typical black spruce habitats that comprise a substantial component of the Canadian boreal forest (Burton et al. 2003). A combination of morphological (light microscopy) and molecular (polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP)) techniques were used to characterize the structure and diversity of ECM fungal communities and to assess potential differences between wetland and upland habitats that varied with respect to dominant vegetation and soil properties. PCR-RFLP results were also used to evaluate intraspecific variation (genotypic variation within characterized ECM morphotypes) for the ECM fungal community. We expected that some ECM fungi would be associated with black spruce across all three habitats, but that variable moisture and soil conditions and associated vegetation changes would contribute to community differences at both the morphotype and genotype levels. We also predicted lower diversity in the wetland habitats due to the stress imposed by water saturation of soils. Insights into ECM community variation across environmental gradients should provide a better understanding of how climate change will impact boreal forest landscapes and whether ECM systems might facilitate or mediate the movement of plant associations in response to change.

Materials and methods

Study area

The study area was located in the Sub-Boreal Spruce (SBS) biogeoclimatic zone in the central interior of British Columbia. The SBS ranges from 51°30′–59°N latitude and an elevation of 660–1140 m (Meidinger et al. 1991; DeLong and Fahlman 1996). Climate is characterized by severe and snowy winters, warm, moist, and short summers, and moderate annual precipitation (Meidinger et al. 1991).

Site descriptions

Three replicate sites were identified within each of three forest habitat types: black spruce dominated wetlands (W), mixed black spruce – tamarack wetlands (T), and mixed black spruce – lodgepole pine upland forests (U). The W sites, classified as black spruce – water sedge – peat moss bogs / poor fens (Wb05, MacKenzie and Moran 2004), were located approximately 60 km northwest of Prince George,

British Columbia, in the moist, cool subzone variant (SBSmk1) of the SBS. These bogs were dominated by mature black spruce stands with an understory of scrub birch (*Betula glandulosa* var. *glandulosa* Michx.), willow (*Salix* spp.), Labrador tea (*Ledum groenlandicum* Oeder), bog cranberry (*Vaccinium oxycoccos* L. MacM.), and bog rosemary (*Andromeda polifolia* L.).

The T sites, classified as tamarack – water sedge – fen moss bogs / poor fens (Wb06, MacKenzie and Moran 2004), were located approximately 40 km west of Prince George, in the dry, warm (SBSdw3) subzone variant of the SBS. Tamarack and black spruce were the dominant tree species, and the shrub understory consisted mainly of scrub birch, willow, and Labrador tea. In both wetland habitat types, site microtopography consisted of raised mounds (hummocks of partially decomposed moss that covered woody debris) amongst depressions (hollows) that were often below the level of the water table. *Sphagnum* moss covered most of the open areas, with other mosses and lichens (*Cladina* and *Peltigera* species) on drier hummocks and woody debris. Soils were classified as Typic Humisols, a subgroup of the Organic soils (Soil Classification Working Group 1998).

The U sites were located adjacent to the black spruce dominated wetlands (W sites). These sites were dominated by mature black spruce and lodgepole pine, with a small component of hybrid white spruce (*Picea glauca* × *Picea engelmannii* Parry ex Engelm.), subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), or sitka alder (*Alnus crispa* var. *sinuata* (Reg.) Rydb.). The understory contained a diversity of shrubs and herbs, including prickly rose (*Rosa acicularis* Lindl.), pink spirea (*Spiraea douglasii* subsp. *menziesii* Hook.), black twinberry (*Lonicera involucrata* (Richards.) Banks ex Spreng.), highbush cranberry (*Viburnum edule* (Michx.) Raf.), twinflower (*Linnaea borealis* L.), dwarf blueberry (*Vaccinium caespitosum* Michx.), trailing raspberry (*Rubus pubescens* Raf.), kinnikinnick (*Arctostaphylos uva-ursi* L. Spreng.), round-leaved rein-orchid (*Platanthera orbiculata*), rattlesnake plantain (*Goodyera oblongifolia* Raf.), one-sided wintergreen (*Orthilia secunda* (L.) House), pink wintergreen (*Pyrola asarifolia* Michx.), and bunchberry (*Cornus canadensis* L.); mosses and lichens formed the forest floor. Upland soils were classified as Orthic Gray Luvisols, which characteristically develop in well to imperfectly drained sites with sandy loam to clay soils under boreal or mixed forests (Soil Classification Working Group 1998).

Seedling, soil, and sporocarp sampling

Sampling plots (50 m × 50 m) were established at least 10 m inside site boundaries at each of the nine sites. All regenerating (no obvious signs of vegetative layering) black spruce seedlings (15–30 cm in height) were located within each plot. Five seedlings were randomly selected from each plot (total of 45 seedlings) and harvested (along with the surrounding soil) between 26 July and 2 August 2001, when optimal fine root growth and ECM development was expected to have occurred. Seedlings were placed in pots, bagged, and stored at 5 °C until ECM characterization was performed.

Organic soil samples were collected from several locations within each wetland plot, combined for each plot, and stored in plastic bags. Two or three soil pits were excavated within upland forest plots, and samples from the forest floor and mineral horizons were collected, combined for each plot, and bagged. Soil samples were dried and stored at 22 °C for total carbon (C), total N, extractable phosphorus (P), and pH analyses (Kalra and Maynard 1991).

Fungal sporocarps were collected from all study sites at the time of seedling harvest. After descriptions and fungal identities were recorded, samples were taken from the spore-producing tissues and stored in microcentrifuge tubes at –20 °C for DNA analysis and subsequent comparisons to ECM root tip data.

ECM characterization

Root systems were gently washed free of soil, mosses, and debris in tap water, and cleaned roots were floated over a grid of 2 cm^2 cells in a tray of water. Root fragments 2 cm long were randomly selected from cells until approximately 200 root tips had been sampled. One unbranched root tip was considered to be one ECM. Only roots that appeared turgid with intact meristems were examined.

ECM tips were initially described using a dissecting microscope and classified according to color, texture, lustre, dimensions, tip shape, branching pattern, and presence or absence of rhizomorphs (mycelial strands) following standard techniques of Agerer (1987–2002), Ingleby et al. (1990), and Goodman et al. (1996). Root squash mounts were subsequently examined using bright field microscopy, and descriptions of mantle features, emanating hyphae, rhizomorphs, and other characteristics were used to further categorize the different ECM morphotypes. Root tips that appeared uncolonized or lightly colonized (due to the lack of a well-developed mantle) were classified as nonmycorrhizal. Identification to family or genus was made based on similarities in features to published descriptions (Agerer 1987–2002; Ingleby et al. 1990; Goodman et al. 1996); when identification was not possible, a descriptive name was assigned.

DNA extraction, amplification, and restriction endonuclease digestion

From each seedling, a proportionally representative sample of 20 root tips (10% of each morphotype) was stored at –20 °C for molecular analysis. DNA was extracted from tips using a modified CTAB (2× hexadecyl trimethyl ammonium bromide) extraction protocol (Mah et al. 2001). PCR amplification of the internal transcribed spacer (ITS) region of fungal rDNA was conducted using Platinum® *Taq* DNA polymerase (Invitrogen, Burlington, Ontario) along with the universal oligonucleotide primer ITS1 (White et al. 1990) and the fungal-specific primer NL6Bmun (Egger 1995). Reaction tubes were placed in a PTC-100™ programmable thermal controller (MJ Research, Inc., Waltham, Massachusetts), programmed to the following settings: denaturation at 94 °C for 30 s and 93 °C for 35 s, annealing at 50–52 °C for 53 s, and extension at 72 °C beginning at 30 s and increasing by 5 s per cycle for 35 cycles. PCR products were digested using the restriction endonucleases *Alu*I, *Hin*fI, and *Rsa*I (Invitrogen). Resulting fragments were run with 1 kb DNA ladder (Invitrogen) for approximately 3 h on high-resolution gels (1.0% agarose plus 1.5% low melting point agarose in 10% Tris–borate EDTA (TBE) buffer) containing ethidium bromide for visualization at ~115 mV. Digital images were

saved using the Gel Print 2000I photographic system (BioPhotonic Corp., Dexter, Michigan).

Data analysis

Soil nutrient content (C, N, and P) and pH were compared between habitats (and between different soil horizons in the U sites) using one-way analysis of variance (ANOVA) (Systat version 8.0, SPSS Inc., Chicago, Illinois). Mean comparisons were tested using Fisher's least significant difference (LSD) test ($\alpha = 0.05$).

