

# Properties of soils influenced by ectomycorrhizal fungi in hybrid spruce [*Picea glauca* × *engelmannii* (Moench.) Voss]

K. R. Glowa<sup>1</sup>, J. M. Arocena<sup>1,2,3</sup>, and H. B. Massicotte<sup>1</sup>

<sup>1</sup>College of Science and Management, <sup>2</sup>Canada Research Chair – Soil and Environmental Sciences, University of Northern British Columbia, Prince George, British Columbia, Canada V2N 4Z9.

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Glowa, K. R., Arocena, J. M. and Massicotte, H. B. 2004. **Properties of soils influenced by ectomycorrhizal fungi in hybrid spruce [*Picea glauca* × *engelmannii* (Moench.) Voss]**. Can. J. Soil Sci. **84**: 91–102. Soil properties of rhizosphere zones in coniferous forests are influenced by the presence of ectomycorrhizae. To elucidate the role of ectomycorrhizae (ECM) on the alteration of chemical and mineralogical properties of soils, soil pH, total C and N, cation exchange capacity, and the contents of mica, chlorite, and kaolinite, 2:1 type expandable clays, and amorphous minerals were compared in two soils, soils influenced by ectomycorrhizal fungi (ECS) and non-ectomycorrhizosphere soils (N-ECM) of *Picea glauca* × *engelmannii* (Moench.) Voss. Specifically, the two ECS soils were dominated by (1) *Piloderma* spp. (ECS-A) and (2) *Inocybe lacera*-like and *Hebeloma*-like morphotypes or where *Piloderma* spp. colonization was <1% (ECS-B). Our results showed that pH was lower in ECS compared to N-ECM samples. Total C and N were significantly higher in ECS soils than N-ECM samples. Cation exchange capacity as well as exchangeable K<sup>+</sup>, and Na<sup>+</sup> were higher in ECS compared to N-ECM soils. X-ray diffraction analysis showed that the amount of 2:1 expanding clays (vermiculite and smectite) was higher in ECS than N-ECM samples and results suggest that there is an enhanced transformation of mica and chlorite to 2:1 type expandable clays in ECS samples when compared to N-ECM samples. The differences in chemical and mineralogical properties between ECS and N-ECM soils, in our study, support earlier studies that show ectomycorrhizal fungi can alter the properties of soils in the rhizosphere zone.

**Key words:** ectomycorrhizosphere soils, soil properties, *Piloderma* spp.

Glowa, K. R., Arocena, J. M. et Massicotte, H. B. 2004. **Propriétés des sols affectées par les ectomycorhizes de l'épinette hybride [*Picea glauca* × *engelmannii* (Moench.) Voss]**. Can. J. Soil Sci. **84**: 91–102. Les propriétés du sol dans la rhizosphère des forêts de conifères subissent l'influence des ectomycorhizes. Pour élucider le rôle de ces derniers sur l'altération des propriétés chimiques et minéralogiques du sol, les auteurs ont comparé le pH, la concentration totale de C et de N, la capacité d'échange des cations ainsi que la concentration de mica, de chlorite, de kaolinite, d'argile expansible 2:1 et de minéraux amorphes de deux sortes de sol, le premier influencé par les ectomycorhizes (SEM) et le second prélevé dans la partie de la rhizosphère de *Picea glauca* × *engelmannii* (Moench.) Voss échappant à cette influence (N-SEM). Plus précisément, les deux SEM examinés renfermaient surtout (1) *Piloderma* spp. (SEM-A) et (2) des structures morphologiques ressemblant à celles de *Inocybe lacera* et de *Hebeloma* ou moins de 1 % de *Piloderma* spp. (SEM-B). Les résultats indiquent que les échantillons de SEM ont un pH plus faible que ceux de N-SEM. La concentration de C et de N est sensiblement plus élevée dans les SEM que les N-SEM. Les échantillons de SEM présentent une meilleure capacité d'échange des cations et plus de K<sup>+</sup> et de Na<sup>+</sup> échangeables. L'analyse par diffraction des rayons X indique que les échantillons de SEM contiennent plus d'argile expansible 2:1 (vermiculite et smectite) et une plus grande quantité de mica et de chlorite semble s'y transformer en ce type d'argile, comparativement à ce qui se passe dans les échantillons de N-SEM. La variation des propriétés chimiques et minéralogiques entre les SEM et N-SEM observée dans le cadre de cette étude appuie les résultats de recherches antérieures indiquant que les ectomycorhizes influent sur les propriétés du sol dans la rhizosphère.

**Mots clés:** Sols de la rhizosphère à ectomycorhizes, propriétés du sol, *Piloderma* spp.

Biogeochemical processes involved in soil mineral weathering occur largely in soil microenvironments influenced by microorganisms. Soil processes in these microenvironments can vary considerably because of the diversity in biological associations. Moreover, soil properties in these microenvironments such as pH (Nye 1981), cation-exchange equilibria (Chung and Zasoski 1994), organic carbon/acid concentrations (Gardner et al. 1982), monosaccharide contents (Dormaar 1988), grain-

size distribution, moisture, and mineral composition are usually different from those of bulk soils. A majority of earlier studies is confined to the composite effects of a multitude of organisms and roots on soil properties (e.g., Fitter and Garbaye 1994; Grayston et al. 1996; Courchesne and Gobran 1997).

However, we suggest that organism-specific effects on soil properties need to be examined further to better understand soil processes in the rhizosphere zone. This view con-

<sup>3</sup>To whom correspondence should be addressed (e-mail: [arocenaj@unbc.ca](mailto:arocenaj@unbc.ca)).