ECM frequency of occurrence (number of seedlings on which each morphotype was found) and abundance (proportion of each morphotype on a seedling) was calculated for each seedling, site, and habitat type. The relative abundance of each morphotype was plotted against species rank order to visualize and compare ECM community structure between habitats (Magurran 1988; Taylor et al. 2000). Mean abundance values for individual morphotypes (calculated per seedling and averaged within each habitat) were compared between habitats using one-way ANOVA and Fisher's LSD test ($\alpha = 0.05$). ECM diversity was measured using the Margalef, Shannon, Shannon evenness, and Simpson indices (Magurran 1988). Diversity indices were calculated from morphological data for each seedling and averaged within habitats. To determine differences between habitats, indices were compared using one-way ANOVA and Fisher's LSD test ($\alpha = 0.05$).

RFLP gel images were analyzed using RFLPscan version 3.12 (Scanalytics 1994). Individual DNA fragments were marked and their sizes (base pairs) calibrated against the 1 kb standards (1018, 514, 356, 344, 298, 220, 201, 154, 134, and 75 bp fragments) using the Desmile calibration method with log piecewise linear curve fitting. Fragments smaller than 80 bp were excluded from analysis to reduce the possibility of primer dimer products being included. Fragment patterns for individual samples were imported into both individual morphotype and seedling databases in RFLPscan Database version 3.12 (Scanalytics 1994). Fragment patterns obtained from digestion of field-collected sporocarp samples were imported into a separate database for comparison.

Within each RFLPscan database, pairwise comparisons of all band patterns were compiled for each of the three enzymes. Band patterns were matched at a 5% threshold, a level determined to represent the best balance between type I (incorrectly calling the same fragment different) versus type II (incorrectly calling different fragments the same) errors (Baldwin 1999). Values ranged from zero to one within each enzyme and from zero to three between two samples, with three indicating an identical ECM. The fragment pattern data were imported into a spreadsheet, where samples were first sorted into morphotypes, then into genotypes. A genotype was defined as a set of fragment patterns that, when compared to other sets, is within a 5% threshold of similarity for all three enzyme digests. Partially digested or potentially contaminated samples were removed from the total sample and average fragment sizes were calculated for each genotype. Cluster analysis of the similarity matrix using the neighbour-joining method was performed using PHYLIP (Phylogeny Inference Package) version 3.573*c* (J. Felsenstein, University of Washington), and resulting phylograms were viewed in TreeView version Win 3.2 (Roderick D.M. Page, Institute of Biomedical and Life Sciences, University of Glasgow). Individual databases were merged into one large database, which was compared to reference ECM root tip (K. Egger, unpublished data) and sporocarp databases to confirm the classification and taxonomic naming of ECM morphotypes and to attempt to identify unknowns based on clustering with known types. From all successfully digested root tips, pairwise matching of restriction fragment patterns was also conducted using Dice's index (Dice 1945), which was then converted to a distance matrix (1 – Dice's index) to calculate the phi (Φ) index (Mah et al. 2001). Phi values were used to assess molecular diversity within each morphotype (intraspecific diversity) and between habitats (data pooled for sites) using one-way ANOVA and Fisher's LSD ($\alpha = 0.05$).

Results

Seedling and soil characteristics

No differences in seedling characteristics (i.e., height and age) were observed between habitats. Although only seedlings considered to be naturally regenerating (true seedlings) were selected for harvest, almost half (44.4%) of the harvested seedlings were found to originate from branches of trees that had developed underground adventitious roots and formed new seedlings (layered seedlings). These were evenly distributed across habitats.

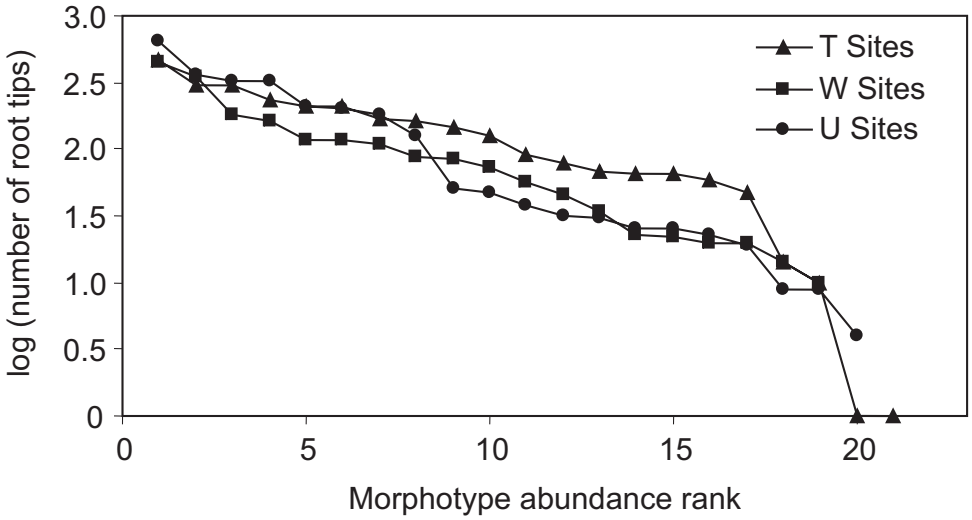
Soil nutrient content (C, N, and P) varied significantly between the wetland (both T and W) and upland habitats. Total C and total N content were greater ($p = 0.000$ and 0.001, respectively) in the wetland soils, whereas the C:N ratio and available P were greater ($p = 0.036$ and 0.025, respectively) in the upland forest floor soils. All soil samples were acidic (ranging from pH 3.8 to 6.9), and pH did not vary significantly between wetland and upland soils.

ECM morphotype occurrence, frequency, and abundance

Overall, 85% of all root tips examined were ectomycorrhizal. The majority (~30) of the 33 ECM morphotypes described from 8858 root tips were likely basidiomycetes. Twenty-seven morphotypes were assigned to families including the Thelephoraceae, Cortinariaceae, and Russulaceae, or to genera such as *Cenococcum*, *Mycelium radicis atrovirens* (*MRA*), *Amphinema*, *Lactarius*, *Tomentella*, *Piloderma*, and *Hebeloma*. Six morphotypes could not be assigned with confidence to any taxonomic group. Detailed morphological descriptions are in Robertson (2003). Nonmycorrhizal tips (1337) were found on 66.7% of all seedlings (including seedlings from each habitat and from all sites) with significantly greater numbers in the W habitat ($p = 0.000$).

Between 2 and 8 (mean of 4.8) ECM morphotypes were identified per seedling. The mean number of morphotypes varied significantly ($p = 0.005$) between habitats, with more occurring in the two mixed-species habitats (mean of 5.2 for both the T and U habitats) than in the black spruce dominated habitat (mean of 4.0 morphotypes). The number of morphotypes found on true seedlings (5.16 ± 0.24, $n = 25$) was significantly greater ($p = 0.037$) than the number that occurred on layered seedlings (4.35 ± 0.29, $n = 20$).

Fig. 1. Comparison of rank-abundance of ECMs of black spruce in three habitats (black spruce – tamarack wetlands (T), black spruce dominated wetlands (W), and black spruce – pine upland forests (U)), using log abundance plotted against ranked morphotype abundance for each habitat.



ECM communities in each habitat consisted of a few dominant fungal species and many rare species (Fig. 1). ECM morphotype log rank – abundance curves appear fairly even for wetland habitats; for the upland habitat, the curve tends toward a broken stick distribution (i.e., more distinct drops with decreasing ECM abundance), which can be interpreted as representing the lack of a relationship between different ECM types, with each species obtaining a random fraction of the total resource (Magurran 1988). Wetland habitats were dominated by *Cenococcum*, *Lactarius* 1, *Amphinema* (T sites), and Thelephoraceae-like 4 (W sites) morphotypes, whereas *Cenococcum*, Cortinariaceae 2, Russulaceae 2, and *Amphinema* morphotypes dominated in the upland habitat.