**Abbreviations:** CEC, cation exchange capacity; ECM, ectomycorrhizae; ECS, soils influenced by ectomycorrhizal fungi; N-ECM, non-ectomycorrhizal soils; XRD, X-ray diffraction

**Table 1. Mean abundance (%) of ectomycorrhizae present in the ectomycorrhizosphere A (ECS-A) and B (ECS-B) soils for hybrid spruce (for each soil group, n = 6)**

Ectomycorrhizae	ECS-A	ECS-B
<i>Amphinema byssoides</i> -like	0.10 (0.24) <sup>z</sup>	18.7 (20.33)
Brown, finely grainy 1	– <sup>y</sup>	0.24 (0.58)
Brown, finely grainy 2	–	0.57 (1.17)
<i>Cenococcum geophilum</i> Fr.	5.43 (1.78)	9.73 (5.01)
E-strain-like 1	–	0.05 (0.12)
<i>Hebeloma</i> -like	0.40 (0.98)	13.3 (9.07)
<i>Hysterangium</i> -like	–	1.64 (3.59)
<i>Inocybe lacera</i> -like	0.17 (0.40)	29.6 (24.93)
ITE 6-like	–	3.64 (3.60)
<i>Lactarius</i> -like	–	0.16 (0.40)
<i>Leccinum</i> -like	0.20 (0.31)	0.20 (0.48)
<i>Mycelium radicans atrovirens</i> (MRA)	0.65 (0.69)	4.26 (5.06)
<i>Piloderma</i> sp.	93.0 (1.74)	0.73 (0.96)
<i>Russula</i> -like 1	–	0.30 (0.75)
<i>Russula</i> -like 2	–	0.50 (0.80)
<i>Piloderma</i> -like	0.10 (0.24)	11.3 (20.35)
Tan Brown, finely grainy	–	0.08 (0.18)
<i>Tomentella</i> -like 1	–	0.95 (1.43)
<i>Tomentella</i> -like 2	–	0.16 (0.40)
<i>Tuber</i> -like	–	2.84 (3.08)
<i>Russulaceae</i> -like 1	–	1.09 (1.30)
Avg. number of root tips counted per sample	883 (94.7)	914 (61.0)
Percentage of mycorrhizal tips per sample	54.9 (9.37)	48.9 (7.46)
Percentage of non-living tips per sample	44.9 (9.72)	49.0 (8.82)
Percentage of non-mycorrhizal tips per sample	0.17 (0.41)	2.18 (3.38)

<sup>z</sup>Values in parentheses are standard deviations.

<sup>y</sup>– denotes absence of ectomycorrhizae.

curs with Berthelin (1983) who proposed that, with respect to mineral weathering, microorganisms exert a specific influence when in association with certain trees. Several studies have examined the effects of fungi as well as root-fungal symbioses (mycorrhizae) on soils. For example, *Laccaria laccata* inoculated on pine seedlings was able to weather phlogopite to vermiculite (Leyval and Berthelin 1991), and *Paxillus involutus* and *Rhizopogon luteolus* fungi have been reported to release the NH<sub>4</sub><sup>+</sup> ions trapped in the interlayer of vermiculite (Paris et al. 1994). *Paxillus involutus* has also been shown to produce oxalic acid by using bicarbonate ions (Lapeyrie 1988) whereas *Hysterangium crassum* on Douglas-fir was found to extract Fe and Al from andesite by providing high amounts of oxalate for chelation (Cromack Jr. et al. 1979). Munir et al. (2001) indicated that wood-rotting basidiomycetes oxidize glucose to oxalate in order to generate energy. Wallander and Wickman (1999) showed that *Pinus sylvestris* (L.) seedlings colonized by *Suillus variegatus* appeared to be more efficient in the uptake of K from biotite than from microcline. In Sweden, Jongmans et al. (1997) found that hyphae of *S. granulatus* and *Piloderma croceum* ectomycorrhizae could penetrate the calcium feldspars in granitic rocks; they postulated that this provides an opportunity for a direct flow of nutrients from the mineral to the plant. In a Mediterranean type climate ecosystem, de los Rios et al. (2002) reported that fungi in crustose lichens combined physical attachment with potassium depletion to weather the laminar, micaceous minerals in granite. Etienne and Dupont (2002) have attributed the increase in porosity, flaking and etching in rocks to fun-

gal groups such as *Penicillium* and *Aspergillus* that inhabit basaltic rocks in cold oceanic Icelandic environments.

Hybrid spruce [*Picea glauca* × *engelmannii* (Moench.) Voss] is an ecologically and economically important tree species in boreal and sub-boreal forests of North America. It associates with a wide diversity of ECM fungal species (Mah et al. 2001) and is believed to benefit from these symbioses especially in terms of nutrient availability and uptake.

In this study, chemical and mineralogical properties of soils influenced by hybrid spruce ECM were investigated to determine the effect of different ECM in the alteration of soil properties. Specifically, soil pH, total C and N, cation exchange properties, the contents of mica, chlorite, kaolinite, feldspars, 2:1 expanding clays and amorphous minerals were compared between two ectomycorrhizosphere soils and non-ectomycorrhizosphere soil in the Ae horizon of a Gray Luvisol in central British Columbia.

## MATERIALS AND METHODS

### Description of Study Area

The study area was situated within the sub-boreal forest and located near the campus of the University of Northern British Columbia in Prince George, British Columbia, Canada (53°54'N 123°49'W) at approximately 790 masl. The mean annual air temperature is 3.3°C with an average annual precipitation of 500–800 mm. The soil is a Gray Luvisol with L, F, and H layers, light colored Ae horizons, AB horizons, and Bt horizons (Dawson 1989). The study area was dominated by *Picea glauca* × *engelmannii* (Moench.) Voss with varying or