Table 1 shows the comparison of the frequency of occurrence and relative abundance of all ECM morphotypes between habitats. Abundance varied significantly between habitats for 6 of the 14 ECM morphotypes that occurred on four or more seedlings. Russulaceae 2 and *Piloderma* were significantly more abundant ($p = 0.020$ and $p = 0.001$, respectively) on upland seedlings than on wetland (T or W site) seedlings. *Tomentella* and *Tomentella*-like 1 were more abundant ($p = 0.011$ and $p = 0.037$, respectively) in the black spruce – tamarack wetlands than in either of the other habitats. *Lactarius* 1 ECM abundance was also significantly greater ($p = 0.047$) in the black spruce – tamarack wetlands than in the upland habitat (where it was not found) and greater (but not significant) than in the spruce-dominated wetlands. Thelephoraceae-like 4 was the only morphotype that was significantly greater in the spruce-dominated wetlands than in either of the mixed-species habitats ($p = 0.025$). Although differences in ECM frequency occurred between habitats for some commonly occurring morphotypes such as *Cenococcum*, Cortinariaceae 1, Cortinariaceae 2, *Amphinema*, and *MRA* 1, mean abundance values did not vary significantly.

Molecular fragment pattern analysis and diversity within morphotypes

From the original ECM root tips sampled for PCR-RFLP analysis (888), 51% (454) yielded restriction fragment patterns suitable for molecular analysis. The success rate from colonized tips (755) was 60% and varied with morphotype. Types such as *Amphinema* and *Piloderma* had higher success rates (>75%) than Cortinariaceae and Thelephoraceae-like types (37%–48%). With respect to nonmycorrhizal tips (133 samples), 24% (32) amplified and produced fungal DNA fragment patterns. Of these, 26 matched patterns of *Cenococcum* (genotypes 1 and 2), *MRA* 1 (genotypes 2 and 3), *Amphinema* (genotype 3), Russulaceae 1 (genotype 1), Russulaceae 2 (genotypes 1 and 6), and Thelephoraceae-like 2 (genotype 2).

Cluster analysis of fragment patterns generally confirmed the morphological classification of ECM morphotypes (tree not shown), but fungal identification (as related to taxonomic placement) was generally not improved. Comparisons with ECM root tip reference databases also helped to establish taxonomic groupings. For example, one group classified as nonmycorrhizal tips clustered with a reference sample of E-strain, allowing tentative identification of these ECMs as a type not morphologically described in this study. When fragment patterns generated from ECM root tips were compared to those for field-collected sporocarps, few matches were found. Two exceptions were *Lactarius deliciosus* (Fries) S.F. Gray, which grouped within the *Lactarius* 1 genotype 1 clade, and *Lactarius torminosus* (Schaeff. ex Fr.) Gray, which grouped within the *Lactarius* 3 genotype 1 clade.

Averaged restriction fragment lengths for all ECM genotypes are presented in Table 2. Most morphotypes consisted of several genotypes. Morphotypes with the most genotypes included *Amphinema* (six), Cortinariaceae 2 (six), and Russulaceae 2 (seven); *Cenococcum* (two); Thelephoraceae-like 1 and *Piloderma* only exhibited one genotype. In a few cases, delimitation of genotypes was based on fragment size variation in only one of the three enzyme digests. For example, *Amphinema* genotypes 1, 2, and 3 varied only by the *Alu*I pattern; genotype 5 differed from genotype 1 only by the *Rsa*I enzyme. The single genotype of *Lactarius* 3 varied from *Lactarius* 1 genotype 1 only by the *Hin*fI pattern, as both profiles showed fragments that were not digested by the

Table 1. Treatment effects, percent abundance (mean ± SE), and frequency of occurrence for ectomycorrhizal (ECM) morphotypes of black spruce growing in three habitats.

	Treatment effect		Black spruce – tamarack wetland (T)		Black spruce wetland (W)		Black spruce – pine upland forest (U)	
ECM morphotype	*F*	*P*	Abundance	Frequency (%)	Abundance	Frequency (%)	Abundance	Frequency (%)
Cenococcum	0.966	0.389	10.2 (4.6)	46.7	18.2 (5.9)	80.0	10.7 (2.3)	80.0
Cortinariaceae 2	1.515	0.232	4.9 (3.3)	40.0	3.9 (1.6)	46.7	10.9 (3.8)	80.0
Russulaceae 2	4.295	**0.020**	4.3 (7.1)b	33.3	3.0 (1.8)b	26.7	21.8 (6.8)a	53.3
MRA 1	1.599	0.214	2.2 (1.4)	33.3	2.2 (1.2)	33.3	6.1 (2.5)	53.3
Amphinema	1.130	0.333	10.1 (7.9)	33.3	4.0 (2.4)	20.0	12.1 (4.2)	46.7
Lactarius 1	3.293	**0.047**	15.7 (6.6)a	66.7	6.0 (3.7)ab	26.7	0.0 (0.0)b	0.0
Cortinariaceae 1	0.413	0.664	5.6 (3.3)	20.0	2.4 (1.9)	33.3	4.2 (2.0)	40.0
Thelephoraceae-like 1	3.165	0.052	7.0 (3.4)	46.7	1.1 (1.1)	13.3	0.3 (0.3)	6.7
Tomentella-like 1	3.567	**0.037**	5.4 (2.6)a	33.3	0.5 (0.5)b	6.7	0.3 (0.3)b	13.3
Piloderma	9.099	**0.001**	0.0 (0.0)b	0.0	0.0 (0.0)b	0.0	7.0 (2.3)a	46.7
Thelephoraceae-like 4	4.013	**0.025**	0.0 (0.0)b	0.0	11.7 (5.8)a	33.3	0.1 (0.1)b	6.7
Tomentella	4.987	**0.011**	7.9 (3.5)a	40.0	0.0 (0.0)b	0.0	0.0 (0.0)b	0.0
Cottony gold-brown	1.591	0.216	2.6 (1.8)	13.3	0.7 (0.5)	13.3	0.0 (0.0)	0.0
Thelephoraceae 2	1.292	0.285	0.0 (0.0)	0.0	0.7 (0.7)	6.7	0.7 (0.7)	20.0
Lactarius 3			0.0 (0.0)	0.0	0.0 (0.0)	0.0	6.6 (5.2)	20.0
Cottony halo			0.0 (0.0)	0.0	6.0 (3.3)	20.0	0.0 (0.0)	0.0
Russulaceae 1			4.1 (2.7)	20.0	0.0 (0.0)	0.0	0.0 (0.0)	0.0
Thelephoraceae-like 2			2.2 (1.5)	13.3	0.0 (0.0)	0.0	1.6 (1.6)	6.7
Russulaceae 4			0.0 (0.0)	0.0	0.8 (0.5)	13.3	1.7 (1.7)	6.7
Tomentella-like 3			1.6 (0.9)	20.0	0.0 (0.0)	0.0	0.0 (0.0)	0.0
Thelephoraceae-like 3			0.5 (0.4)	13.3	0.0 (0.0)	0.0	1.1 (1.1)	6.7
Brown 1			0.3 (0.3)	6.7	0.0 (0.0)	6.7	1.0 (1.0)	6.7
Thelephoraceae 3			0.3 (0.3)	6.7	3.7 (3.7)	6.7	0.0 (0.0)	0.0
Lactarius 2			3.1 (2.8)	13.3	0.0 (0.0)	0.0	0.0 (0.0)	0.0
Creamy rhizomorphic clamp			2.0 (2.0)	6.7	0.0 (0.0)	0.0	0.8 (0.8)	6.7
Orange 1			0.0 (0.0)	0.0	1.5 (1.5)	6.7	0.6 (0.6)	6.7
Cortinariaceae 3			0.0 (0.0)	0.0	2.8 (2.8)	6.7	0.0 (0.0)	0.0
Russulaceae 3			2.2 (2.2)	6.7	0.0 (0.0)	0.0	0.0 (0.0)	0.0
MRA 2			0.0 (0.0)	0.0	0.0 (0.0)	0.0	0.8 (0.8)	6.7
Brown 3			0.0 (0.0)	0.0	0.0 (0.0)	0.0	0.8 (0.8)	6.7
Hebeloma			0.0 (0.0)	0.0	0.7 (0.7)	6.7	0.0 (0.0)	0.0
Tomentella-like 2			0.0 (0.0)	0.0	0.3 (0.3)	6.7	0.0 (0.0)	0.0
Thelephoraceae 1			0.0 (0.0)	6.7	0.0 (0.0)	0.0	0.0 (0.0)	0.0
Nonmycorrhizal	9.785	**0.000**	5.2 (1.7)b	60.0	30.0 (5.7)a	93.3	10.0 (4.1)b	46.7

Note: Habitat differences based on morphotype abundance (calculated from seedling measures) were determined using one-way ANOVA. Mean comparisons were tested using Fisher's least significant difference test. Means followed by the same letter within rows are not significantly different ($\alpha = 0.05$).