**Table 2. Morphological descriptions of the dominant ectomycorrhizae of hybrid spruce in Ae horizon of Luvisol**

Ectomycorrhizae	Branching pattern, surface texture, lustre, color	Mantle characteristics, outer mantle (OM), inner mantle (IM)	Emanating hyphae (EH), mycelial strands (MS), Cystidia (C)
<i>Amphinema byssoides</i> -like	Irregularly branched to monopodial pinnate, felty to cottony, matte to reflective, white to dingy yellow/brown	All cells turning distinctly yellow in 10% KOH, mantle thickness variable; OM felt to net prosenchyma, hyphae smooth to finely verrucose, 2.5–4 µm wide, large clamped septa; IM net prosenchyma to synenchyma, hyphae smooth, 2.5–5 µm wide, septa not clamped	EH abundant, 2.5–3.5 µm wide, white-yellow to dingy yellow/brown, smooth to verrucose, clamped septa, H-shaped anastomoses with clamp, frequently branched, distinctly yellow in 10% KOH; MS abundant, loose, to smooth undifferentiated, hyphae as per EH
<i>Cenococcum geophilum</i> Fr.	Simple to monopodial pinnate (rare), finely to coarsely grainy, shiny, black	Mantle 5–25 µm thick; OM net synenchyma, hyphae 4–7 µm wide, with distinct “stellar” pattern; IM net synenchyma, hyphae 1–4 µm	EH common, straight, thick walls, 3–6 µm wide, septate (no clamps), smooth, dark brown-black
<i>Hebeloma</i> -like	Monopodial pinnate, cottony, matte to reflective, white to slightly brown/white	Mantle 5–30 µm thick; OM net prosenchyma, hyphae 3–6 µm wide, clamped septa present, common enlarged junctions; IM netsynenchyma, hyphae 2–6 µm wide, no septa	EH common, straight, 2–6 µm wide, white, medium sized verrucose ornamentation, clamped septa frequent, H-shaped anastomoses without clamp. MS loose to smooth-undifferentiated, hyphae as per EH
<i>Hysterangium</i> -like	Monopodial pinnate, wooly, reflective, white	Mantle 10–25 µm thick; OM felt to net prosenchyma, hyphae 2.5–4 µm wide, crystal-like deposits, clamped septa frequent; IM netprosenchyma to net synenchyma, hyphae 2–4 µm, no ornaments, septa not clamped	EH rare, 2.5–4 µm wide, large crystalline ornamentation, clamped septa frequent; MS, observed hyphae as per EH; No C
<i>Inocybe lacera</i> -like	Not branched, smooth to finely grainy, matte to shiny, tan brown	Mantle 10–20 µm thick; OM felt to net prosenchyma, hyphae 0.5–3 µm wide, oil-like contents present, septate, enlarged junctions with multiple septa close together; IM net synenchyma, hyphae 1–2 µm	EH not obvious
ITE.6-like	Simple, smooth, shiny, brown to tan brown	Mantle 8–15 µm thick; OM net synenchyma, hyphae 1–3 µm wide, hyphal cells stain rose to bright pink in toluidine blue; IM net synenchyma, hyphae 1.5–3 µm wide	EH rare to common, hyphae 3–4 µm, often verrucose to crystalline, clamped septa, contact anastomoses without no clamps; MS numerous, strand hyphae as per EH
<i>Mycelium radicans atrovirens</i> (MRA)	Simple, finely grainy to slightly felty, shiny, black/dark brown, root apex sometimes hyaline	Mantle 10–15 µm thick; OM net prosenchyma with typically inflated outer hyphal cells, hyphae 1–5 µm wide; IM net synenchyma, hyphae 2.5–3 µm wide	EH common, 2–3 µm wide, finely verrucose, septate, brown/black, no clamps
<i>Piloderma</i> sp.	Irregular systems, coarsely felty, matte, bright yellow	Mantle 10–40 µm thick; OM felt prosenchyma, finely verrucose to crystalline, hyphae 2.5–3 µm wide, septate; IM net prosenchyma, hyphae 1.5–2.5 µm wide	EH abundant, ~3 µm wide, bright yellow, verrucose, septate, H-shaped anastomoses with septa, no clamps; MS numerous, strand hyphae as per EH
<i>Piloderma</i> -like	Simple to monopodial pinnate, cottony, reflective, white to dull white to golden yellow	Mantle 10–40 µm thick; OM net prosenchyma, with medium to large crystalline/needle-like deposits, hyphae 2–3 µm wide, septate; IM net synenchyma,	EH abundant, 2–4 µm wide, rare medium to large crystal or needle-like deposits, septate, H-shaped no clamps; MS numerous, smooth anastomoses, undifferentiated, strand hyphae as per EH

Table 2. Continued

<i>Russulaceae</i> -like 1	Simple monopodial pinnate, felty, matte, cream yellow/gray	Mantle 15–30 µm thick; OM non-interlocking to interlocking irregular to regular synchyma, cells 3–42 µm wide; IM net synchyma, cells 2–9 µm wide	EH rare to common, 3–6 µm wide, clamped septa
<i>Tuber</i> -like	Simple to monopodial pinnate, felty, smooth, matte, cream yellow/gray to tan brown	Mantle 15–30 µm thick; OM non-interlocking to interlocking irregular synchyma, cells 3–17 µm wide; IM net synchyma, cells 1–4 µm wide	EH and MS absent; C rare to common, bristle-like to awl shaped, 40–300 µm long, apex width 2–3 µm median width 2–5 µm, basal width 4–6 µm, wall thickness 1–1.5 µm; C variation: some tips with smaller C (20–100 µm long, apex width 1–2 µm, median width 1–2 µm wide, basal width 2–4 µm)

equal amounts of *Abies lasiocarpa* (Hook.) Nutt., *Pinus contorta* var. *latifolia* Dougl. ex Loud., and *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco. The understory vegetation was a mixture of shrubs such as thimbleberry (*Rubus parviflorus* Nutt.), Prince's-pine [*Chimaphila umbellata* (L.) W. Bart.], wildflowers [e.g., wild sarsaparilla (*Aralia nudicaulis* L.), bunchberry (*Cornus canadensis* L.), round-leaf violet (*Viola orbiculata* L.)] as well as minor amounts of oak fern (*Gymnocarpium dryopteris* (L.) Newman) and clubmosses (*Lycopodium complanatum* L. and *L. dendroideum* Michx.). Sampling sites were restricted to areas dominated by hybrid spruce and were selected based on recognizable presence of the fungus *Piloderma* in the forest floor (L-H).