*Rsa*I enzyme, even though length variation was observed. For some ECM genotypes, differences were observed in two of the enzyme profiles. For example, Russulaceae 1 genotypes 1 and 2 showed very similar fragment patterns with the *Hin*fI enzyme, but different patterns with the *Alu*I and *Rsa*I enzymes. Most genotypes differed from one another in all three enzymes, particularly when compared between morphotypes. Intraspecific diversity (Φ index values) was highest for *Tomentella*-like 1, Thelephoraceae-like 4, *MRA* 1, Russulaceae 2, *Lactarius* 1, and *Amphinema*; *Piloderma* and *Cenococcum* had the lowest Φ index values.

When multiple genotypes occurred within a morphotype, often a single genotype dominated. For example, *Cenococcum* genotype 1 accounted for 83% of *Cenococcum* samples, *MRA* 1 genotype 2 represented 59%, and Russulaceae 2 genotype 1 accounted for 46%. Within other morphotypes such as *Amphinema*, a more even distribution of the six genotypes was observed.

Genotype distribution across habitats

Some ECM morphotypes (such as *Cenococcum*, *MRA* 1, and Cortinariaceae 1) that were found in all three habitats consisted of genotypes that were also fairly evenly distributed across all habitats (Table 2). In contrast, other broadly distributed ECM morphotypes (such as *Amphinema*, Cortinariaceae 2, and Russulaceae 2) had more uneven patterns of genotype occurrence. Although all three habitats had some representation, each *Amphinema* and Cortinariaceae 2 genotype occurred in only one or two of the three habitats, never all. Cortinariaceae 2 had two genotypes in two of three habitats, with the remainder (four) in single habitats. Five of the Russulaceae 2 genotypes only occurred at U sites, with

one other genotype at T sites and one genotype at both the W and U sites.

When larger taxonomic groups were considered, other habitat distribution patterns were observed. For the Cortinariaceae group, the number of genotypes was fairly even across all habitats. In contrast, more *Lactarius* genotypes were in the T habitat (four) than in either the W (two) or U (one) habitats. Half of Russulaceae genotypes occurred in the U habitat (eight), followed by the T (five) and W (two) wetland habitats. The majority of genotypes (88%) from the Thelephoraceae, *Tomentella*, and brown types were found in the wetland habitats; *Tomentella* genotypes appeared restricted to the T sites. In some instances, ECM genotype distribution patterns mirrored ECM morphotype occurrence (where ECM are present at some sites and not others).

Habitat effects on ECM morphotype and genotype diversity

All diversity indices (Margalef, Shannon, Shannon evenness, Simpson, and Φ) indicated that ECM community diversity was greatest in the black spruce – pine upland (U) forests and least in the spruce-dominated wetland (W), with intermediate diversity in the spruce – tamarack wetland (T) habitat (Table 3). Species richness (Margalef index) varied significantly ($p = 0.038$) between the black spruce dominated wetlands and both mixed habitats, which had similar and higher richness values. Shannon index values were also significantly greater ($p = 0.028$) in the black spruce – pine upland habitat than in the black spruce dominated wetland habitat, whereas tamarack – black spruce wetlands had intermediate values. Shannon evenness and Simpson indices did not vary significantly, but supported similar trends. Molecular diversity did not differ significantly between habitats, although Φ index values were higher in the T and U habitats than in the spruce-dominated wetlands.

Discussion

ECM morphotype occurrence and abundance

Although the ECM status of black spruce has been confirmed by others (Malloch and Malloch 1981; Thormann et al. 1999), this is the first study to report on the composition and diversity of black spruce ECM communities across a range of habitats representing a moisture gradient. The 33 ECM morphotypes characterized for black spruce is within an expected range (20–35 fungal species) for small monoculture forests in relatively homogeneous environments (Bruns 1995). In central British Columbia, Mah et al. (2001) described 24 ECM morphotypes on naturally regenerating and planted hybrid spruce seedlings from clearcut or burned and mature SBS forests, while Kranabetter et al. (1999) reported as many as 74 morphotypes (an average of 52) on 2-year-old outplanted white spruce, subalpine fir, and lodgepole pine seedlings. Flynn et al. (1998) described 13 morphotypes on naturally regenerating Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings from Scottish plantation forests. Reported variation in ECM diversity may be attributed to differences in host receptivity to ECM fungi, inoculum potential at sites, soil and environmental factors, sampling intensity, or differences in techniques used by the investigators. The relatively high number of morphotypes in the present study reflects variation in the ECM community composition across the three habitats (including host species composition) and suggests the importance of sampling across the ecological range of any host to capture associated differences.

Many of the ECM communities described in this study consist of fungal taxa that have extensive distributions with little host specificity and that often associate with N-limited, acidic forest soils (Molina et al. 1992; Goodman and Trofymow 1998). Although most ECM morphotypes were basidiomycetes, ascomycete fungi (predominantly *Cenococcum* and *MRA* 1) accounted for almost 19% of the overall community. Malloch and Malloch (1981) found that *Cenococcum* was frequently associated with black spruce roots collected from upland forests in northeastern Ontario, and Thormann et al. (1999) identified *Cenococcum* and *MRA* on black spruce roots from bogs and fens in northern Alberta. *Cenococcum geophilum* Fr. is considered a cosmopolitan fungus with a wide host range and broad environmental tolerances (Goodman and Trofymow 1998). Less is known about fungi in the MRA species complex, but some *MRA* types have been described as exhibiting both mycorrhizal and pathogenic characteristics (Summerbell 1989; De la Bastide and Kendrick 1990). ECM abundance did not vary significantly across the moisture gradient for either *Cenococcum* or *MRA*, but their presence in both wetland types suggests a level of tolerance by these fungi for wetter habitat associations.

ECM abundance for the Cortinariaceae and *Amphinema* morphotypes was similar between wet and dry sites; other results support this lack of habitat specificity exhibited by *Amphinema*, but not for Cortinariaceae types. Lilleskov et al. (2002) found that along a N deposition gradient, *Amphinema* (*A. byssoides* Pers.: Fr.) – white spruce mycorrhizas occurred on sites having low to intermediate soil N in contrast to *Cortinarius* species that were confined to low-N sites. Both upland and wetland habitats in this study were likely N limited (the highest N content found on any site was 2.6%) and could, therefore, support both *Amphinema* and Cortinariaceae ECM fungi.

Several Russulaceae, Thelephoraceae, and *Piloderma* morphotypes exhibited habitat specificity. Fungi belonging to Russulaceae and *Lactarius* types were identified across all habitats; however, Russulaceae 2 was significantly more abundant at the drier upland sites than was *Lactarius* 1 (*Lactarius deliciosus*), which was most abundant at the spruce – tamarack wetlands and not found in the upland habitats (even though it has been described as being environmentally tolerant and lacking substrate specificity) (Goodman and Trofymow 1998). Remaining Russulaceae and *Lactarius* morphotypes occurred in relatively low abundance, usually specific to single upland or black spruce – tamarack habitats. In contrast, Lilleskov et al. (2002) found that *Lactarius* – white spruce ECM were less abundant at low-N than at high-N sites. Although our sites appeared to be N limited, total N was greater in the wetland habitats, where it was likely retained in organic forms. We do not know if *Lactarius* or Russulaceae fungi are able to use organic forms of N or to adapt to N-limiting conditions.

Thelephoraceae morphotypes (including *Tomentella* and several unidentified brown types) were identified most often in

Table 2. Approximate fragment sizes of the amplified internal transcribed spacer (ITS) region for black spruce ectomycorrhizal (ECM) morphotypes and genotypes from three habitats (black spruce – tamarack (T) wetlands, black spruce dominated (W) wetlands, and black spruce – pine (U) upland forests).