### Sample Collection

Soil samples were collected from June to late October 1998 from the base of spruce trees with a 12- to 50-cm diameter at breast height. Samples adjacent to various trees, or trees in the same general area, were combined to obtain six composite sub-samples (~ 0.50 kg each) for each non-ectomycorrhizosphere (N-ECM), ectomycorrhizosphere A (ECS-A) and ectomycorrhizosphere B (ECS-B) soil. ECS-A samples were characterized by the presence of yellow *Piloderma* spp. ECM whereas ECS-B samples were taken from around ectomycorrhizal roots colonized by fungi other than *Piloderma*. We selected *Piloderma* spp. because it is commonly observed on hybrid spruce and its yellow color was readily recognizable in the field.

Representative samples for each of the three groups were collected at the base of each tree following the removal of the humus layer from a sampling area of approximately 2 m<sup>2</sup>; removal of the humus exposed the rooting zone in the LFH/Ae horizon boundary. Roots were selected by tracing the rootlets to the main lateral roots of the hybrid spruce. Roots dominated by *Piloderma* spp. colonization (ECS-A), and roots exhibiting colonization by other ECM fungi (<1% *Piloderma* spp.) (ECS-B) were collected with soil attached. Non-ectomycorrhizosphere (N-ECM) soil was collected where rootlets and associated rhizomorphs were visually absent. Roots and soil were placed in plastic bags and stored at 4°C until processing. In the laboratory, ECS samples were collected from the layer of soil (≤ 3 mm thick) that adhered to the rootlets by agitating the rootlets on a 2-mm sieve until most of the soil from the roots was collected. Six composite sub-samples for each of ECS-A, ECS-B, and N-ECM were analyzed for mycorrhizal colonization, as well as physical, chemical and mineralogical composition. In this study, ECS-A and ECS-B, as a group of ectomycorrhizosphere soils, are referred to as ECS soils. The ECS soils were air dried for subsequent analysis. The rootlets from ECS soils were stored at 4°C for further mycorrhizal assessment.

### Mycorrhizal Colonization

Morphological descriptions of ECS-A and ECS-B root samples were made using bright field microscopy and with reference to Goodman et al. (1996), Ingleby et al. (1990) and Agerer (1987–1998). Roots were placed in a glass dish (~30 cm × 20 cm × 5 cm) and cut into short pieces; roots were then randomly selected, examined microscopically, and described accord-

**Table 3. Mean values for physical and chemical properties of two ectomycorrhizosphere (ECS-A and ECS-B) and non-ectomycorrhizosphere (N-ECM) soils of hybrid spruce (for each soil group,  $n = 6$ )**

Soil properties	ECS-A	ECS-B	N-ECM
Particle size dist. (g kg <sup>-1</sup> )			
Sand	550 (112) <sup>z</sup>	529 (80)	482 (92)
Silt	382 (92)	398 (65)	450 (73)
Clay	68 (21)	73 (16)	68 (20)
pH	4.1a (0.13)	4.3a (0.15)	5.1b (0.17)
Total C (g kg <sup>-1</sup> soil)	19.7a (4.29)	21.3a (6.44)	6.05b (1.32)
Total N (g kg <sup>-1</sup> soil)	0.85a (0.14)	0.87a (0.17)	0.39b (0.06)
C/N ratio	23a (2.21)	24a (5.63)	15b (1.99)
CEC (cmol <sub>c</sub> kg <sup>-1</sup> soil)	5.87a (1.10)	6.61a (0.83)	3.90b (0.61)
Ex. cations (cmol <sub>c</sub> kg <sup>-1</sup> soil)			
Ca	2.65ab (0.67)	3.34a (0.76)	2.36b (0.69)
Mg	0.84 (0.24)	1.11 (0.27)	0.78 (0.34)
K	0.29a (0.06)	0.32a (0.04)	0.17b (0.04)
Na	0.12a (0.03)	0.12a (0.02)	0.07b (0.01)
Base saturation (%)	67a (11.6)	68a (7.9)	88b (16.32)

<sup>z</sup>values in parentheses are standard deviations.

a, b Across each row, means followed by the same letter are not significantly different ( $P > 0.05$ ).

ing to branching pattern, surface texture, lustre, color, mantle characteristics, emanating hyphae and other features in order to determine ECM morphotypes. Root tips with wrinkled, brown or decayed cells were categorized as non-living. Damaged or broken root tips were not counted. Abundance (proportion) of each ECM morphotype was estimated when either a total of 500 live mycorrhizal root tips or 1000 non-living root tips were counted per sample, whichever came first.