	Habitat					Approximate fragment sizes (bp)													
ECM genotype	T	W	U	No.	Size (bp)	*Alu*I					*Hin*fI					*Rsa*I			
Cenococcum																			
Genotype 1	+	+	+	45	790	440	150	110	80		275	165	130	100		920			
Genotype 2		+		8	915	400	240	150	115		435	290	165			950			
***MRA* 1**																			
Genotype 1			+	3	870	640	145	120			385	210	145			790	180		
Genotype 2	+	+	+	14	855	650	150	110			445	250	160			630	170		
Genotype 3	+	+	+	6	935	655	150	115			440	185	165	120		790	190		
Russulaceae 1																			
Genotype 1	+	+	+	7	770	365	175	160	110		280	165	150			730	175		
Genotype 2	+			2	860	445	230	190	110		285	165	155			835	175		
Genotype 3	+			5	920	425	190	130	115		350	165	150	125	105	1000			
Russulaceae 2																			
Genotype 1		+	+	26	950	690	190	110			335	290	165	150		555	195	175	
Genotype 2			+	4	1000	740	190	115			335	295	170	155		610	205	185	
Genotype 3			+	4	980	710	190	115			335	290	165	150		775	200		
Genotype 4	+			12	980	530	310	150	110		295	200	165	140	100	465	200	165	105
Genotype 5			+	1	955	600	260	150			365	185	165	145		995			
Genotype 6			+	5	1015	620	185	150	110		360	345	160	145		975			
Genotype 7			+	3	860	370	195	110			320	290	165	150		980			
Russulaceae 3																			
Genotype 1	+			1	1040	590	190	170	110		360	285	170	155		615	300	175	
Russulaceae 4																			
Genotype 1			+	4	1040	465	285	190	115		415	310	170	155		1015			
***Lactarius* 1**																			
Genotype 1	+	+		21	1070	520	290	190	110		415	350	165	150		1015			
Genotype 2	+			3	950	430	245	190	115		325	215	165	145		1020			
Genotype 3		+		11	1000	470	280	185	105		335	285	170	155		555	465		
***Lactarius* 2**																			
Genotype 1	+			3	950	510	190	110			350	330	170	155		935	100		
Genotype 2	+			3	980	520	190	115	85		340	320	165	155		1055			
***Lactarius* 3**																			
Genotype 1			+	16	1070	515	285	185	110		350	315	165	150	100	1040			
Thelephoraceae 3																			
Genotype 1		+		4	980	405	230	220	120		340	185	130	90		625	410	175	
Thelephoraceae-like 1																			
Genotype 1	+			8	950	420	185	150	110	95	320	225	165	150		855	175		
Genotype 2		+		4	950	430	185	150	95		350	300	165	150		1025			
Thelephoraceae-like 2																			
Genotype 1			+	4	1010	560	190	155	110		320	260	170	150	85	840	185		
Genotype 2	+			3	1000	525	235	180	110		330	255	165	150		830	210		

Table 2 (*continued*).

ECM genotype	Habitat T	W	U	No.	Size (bp)	Approximate fragment sizes (bp) *Alu*I					*Hin*fI					*Rsa*I		
Thelephoraceae-like 3																		
Genotype 1	+			1	965	425	185	150	120		360	320	165	150		1025		
Thelephoraceae-like 4																		
Genotype 1		+		7	1000	475	280	185	125		325	280	170	150		545	455	
Genotype 2		+		3	900	395	260	185	110		320	215	155	145		580	180	160
Genotype 3		+		3	965	465	245	185	110		315	295	160	145		975		
Tomentella																		
Genotype 1	+			6	1000	370	190	125	105	90	325	265	165	155	110	945	180	
Genotype 2	+			2	1000	480	380	185			315	285	235	120		1025		
Genotype 3	+			1	990	430	190	150	115	95	360	320	165	155		790	205	
***Tomentella*-like 1**																		
Genotype 1	+			6	965	435	230	185	120	110	315	190	165	150		990		
Genotype 2	+			1	1010	525	250	190	160	115	365	170	155			910	185	
***Tomentella*-like 3**																		
Genotype 1	+			1	875	415	185	120	110	90	220	190	165	150		980		
Cortinariaceae 1																		
Genotype 1		+	+	6	1060	670	185	145	110		370	340	140	120		935	175	
Genotype 2	+	+	+	9	1040	620	185	145	110		360	345	165	155		850	175	
Genotype 3	+			2	1040	605	185	145	110		355	345	165	150		1060		
Cortinariaceae 2																		
Genotype 1	+	+		7	955	430	185	145	115	80	335	290	170	155		785	175	
Genotype 2		+	+	11	940	355	235	185	150	130	360	170	150			910	175	
Genotype 3	+			2	1045	735	225				370	260	165	150		835	225	165
Genotype 4			+	5	1075	440	330	220	185		340	300	170	155		1090		
Genotype 5			+	3	1035	440	190	150	110	90	370	350	165	150		905	180	
Genotype 6			+	2	985	450	185	145	110	85	335	305	160	150		1030		
Cortinariaceae 3																		
Genotype 1		+		10	930	665	165	115			320	290	165	155		520	395	
Hebeloma																		
Genotype 1		+		2	895	360	240	180	135		335	275	130			840	195	
Piloderma																		
Genotype 1			+	17	920	365	260	190	110		315	180	165	155		850	175	
Amphinema																		
Genotype 1			+	16	900	365	190	140	110		325	295	165	155		780	175	
Genotype 2	+			14	950	575	185	110	85		325	290	165	155		790	175	
Genotype 3		+		10	940	365	235	150	125	100	335	285	165	110		775	175	
Genotype 4		+	+	5	950	460	363	160			315	285	165	150		945		
Genotype 5			+	10	940	365	190	140	115		320	285	160	145		920	180	
Genotype 6	+			6	920	275	240	185	175	110	370	170	155			1085		
Cottony halo																		
Genotype 1		+		6	1040	425	250	190	115	105	345	330	165	155		1035		
Genotype 2		+		8	1055	430	255	190	130	95	355	330	165	150		885	175	

Table 2. (*concluded*).

ECM genotype	Habitat			No.	Size (bp)	Approximate fragment sizes (bp)											
	T	W	U			*Alu*I				*Hin*fI				*Rsa*I			
Genotype 3		+		2	1000	395	260	185	110	100	320	220	165	155	85	625	200	175
Creamy rhizomorphic clamped																		
Genotype 1	+			5	940	595	190	110			295	240	165	155	85	985		
Genotype 2			+	4	950	430	185	145	110		295	230	165	145	115	1025		
Orange 1																		
Genotype 1			+	2	940	430	185	150	125	115	95	220	170	140	115		1070	
Brown 1																		
Genotype 1	+			2	900	590	420					215	180	165	150		985	
Brown 3																		
Genotype 1			+	2	1040	365	295	185	125	90		370	335	165	150		1030	
E-strain																		
Genotype 1	+	+	+	6	830	360	255	180	105			325	175	140			950	

Note: DNA fragments from amplified fungal rDNA using the ITS1 and NL6Bmun primers, and digestion with the restriction endonucleases *Alu*I, *Hin*fI, and *Rsa*I. "No." refers to the number of tips amplified. Size refers to the length of undigested rDNA.

both wetland habitats; three frequently occurring morphotypes (*Tomentella*, *Tomentella*-like 1, and Thelephoraceae-like 4) were significantly more abundant at wetland than at dry upland sites. *Tomentella* closely matched an uncommon variant of the "*Tomentella*-like with cystidia" morphotype described by Danielson and Pruden (1989) on urban blue spruce (*Picea pungens* Engelm.) and white spruce. The color, mantle features, and cystidia of *Tomentella*-like 1 were similar to those of dark-mantled types of *Tomentella* (Agerer 1987–2002; Lilleskov et al. 2002) and those described from black spruce peatlands in northern Alberta (Thormann et al. 1999). Thelephoraceae – conifer associations are well known and important components of ECM communities (Kõljalg et al. 2000). Many form inconspicuous resupinate fruit bodies on dead plants, wood, and soil debris (Kõljalg 1996), and some have been functionally classified as decomposers (Read and Perez-Moreno 2003). The ability of some fungi in the thelephoroid group to revert to a free-living saprotrophic state may give these fungi a competitive advantage over nondegrading ECM fungi in nutrient-poor habitats (Hibbett et al. 2000). Although much remains to be explored with respect to this group of fungi, Thelephoraceae had a propensity towards forming numerous symbioses with black spruce in the very wet sites.