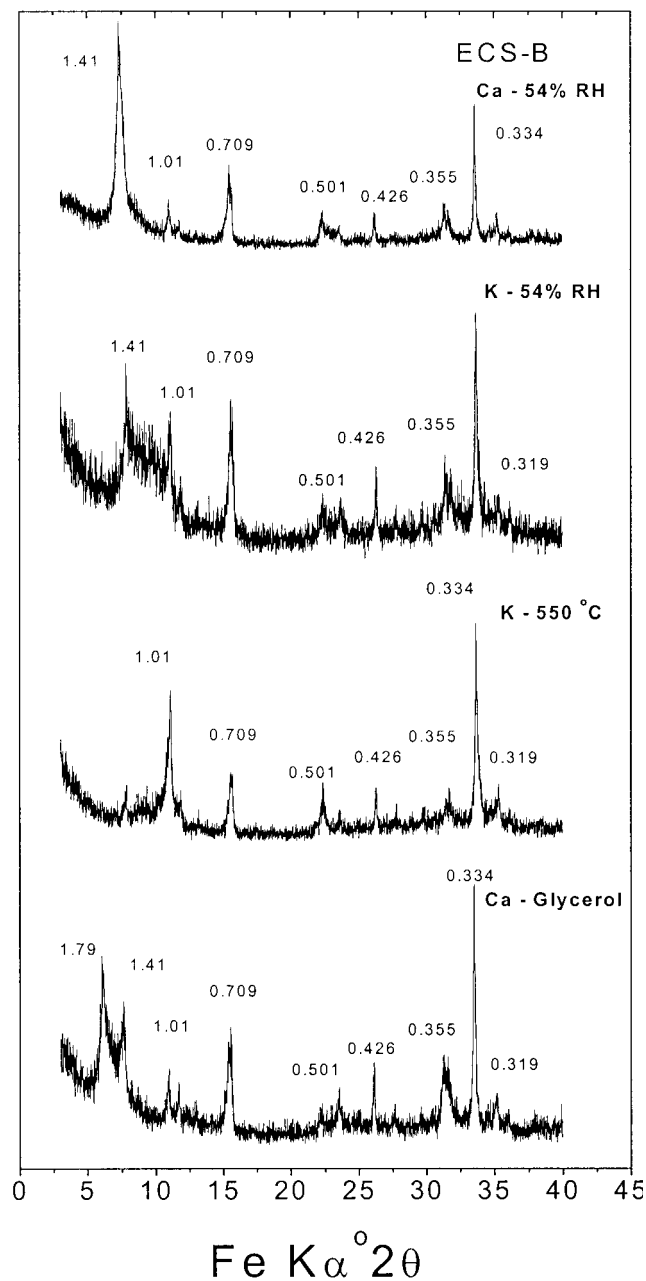
### Physical and Chemical Analyses

The particle size analysis was conducted on air-dried soil samples (<2 mm in diameter), subjected once for 3–4 min of ultrasonic dispersion using Braunsonic™ probe operated at 300–400 W of power. The treated samples were suspended in a 2-L beaker after wet-sieving to separate the sand (50–2000 μm) from the clay (<2 μm) and silt (2–50 μm) fractions. We separated the clay from the silt fraction by successive dispersion/sedimentation technique using the principle of Stoke's Law (Sheldrick and Wang 1993). Soil pH was measured in a suspension of 1:1 soil:water ratio using Orion pH meter. The cation exchange capacity (CEC) and exchangeable cations in the soils were determined using NH<sub>4</sub>OAc buffered at pH 7.0 (Kalra and Maynard 1991). The amounts of exchangeable Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> and Na<sup>+</sup> were determined from the NH<sub>4</sub>OAc-extract using inductively coupled plasma-atomic emission spectrometry (ICP-AES). Six composite sub-samples for each of the three types (total 18) were used in the chemical analyses. Total elemental analysis of clay and sand fractions was conducted on microwave-digested samples using ICP-AES and reference soil samples (SO-2 and SO-3) from the Canadian Certified Reference Materials Project were used as quality control standards.

### Mineral Composition

The mineral composition of the sand and clay fractions of N-ECM and ECS soils was determined by X-ray diffraction (XRD) using a Rigaku X-ray diffraction unit. A sample of

the clay fraction was prepared following the paste method (Theisen and Harward 1962) for both K- and Ca-saturated samples. The Ca-saturated clay sample was solvated with glycerol (Gly) while the K-saturated sample was heated at 550°C. Ca- and K-saturated samples were also scanned at ambient temperature and relative humidity. Identification of minerals in the clay fraction was based on the following criteria: (1) mica, 1.0 nm reflection in all treatments; (2) chlorite, 1.4 nm reflection in all treatments; (3) kaolinite in the presence of chlorite, 0.712 nm reflection that disappeared after 550°C heat treatment of K-saturated clay, and the doublet reflection at 0.357 and 0.354 nm regions; and (4) 2:1 type expandable clays, 1.7 nm reflection in Ca-Gly. We estimated the amounts of mica (muscovite) from the total K content and chlorite from total Mg content in the clay fraction. We assumed a 98.7 g K kg<sup>-1</sup> muscovite and 89.7 g Mg kg<sup>-1</sup> chlorite based on the chemical composition K(Si<sub>3</sub>Al)Al<sub>2</sub>O<sub>10</sub>(OH)<sub>2</sub> for muscovite (Fanning et al. 1989), and (Mg, Fe, Al)<sub>6</sub>(SiAl)<sub>4</sub>O<sub>10</sub>(OH)<sub>8</sub> for chlorite (JCPDS Card # 7-78). The amount of 2:1 expandable clays (vermiculite and smectite) was estimated from CEC<sub>Ca</sub> and CEC<sub>K</sub> of the clay fraction following the guidelines of Alexiades and Jackson (1965). CEC<sub>Ca</sub> was the CEC of the clay measured by the amount of Ca replaced by MgCl<sub>2</sub>, while CEC<sub>K</sub> was determined from the amount of K<sup>+</sup> replaced by NH<sub>4</sub>Cl after overnight heating of the K-saturated clay at 110°C. Kaolinite content was estimated from a modified procedure of Warren and Dudas (1992) by subtracting the integrated area under 0.712 nm peak in K-550°C sample from the integrated area of the same reflection in Ca-saturated sample scanned at ambient conditions. The amounts of amorphous Al<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub>, and SiO<sub>2</sub> were estimated from Al, Fe and Si determined from acid ammonium oxalate (McKeague 1967). The amount of crystalline Fe (as goethite—FeOOH) was estimated to be the difference between the total Fe content corrected for Fe in chlorite minus the Fe in ammonium oxalate extract.



**Fig. 1.** X-Ray diffraction patterns of the clay fraction from ECS-B samples subjected to four pre-treatments. Figure shows the relative intensities of the 1.7-, 1.4-, 1.0-, 0.71-, 0.42-, 0.35-, and 0.33-nm reflections.

### Statistical Analysis

We analyzed the data by ANOVA using Statistica™ version 5 (Statsoft Inc. 1995). The distribution of data was checked for normality using the Shapiro-Wilks W-test statistic and for the homogeneity of variance using Levene's test. Post hoc comparison of significantly different means was made using planned LSD test statistics. All outliers and extremes in data were removed using box plots technique outlined in Statistica™.

## RESULTS

### Ectomycorrhizal Colonization

The average number of root tips counted in ECS-A and ECS-B samples was 883 and 914, respectively (Table 1). Of these, 54.9 and 48.9% were considered mycorrhizal, 44.9 and 49.0% were scored as non-living, and 0.17 and 2.18% were classified as non-mycorrhizal in ECS-A and ECS-B samples, respectively. Based on morphological assessment, eight ECM morphotypes in ECS-A samples and 21 ECM morphotypes in ECS-B samples were characterized (Table 1). Ectomycorrhizal colonization was 0% in N-ECM soils.

In ECS-A samples, 93% of the ECM consisted of *Piloderma* spp., 5.4% *Cenococcum geophilum*, and 1.6% represented the remaining six morphotypes (Table 1). In ECS-B samples, seven morphotypes comprised 90.5% of all ECM. *Inocybe lacera*-like was the most abundant (29.6%), followed by *Amphinema byssoides*-like morphotype (18.7%), *Hebeloma*-like (13.3%), *Piloderma*-like (11.3%), *Cenococcum geophilum* (9.7%), *Mycelium radialis atro-virens* (MRA) (4.3%), and ITE 6 (Ingleby et al. 1990) (3.6%). Tuber-like morphotypes made up 2.8% and *Piloderma* spp. were present at 0.7%, a level much lower than that found in ECS-A in samples. Twelve other ECM morphotypes comprised <5% of all ECM in ECS-B samples (Table 1). Descriptions for the 11 most commonly found ECM are given in Table 2.

### Physical and Chemical Properties

Particle size composition of the soil samples ranged from 68–73 g kg<sup>-1</sup> clay, 382–450 g kg<sup>-1</sup> silt, and 482–550 g kg<sup>-1</sup> sand (Table 3). The content of silt and sand fractions varied respectively, at 450 and 482 g kg<sup>-1</sup> for N-ECM samples, 398 and 529 g kg<sup>-1</sup> for ECS-B samples, and 382 and 550 g kg<sup>-1</sup> for ECS-A samples. The pH value in both ECS soils was similar and significantly lower than N-ECM samples. Mean soil pH ranged from 4.1 in ECS-A to 5.1 in N-ECM samples. The content of total C and N was significantly lower in N-ECM compared to ECS soils, which were similar (Table 3). The C:N ratio was also significantly lower in N-ECM compared to ECS samples and ranged from 15 in N-ECM to 24 in ECS-B soils. The CEC in ECS-A and B soils, at 5.87 and 6.61 cmol<sub>c</sub> kg<sup>-1</sup>, respectively, were significantly higher than N-ECM samples at 3.90 cmol<sub>c</sub> kg<sup>-1</sup>. The amount of exchangeable cations followed the order: exchangeable Ca<sup>2+</sup> > Mg<sup>2+</sup> > K<sup>+</sup> > Na<sup>+</sup>. The amount of exchangeable K<sup>+</sup> and Na<sup>+</sup> in ECS soils was significantly higher than in N-ECM soils. The exchangeable Ca<sup>2+</sup> in N-ECM soils (2.36 cmol<sub>c</sub> kg<sup>-1</sup>) was significantly lower than ECS-B (3.34 cmol<sub>c</sub> kg<sup>-1</sup>) but similar to ECS-A (2.65 cmol<sub>c</sub> kg<sup>-1</sup>). Exchangeable Mg<sup>2+</sup> was similar in all samples. The base saturation (%) was significantly different between ECS and N-ECM soils at 67 and 88%, respectively.

### Mineral Composition

Based on XRD analysis of clay samples, the following minerals were identified in all samples: mica, chlorite, 2:1 expandable clays, kaolinite and quartz (Fig. 1). The XRD peaks at 1.0 and 0.5 nm indicate that muscovite was the

**Table 4.** Mean content (g kg<sup>-1</sup>) of selected phyllosilicates and oxides in clay fraction in non-ectomycorrhizosphere (N-ECM) and ectomycorrhizosphere (ECS-A and ECS-B) soils of hybrid spruce (for each soil group, *n* = 6)

Minerals	ECS-A	ECS-B	N-ECM
Mica	178 (13) <sup>z</sup>	182 (18)	194 (23)
Chlorite	99 (26)	103 (33)	103 (31)
Kaolinite <sup>y</sup>	40 (25)	40 (32)	70 (67)
Amor. Al <sub>2</sub> O <sub>3</sub>	2.0 (0.6)	2.0 (0.6)	3.0 (0.7)
Amor. Fe <sub>2</sub> O <sub>3</sub>	3.0 (0.3)	4.6 (1.0)	4.0 (1.0)
Amor. SiO <sub>2</sub>	1.0 (0.2)	1.0 (0.1)	1.0 (0.1)
FeOOH <sup>x</sup>	15 <sup>a</sup> (2.0)	18 <sup>b</sup> (2.0)	18 <sup>ab</sup> (0.5)
Vt + Sm <sup>w</sup>	105 (10)	94 (7.0)	95 (22)
(Vt + Sm)/Mi	0.56 (0.04)	0.55 (0.08)	0.48 (0.09)
(Vt + Sm)/Ch	1.13 (0.37)	1.07 (0.42)	1.03 (0.47)

<sup>z</sup>Values in parentheses are standard deviations.

<sup>y</sup>*n* = 4.

<sup>x</sup>FeOOH (goethite) assumed as the dominant crystalline Fe oxide.

<sup>w</sup>Vt = vermiculite, Sm = smectite, Mi = mica; Ch = chlorite.