Piloderma (*Piloderma fallax* (=*croceum*) Erikss. & Hjortst.) was identified only from the upland sites, almost exclusively on seedlings rooted in coarse woody debris. Others have noted the strong relationship between the occurrence of *Piloderma* and the percent cover of advanced-decay coarse woody debris (Goodman and Trofymow 1998), as well as its association with acidic humus and forest floor – mineral soil boundary layer (Arocena et al. 1999). *Piloderma* species may be able to adapt to relatively acidic, N-poor soil conditions and specialize in efficient N uptake (Lilleskov et al. 2002) or increase nutrient availability through enzymatic degradation of plant material (Griffiths and Caldwell 1992). Nevertheless, despite the abundant coarse woody debris on low-N, acidic wetland sites, where seedlings often grew, *Piloderma* was never found. Factors such as soil saturation, low oxygen, and cold substrates may inhibit *Piloderma* growth on these sites.

Molecular variation within ECM morphotypes

Sixty-five genotypes were delimited from the 29 ECM morphotypes successfully amplified and digested. Others using similar methods have reported comparable orders of magnitude: 22 genotypes were described for the 8 most common ECM morphotypes on hybrid spruce (Mah et al. 2001), 26 genotypes for 11 morphotypes of Douglas-fir (Sakakibara et al. 2002), and 23 genotypes within 18 morphotypes on Norway spruce (Mehmann et al. 1995). In the present study, some restriction fragment patterns (comparing two or three enzymes) of *Amphinema*, *Cenococcum*, *Lactarius* 1, *MRA* 1, and *Piloderma* were similar to those reported by Mah et al. (2001) and Sakakibara et al. (2002).

The number of fragment patterns (genotypes) within morphotypes varied from low for *Piloderma* (one genotype) and *Cenococcum* (two) to high for *Amphinema* (six), Cortinariaceae 2 (six) and Russulaceae 2 (seven). Mah et al. (2001) found similar intraspecific variation (i.e., genotype variation within morphotypes) for *Cenococcum* (one) and

Table 3. ANOVA for mean diversity values (standard error in parentheses) for ECM morphotypes (Margalef, Shannon Evenness and Simpson indices) and genotypes (Phi index) showing habitat effects for three black spruce communities.

	Treatment effect				
Diversity index	*F*	*P*	Tamarack–spruce wetland	Black spruce wetland	Pine–spruce upland
Margalef	3.527	**0.038**	0.802 (0.071)a	0.616 (0.059)b	0.814 (0.045)a
Shannon	3.899	**0.028**	1.188 (0.085)ab	0.950 (0.110)b	1.296 (0.070)a
Shannon evenness	1.740	0.188	0.727 (0.038)	0.677 (0.055)	0.795 (0.040)
Simpson	2.949	0.063	2.903 (0.217)	2.456 (0.268)	3.292 (0.243)
Phi	1.436	0.309	0.363 (0.035)	0.307 (0.027)	0.378 (0.031)

Note: Mean comparisons were tested using Fisher's least significant difference test. Means followed by the same letter are not significantly different ($\alpha = 0.05$). *P* values ≤ 0.05 are presented in bold.

Amphinema (six). In contrast, Sakakibara et al. (2002) found low variation within most morphotypes and many that expressed one dominant set of fragment patterns. Consistent with other studies (Gehring et al. 1998; Horton 2002), we report morphotypes having one or two dominant restriction fragment patterns as well as those with more even distributions of genotypes. Differences may result from a lack of variation in the ITS region of some ECMs; however, minor variations in analysis techniques and decisions made during morphotyping (e.g., classification of morphotypes as the same or different), larger sample sizes, or assignment of restriction fragment patterns as genotypes may also alter outcomes. Genotype variation appeared higher in the morphotypes that were less well defined (such as Cortinariaceae and Russulaceae), some of which grouped in several polyphyletic clusters on the neighbour-joining tree and may represent different species.

Intraspecific diversity for ECMs as measured by the Φ diversity index was lowest for *Piloderma* and *Cenococcum* morphotypes and highest for *MRA* 1, Thelephoraceae-like 4, and *Tomentella*-like 1. Although the latter three ECMs did not have the greatest number of genotypes, high Φ values reflect greater pairwise distances between restriction fragment patterns. Russulaceae 2 and *Lactarius* 1 also had high diversity values, whereas *Amphinema* and Cortinariaceae 1 and 2 had intermediate values. The index suggests that, although *Amphinema* and Cortinariaceae 2 were in the group having the highest number of genotypes, their intraspecific variation was less than that for several other morphotypes. Calculating Φ diversity values is less subjective when assessing intraspecific variation than assigning genotypes to restriction fragment patterns.

Habitat effects on ECM community diversity

ECM community diversity was highest in the black spruce – lodgepole pine upland forests and lowest in the black spruce dominated wetlands; differences were significant when measuring species richness (Shannon and Margalef indices). The Shannon evenness and Simpson indices (reflecting morphotype abundance), as well as the Φ index (a measure of molecular diversity), supported this trend but were not significant. Higher species richness in the drier upland habitat than in both wetland habitats may reflect improved edaphic conditions, particularly soils that are not water saturated for most of the growing season. In general, the lack of aeration is expected to slow or inhibit ECM formation in wetland soils (Walker 1987), although in vitro studies of inoculated jack pine (*Pinus sylvestris* Lamb.) seedlings showed that different ECM fungal species varied in their susceptibility to periodic flooding (Stenström 1991). It is unknown whether this contributed to the greater proportion of nonmycorrhizal roots observed in black spruce dominated wetlands than in both upland forests and black spruce – tamarack wetlands in this study. The propensity of black spruce seedlings to establish on *Sphagnum* hummocks, as well as observations that the water table receded during the period of ECM establishment, indicate that oxygen deficiency was likely not a major factor in determining the composition of ECM communities on wetland black spruce.

Soil nutrient availability may also be altered by water saturation. Although total N was greater in wetland soils, much of this was likely in less accessible organic forms because of low rates of decomposition in these cold, wet habitats (Thormann et al. 1999). Fungal symbionts that are physiologically able to capture nutrients from organic sources (Read and Perez-Moreno 2003) may explain the greater diversity in Thelephoraceae types in the wetland habitats. Phosphorus availability, also low compared to levels in upland soils, may be less important than N availability in ECM systems (Smith and Read 1997), but it is expected that ECM fungi in P-limited habitats would also be able to maximize assimilation of P for their plant partners.

Roots of large, dominant companion plants (tamarack and lodgepole pine) can act as refuges for fungal inoculum for growing black spruce roots and most likely contributed greatly to higher diversity in both mixed-forest habitats. ECM establishing on the root systems of alternate hosts such as pine or larch species can increase the inoculum available to neighbouring plants (Walker 1987; Massicotte et al. 1999). Jonsson et al. (1999) have shown the importance of large root systems as a source of inoculum, citing similarities in species composition of ECM fungi colonizing naturally regenerating pine seedlings and mature trees in Swedish boreal forests.

Overall, ECM morphotype abundance showed the expected dominance by a few ECM types, with other morphotypes occurring in progressively fewer numbers (Horton and Bruns 2001; Taylor 2002). Each habitat type, irrespective of moisture or associated host species, had approximately 20 ECM morphotypes, of which about half were dominant fungi; rare types were generally confined to one or two habitats. The ability of ECM fungi to dominate a host's root system has led some to suggest that species richness may be less important than the dominant species present because it includes a level of functional redundancy that is expected to exist within the soil microbial community (Hart et al. 2001). Nev-

ertheless, rare species are a widely reported component of ECM fungal communities and future studies should continue to examine the contributions of rare species to ecosystem processes, particularly in light of changing temperature and precipitation patterns.

Among genotypes of the more generalist ECM fungi, the molecular analysis provided some resolution of habitat specificity. *Cenococcum*, *MRA* 1, and Cortinariaceae 1 morphotypes and genotypes occurred across both wet and dry habitats, whereas genotypes of *Amphinema*, Cortinariaceae 2, and Russulaceae 2 showed more uneven patterns of distribution. Habitat- and site-specific associations have also been reported for *Amphinema* and *MRA* – hybrid spruce ECMs, lodgepole pine ECMs, and tomentelloid types based on RFLP profiles and ITS sequences (Byrd et al. 2000; Kõljalg et al. 2000; Mah et al. 2001).