<sup>b</sup>For each row, means followed by the same letter are not significantly different (*P* > 0.05).

species of mica. The amount of mica was lower in ECS soils compared to N-ECM samples but not significantly (ECS-A < ECS-B < N-ECM) (Table 4). Chlorite content of the samples followed the same trend as mica but contained approximately half the content per kilogram of soil. The sum of 2:1 expandable clays (vermiculite + smectite) was highest in ECS-A soils; ECS-B and N-ECM soils were similar. Kaolinite was higher in the N-ECM samples compared to ECS samples. The ratio of the sum of 2:1 expandable clays over mica and chlorite was lower in N-ECM samples compared to ECS-A and ECS-B samples (Table 4). These trends were also evident in the relative intensities of 1.7, 1.4, and 1.0 nm reflections in the XRD patterns of glycerol solvated clay samples (Fig. 2). The amount of amorphous Al<sub>2</sub>O<sub>3</sub> was lowest in the ECS samples whereas the amount of amorphous SiO<sub>2</sub> was lower but not significant in N-ECM samples compared to ECS samples. Goethite was significantly lower in ECS-A compared to ECS-B samples but ECS soils did not differ significantly from the N-ECM soils.

## DISCUSSION

### Ectomycorrhizal Colonization

*Piloderma* colonization observed in this study appeared concentrated in the organic and upper mineral layers, particularly in the LFH/Ae horizons. This is similar to findings on subalpine fir (*A. lasiocarpa*) ECM (Arocena et al. 1999) but is in contrast to other studies that report *Piloderma* ECM to be confined to organic materials (Brand 1991; Goodman et al. 1996). The functional role of *Piloderma* ECM associations is largely unknown but reports suggest that ECM may be involved in granite weathering (Jongmans et al. 1997). Other ECM such as *S. variegatus* on *P. sylvestris* seedlings reportedly enhanced the uptake of K more from biotite than microcline (Wallander and Wickman 1999). Calcium-rich encrustations on *Piloderma* hyphae might prevent the build-up of toxic levels of calcium and oxalate (Arocena et al. 1999, 2001). *Piloderma* associates with subalpine fir in the same area where it was found on hybrid spruce; it is possible that it has a similar role in the rhizosphere zones of both tree species.

In ECS-B soils, *Inocybe*-, *Amphinema*-, *Hebeloma*-, and *Piloderma*-like ECM dominated roots that had low levels of *Piloderma*. *Inocybe lacera* has been found to associate with *Pinus* and *Betula* species on coal waste sites in Scotland; it is considered to be a tree seedling colonizer (Ingleby et al. 1990). Another colonizer of young seedlings, *Hebeloma*, has been reported on a wide variety of young tree species growing in reasonably fertile brown earth soils, tree nurseries, and glasshouses (Ingleby et al. 1990). In some cases, *Hebeloma* dominates the root systems of *Picea sitchensis* (Bong.) Carr, *Salix* and *Betula* species. It is interesting that, although often occurring on younger trees, these fungi also colonized many fine roots of mature hybrid spruce when *Piloderma* was less abundant.

The *Amphinema* morphotype was abundant in ECS-B but rare in ECS-A samples. *Amphinema byssoides* often occurs between the duff and mineral soil and is known to form associations with other tree species including *A. lasiocarpa*, *Alnus viridis* (Chaix) DC, *Arctostaphylos uva-ursi* (L.) Spreng, *Betula papyrifera* Marsh, *P. contorta*, *Populus tremuloides* Michx., and *Salix commutata* Bebb (Harniman and Durall 1996; Hagerman et al. 1999; Krasowski et al. 1999; Mah et al. 2001).

*Cenococcum geophilum* ECM occurred at low to moderate levels in ECS soils and the abundance was similar to the findings of Arocena et al. (1999) for rhizosphere root samples of subalpine fir. Reports suggest that it occurs between the forest floor and mineral soil in some forests in British Columbia (Harniman and Durall 1996; Hagerman et al. 1999; Mah et al. 2001; Khetmalas et al. 2002). *Cenococcum* is distributed worldwide (Molina et al. 1992) and in British Columbia is known to form associations with *A. lasiocarpa*, *A. viridis*, *A. uva-ursi*, *B. papyrifera*, *P. contorta*, *P. tremuloides*, and *S. commutata* (Harniman and Durall 1996).

ITE 6-like (Kranabetter et al. 1998) and *Tuber*-like ECM were found in low numbers in ECS-B soils. Both types are widely distributed, mostly detected at low levels on a variety of host trees, including *Tsuga heterophylla*, *P. menziesii* and *Pinus ponderosa* in the Pacific Northwest (Molina et al. 1992; Kranabetter and Wylie 1998; Massicotte et al. 1999;