The tendency of the rank-abundance model (Fig. 1) towards a broken stick distribution for ECMs in the upland habitat may be interpreted to reflect greater soil heterogeneity, which is considered a major factor in maintaining high diversity in ECM communities (Bruns 1995; Taylor 2002; Allen et al. 2003). Examination of the spatial distribution of ECM genotypes across environmental gradients, when sample sizes are large, can contribute to increased incidental distribution information that may be lost in studies with smaller sample sizes. Kernaghan (2001) found that their small molecular sample was suitable for confirming ECM identities (through fragment pattern comparisons), but too small to detect genotype variation between sites. The ITS region is expected to show intraspecific variation across large geographic scales due to its rapid rate of evolution and the reproductive isolation of populations in different habitats (Horton 2002). However, based on a small sporocarp sample, intraspecific variation in the ITS region has been reported to be negligible at local scales (Kårén et al. 1997). If some intraspecific variation is tracking adaptation of populations to changing environmental conditions, it seems probable that variation in the ITS region could occur at various geographic scales in response to environmental heterogeneity and selective pressures (Cairney 1999).

The ability to measure ECM richness and abundance is related to sampling effort (number of tips), probability of detection, and the relative abundance of each species (percentage of root tips colonized) (Taylor 2002). Compared to the sample size of many studies, our sample size should have detected most ECM types in the habitats, at least for black spruce in an early stage of development. Horton and Bruns (2001) emphasized the clustered distribution of many ECM fungi and reported that most actually occur in fewer than 10% of all samples. Clustered distributions of ECM morphotypes may be partly due to the patchy distribution of habitat features and may explain why morphotype richness is much lower at the individual seedling level than at the site or habitat level.

In the present study, morphological and molecular analyses suggest a mosaic of black spruce – ECM associations consisting of those occurring in both wetland and dry upland forest types, as well as those limited to one or two of the habitats. This mix of shared and habitat-limited associations most likely results from a combination of factors that include moisture differences, variable site hosts, and other soil attributes; the ability of black spruce to shift between fungal associates over diverse landscape and conditions suggests that this species will be able to accommodate a range of climatic changes. Black spruce currently occupies vast tracts of the boreal and sub-boreal forest, where it is subject to varying moisture and temperature gradients. This study supports the hypothesis that alternate hosts, and their ECM associations, increase and (or) modify those ECM associations of black spruce, although it remains unclear how these might be impacted by climate change. Most certainly it will depend partly on the ability of these alternate hosts to adjust to abiotic and biotic shifts. Nevertheless, based upon this study, black spruce, with ECM fungal associates adapted to both wet and dry habitats, seems to be well positioned to tolerate climate changes. Patterns of adaptation may be determined more by the rate at which climate factors change as well as how the extended biotic community, as a whole, is also able to respond to these changes. A greater understanding of the functional aspects of ECM diversity in forest processes will facilitate our ability to assess the impacts of climate change on forest communities.

Acknowledgements

The authors would like to thank Dr. Paul Sanborn, Dr. Art Fredeen, Dr. Mike Rutherford, and Craig DeLong (British Columbia Ministry of Forests) for discussion and practical input into this project. We also thank Jennifer Catherall for field and lab assistance, Kei Fujimura, Susan Gibson, and Linda Rehaume for lab assistance, and Cameron Grose for assisting with computer issues. Funding for this research was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant to H.B.M. Three anonymous reviewers provided helpful comments on the manuscript.

References

Agerer, R. (*Editor*). 1987–2002. Colour atlas of ectomycorrhizae. Einhorn-Verlag Eduard Dietenberger, Schwäbisch Gmünd, Germany.

Allen, M.F., Swenson, W., Querejeta, J.I., Egerton-Warburton, L.M., and Treseder, K.K. 2003. Ecology of mycorrhizae: a conceptual framework for complex interactions among plants and fungi. Annu. Rev. Phytopathol. **41**: 271–303.

Arocena, J.M., Glowa, K.R., Massicotte, H.B., and Lavkulich, L. 1999. Chemical and mineral composition of ectomycorrhizosphere soils of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) in the Ae horizon of a luvisol. Can. J. Soil Sci. **79**: 25–35.

Baldwin, Q.F. 1999. Effects of prescribed burning upon mycorrhizal fungal diversity inhabiting the roots of two and a half-year-old black spruce (*Picea mariana*): molecular characterization of ectomycorrhizal fungi via PCR/ RFLP. M.Sc. thesis, Memorial University of Newfoundland, St. John's, Nfld.

Brundrett, M. 1991. Mycorrhizas in natural ecosystems. Adv. Ecol. Res. **21**: 171–313.

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

Burton, P.J., Messier, C., Weetman, G.F., Prepas, E.E., Adamowicz, W.L., and Tittler, R. 2003. The current state of boreal forestry and the drive for change. *In* Towards sustainable management of

the boreal forest. *Edited by* P.J. Burton, C. Messier, D.W. Smith, and W.L. Adamowicz. NRC Press, Ottawa, Ont. pp. 1–40.

Byrd, K.B., Parker, V.T., Vogler, D.R., and Cullings, K.W. 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. Can. J. Bot. **78**: 149–156.

Cairney, J.W.G. 1999. Intraspecific physiological variation: implications for understanding functional diversity in ectomycorrhizal fungi. Mycorrhiza, **9**: 125–135.

Chapin, F.S., III., Callaghan, T.V., Bergeron, Y., Fukuda, M., Johnstone, J.F., Juday, G., and Zimov, S.A. 2004. Global change and the boreal forest: thresholds, shifting states or gradual change? Ambio, **33**: 361–365.

Colpaert, J.V., and van Tichelen, K.K. 1994. Mycorrhizas and environmental stress. *In* Fungi and environmental change: symposium of the British Mycological Society. *Edited by* J.C. Frankland, N. Magan, and G.M. Gadd. Cambridge University Press, Cambridge. pp. 109–128.

Dahlberg, A. 2001. Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. New Phytol. **150**: 555–562.

Danielson, R.M., and Pruden, M. 1989. The ectomycorrhizal status of urban spruce. Mycologia, **81**: 335–341.

De la Bastide, P.Y., and Kendrick, B. 1990. The in vitro effects of benomyl on disease tolerance, ectomycorrhizal formation, and growth of white pine (*Pinus strobus*) seedlings. Can. J. Bot. **68**: 444–448.

DeLong, C., and Fahlman, R. 1996. Field guide insert for site identification and interpretation for the southeast portion of the Prince George Forest Region. British Columbia Ministry of Forests, Victoria, B.C.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology, **26**: 297–302.

Egger, K.N. 1995. Molecular analysis of ectomycorrhizal fungal communities. Can. J. Bot. **73**: S1415–S1422.

Feil, W., Kottke, I., and Oberwinkler, F. 1988. The effect of drought on mycorrhizal production and very fine root system development of Norway spruce under natural and experimental conditions. Plant Soil, **108**: 221–231.

Flynn, D., Newton, A.C., and Ingleby, K. 1998. Ectomycorrhizal colonization of Sitka spruce (*Picea sitchensis* (Bong.) Carr) seedlings in Scottish plantation forest. Mycorrhiza, **7**: 313–317.

Gehring, C.A., Theimer, T.C., Whitham, T.G., and Keim, P. 1998. Ectomycorrhizal fungal community structure of Pinyon pines growing in two environmental extremes. Ecology, **79**: 1562–1572.

Goodman, D.M., and Trofymow, J.A. 1998. Distribution of ectomycorrhizas in microhabitats in mature and old-growth stands of Douglas-fir on southeastern Vancouver Island. Soil Biol. Biochem. **30**: 2127–2138.

Goodman, D.M., Durall, D.M., Trofymow, J.A., and Berch, S.M. (*Editors*). 1996. A manual of concise descriptions of North American ectomycorrhizae. Mycologue Publications, Sidney, B.C.

Griffiths, R.P., and Caldwell, B.A. 1992. Mycorrhizal mat communities in forest soils. *In* Mycorrhizas in ecosystems. *Edited by* D.J. Read, D.H. Lewis, A.H. Fitter, and I.J. Alexander. CAB International, Oxon. pp. 98–105.

Hamann, A., and Wang, T. 2006. Effects of climate and climate change on ecosystem and tree species distribution in British Columbia. Ecology. In press.

Hart, M.M., Reader, R.J., and Klironomos, J.N. 2001. Biodiversity and ecosystem function: alternate hypotheses or a single theory? Bull. Ecol. Soc. Am. **82**: 88–90.

Hibbett, D.S., Gilbert, L.-B., and Donoghue, M.J. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature (London), **407**: 506–508.

Horton, T.R. 2002. Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale. Plant Soil, **244**: 29–39.

Horton, T.R., and Bruns, T.D. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black box. Mol. Ecol. **10**: 1855–1871.

Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of ectomycorrhizae. Institute of Terrestrial Ecology, Natural Environment Research Council, HMSO, London. Institute of Terrestrial Ecology Research Publication 5.

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

Kalra, Y.P., and Maynard, D.G. 1991. Methods manual for forest soil and plant analysis. For. Can. Info. Rep. NOR-X-319.

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

Kernaghan, G. 2001. Ectomycorrhizal fungi at tree line in the Canadian Rockies II. Identification of ectomycorrhizae by anatomy and PCR. Mycorrhiza, **10**: 217–229.

Kõljalg, U. 1996. *Tomentella* (Basidiomycota) and related genera in temperate Eurasia. Synop. Fungorum, **9**: 1–213.

Kõljalg, U., Dahlberg, A., Taylor, A.F.S., Larsson, E., Hallenberg, N., Stenlid, J., Larsson, K.-H., Fransson, P.M., Kårén, O., and Jonsson, L. 2000. Diversity and abundance of resupinate thelephoroid fungi as ectomycorrhizal symbionts in Swedish boreal forests. Mol. Ecol. **9**: 1985–1996.

Kranabetter, J.M., Hayden, S., and Wright, E.F. 1999. A comparison of ectomycorrhiza communities from three conifer species planted on forest gap edges. Can. J. Bot. **77**: 1193–1198.

Krestov, P.V., Klinka, K., Chourmouzis, C., and Kayahara, G. 2000. Classification of mid-seral black spruce ecosystems of northern British Columbia. Forest Sciences Department, The University of British Columbia. Scientia Silvica Extension Series 26.

Lieffers, V.J., and Rothwell, R.L. 1987. Rooting of black spruce and tamarack in relation to depth of water table. Can. J. Bot. **65**: 817–821.

Lilleskov, E.A., Fahey, T.J., Horton, T.R., and Lovett, G.M. 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology, **83**: 104–115.

MacKenzie, W.H., and Moran, J.R. 2004. Wetlands of British Columbia: a guide to identification. Research Branch, British Columbia Ministry of Forests, Victoria, B.C. Land Manage. Handb. 52.

Magurran, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, N.J.

Mah, K., Tackaberry, L.E., Egger, K.N., and Massicotte, H.B. 2001. The impacts of broadcast burning after clear-cutting on the diversity of ectomycorrhizal fungi associated with hybrid spruce seedlings in central British Columbia. Can. J. For. Res. **31**: 1–12.

Malloch, D., and Malloch, B. 1981. The mycorrhizal status of boreal plants: species from northeastern Ontario. Can. J. Bot. **59**: 2167–2172.

Massicotte, H.B., Molina, R., Tackaberry, L.E., Smith, J.E., and Amaranthus, M.P. 1999. Diversity and host specificity of ectomycorrhizal fungi retrieved from three adjacent forest sites by five host species. Can. J. Bot. **77**: 1053–1076.

McAfee, B.J., and Fortin, J.A. 1989. Ectomycorrhizal colonization on black spruce and jack pine seedlings outplanted in reforestation sites. Plant Soil, **116**: 9–17.

Meidinger, D., Pojar, J., and Harper, W.L. 1991. Sub-Boreal Spruce zone. *In* Ecosystems of British Columbia. *Edited by* D. Meidinger and J. Pojar. British Columbia Ministry of Forests, Victoria, B.C. pp. 209–221.

Mehmann, B., Braus, G.H., and Brunner, I. 1995. Coincidence between molecularly or morphologically classified ectomycorrhizal morphotypes and fruitbodies in a spruce forest. *In* Biotechnology of ectomycorrhizae. *Edited by* V. Stocchi, P. Bonfante, and M. Nuti. Plenum Press, New York. pp. 41–52.

Molina, R., Massicotte, H., and Trappe, J.M. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. *In* Mycorrhizal functioning: an integrative plant–fungal process. *Edited by* M.F. Allen. Chapman & Hall, New York. pp. 357–423.

Montague, T.G., and Givnish, T.J. 1995. Distribution of black spruce versus eastern larch along peatland gradients: relationship to relative stature, growth rate, and shade tolerance. Can. J. Bot. **74**: 1514–1532.

O'Dell, T.E., Ammirati, J.F., and Schreiner, E.G. 1999. Species richness and abundance of ectomycorrhizal basidiomycete sporocarps on a moisture gradient in the *Tsuga heterophylla* zone. Can. J. Bot. **77**: 1699–1711.

Parker, W.H., Knowles, P., Bennett, F., Gray, A., and Krickl, T. 1983. Habitat-dependent morphological and chemical variation in *Picea mariana* from northwestern Ontario. Can. J. Bot. **61**: 1573–1579.

Pitelka, L.F., and the Plant Migration Workshop Group. 1997. Plant migration and climate change. Am. Sci. **85**: 464–473.

Prosser, J.I. 2002. Molecular and functional diversity in soil microorganisms. Plant Soil, **244**: 9–17.

Read, D.J., and Perez-Moreno, J. 2003. Mycorrhizas and nutrient cycling in ecosystems — a journey towards relevance? New Phytol. **157**: 475–492.

Robertson, S.J. 2003. Morphological and molecular assessment of ectomycorrhizal communities associating with black spruce (*Picea mariana* (Mill.) BSP) in wetland and upland forests in central British Columbia. M.Sc. thesis, University of Northern British Columbia, Prince George, B.C.

Roy, V., Bernier, P.Y., Plamondon, A.P., and Ruel, J.-C. 1999. Effect of drainage and microtopography in forested wetlands on the microenvironment and growth of planted black spruce seedlings. Can. J. For. Res. **29**: 563–574.

Sakakibara, S.M., Jones, M.D., Gillespie, M., Hagerman, S.M., Forrest, M.E., Simard, S.W., and Durall, D.M. 2002. A comparison of ectomycorrhiza identification based on morphotyping and PCR-RFLP analysis. Mycol. Res. **106**: 868–878.

Smith, S.E., and Read, D.J. 1997. Mycorrhizal symbiosis. 2nd ed. Academic Press, London.

Soil Classification Working Group. 1998. The Canadian system of soil classification. 3rd ed (revised). Agric. and Agri-Food Canada Publ. 1646.

Stenström, E. 1991. The effects of flooding on the formation of ectomycorrhizae in *Pinus sylvestris* seedlings. Plant Soil, **131**: 247–250.

Summerbell, R.C. 1989. Microfungi associated with the mycorrhizal mantle and adjacent microhabitats within the rhizosphere of black spruce. Can. J. Bot. **67**: 1085–1095.

Taylor, A.F.S. 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. Plant Soil, **244**: 19–28.

Taylor, A.F.S., Martin, F., and Read, D.J. 2000. Fungal diversity in ectomycorrhizal communities of Norway spruce (*Picea abies* (L.) Karst.) and beech (*Fagus sylvatica* L.) along north–south transects in Europe. *In* Carbon and nitrogen cycling in European forest ecosystems. Ecol. Stud. **142**: 343–365.

Thormann, M.N., Currah, R.S., and Bayley, S.E. 1999. The mycorrhizal status of the dominant vegetation along a peatland gradient in southern boreal Alberta, Canada. Wetlands, **19**: 438–450.

Walker, C. 1987. Sitka spruce mycorrhizas. Proc. R. Soc. Edinburgh, **93B**: 117–129.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols: a guide to methods and applications. *Edited by* M.A. Innis, D.H. Gelfand, J.J. Snisky, and T.J. White. Academic Press, New York. pp. 315–321.

Wurzburger, N., Hartshorn, A.S., and Hendrick, R.L. 2004. Ectomycorrhizal fungal community structure across a bog-forest ecotone in southeastern Alaska. Mycorriza, **14**: 383–389.

Zine El Abidine, A., Bernier, P.Y., Stewart, J.D., and Plamondon, A.P. 1994. Water stress preconditioning of black spruce seedlings from lowland and upland sites. Can. J. Bot. **72**: 1511–1518.