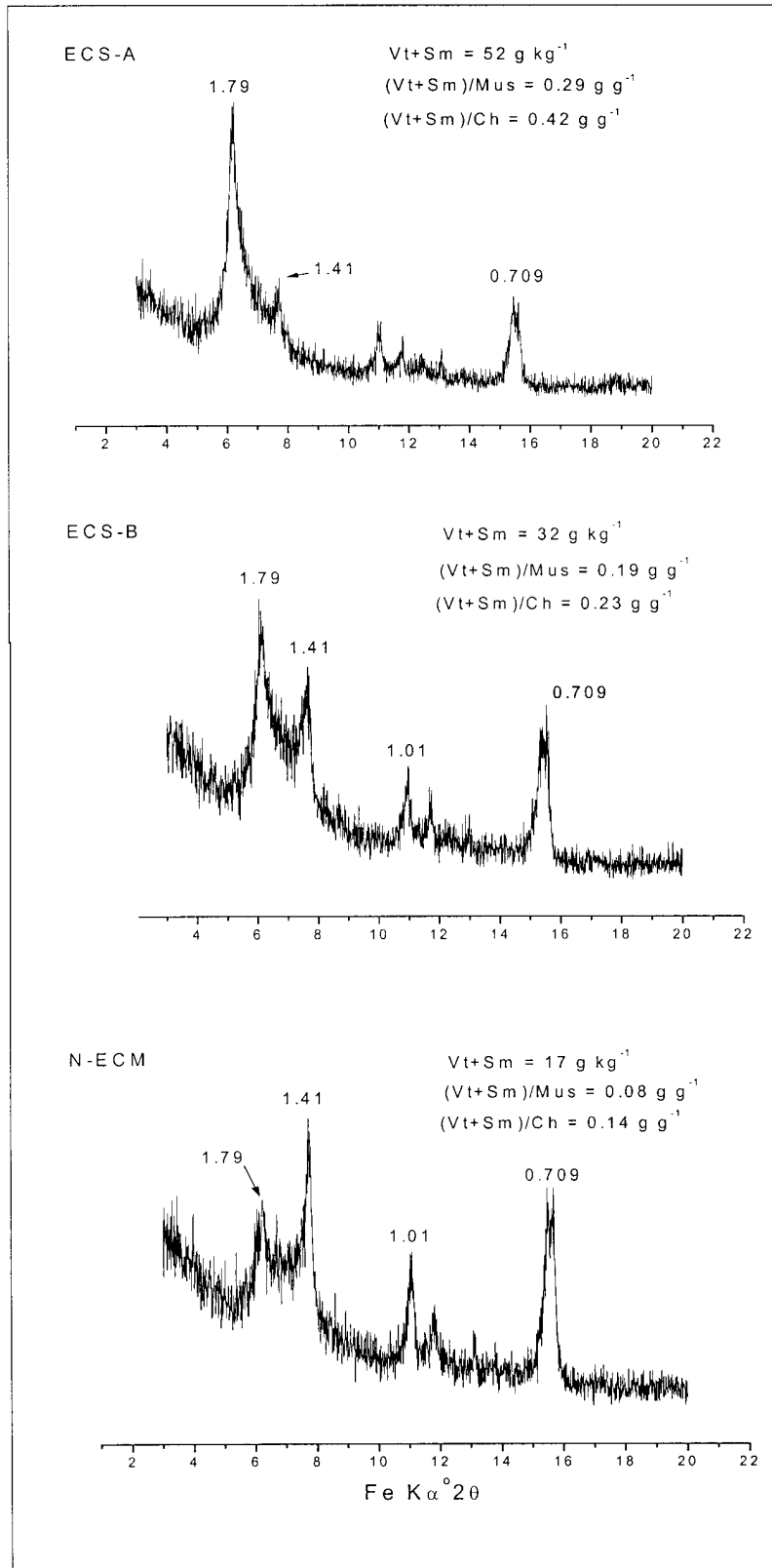


Fig. 2. X-Ray diffraction patterns of Ca-saturated and Glycerol-solvated clay fractions of ECS-A, ECS-B, and N-ECM samples showing the relative intensities of the 1.7- and 1.4-nm reflections and the relative amounts of vermiculite (Vt), smectite (Sm), muscovite (Mus), and chlorite (Ch).

Krasowski et al. 1999; Mah et al. 2001). In Europe, ITE 6 has been observed on young *Picea sitchensis*, and older *P. sylvestris* and *P. menziesii* (Ingleby 1990).

### Chemical Properties of ECS and N-ECM Soils

The lower pH in ECS compared to N-ECM soils observed in this study is consistent with earlier reports. In Douglas-fir stands, Cromack Jr. et al. (1979) reported a pH 4.9 for soils colonized by *H. crassum*; this was significantly lower than pH 6.1 measured in uncolonized soil. ECS soils of subalpine fir also had a lower pH than that of N-ECM soils (Arocena et al. 1999). Berthelin (1983) attributed the low pH in the mycorrhizosphere zone to oxidized inorganics (e.g., sulphur) and the production of organic acids (e.g., oxalic, carbonic, citric, acetic) by the activities of ECM. Singh et al. (2003) and Högberg et al. (2001) reported that respiration from ectomycorrhizal roots is equivalent to up to 65% of total soil respiration. This finding has significant implications with respect to the generation of exudates (e.g., organic acids) that can subsequently lower the pH and further enhance the rates of mineral weathering in ECS compared to N-ECM soils. High rates of  $\text{NH}_4^+$  uptake by plants (Marschner et al. 1987) could also lower pH as roots exude  $\text{H}^+$  into the rhizosphere soil to counteract the depleted positive charge arising from the uptake of  $\text{NH}_4^+$ . Enhanced adsorption of  $\text{NH}_4^+$  and  $\text{K}^+$  by coniferous trees may be due to the presence of ECM (Rygiewicz and Bledsoe 1984; Rygiewicz et al. 1984).

Roots and their associated ECM fungi could also account for higher levels of total C and N in ECS compared to N-ECM soils. Arocena et al. (1999) indicated that fungi (and other organisms) in the ECS were significant sinks for carbon assimilated by the host plant; upon death, their biomass may contribute to the high contents of total C and N in ECS soils. For example, *Suillus luteus* (a mycorrhizal fungus) could utilize up to 30% of the total C assimilated by *P. sylvestris* (Söderström and Read 1987), while Douglas-fir potentially allocates up to 73% of assimilated C to ECM (Fogel and Hunt 1983). In addition, root exudates of *P. sylvestris* were found to be higher in ECS compared to N-ECM soils (Grayston et al. 1996).

The level of total N between ECS and N-ECM soils paralleled that of total C. This reflects the fact that both N and C are a necessary component of the microbial biomass. Ectomycorrhizosphere C:N ratios were significantly higher than N-ECM soils and similar to the results found for subalpine fir (Arocena et al. 1999). The C:N ratios for all samples were close to the threshold of 20:1, indicating higher mineralization rates compared to fresh organic matter where C:N ratios range from 40:1 to 100:1 (Myrold 1998).

Since the clay content in ECS and N-ECM samples was similar, the higher CEC in ECS compared to N-ECM soil might be attributed to higher amounts of organic matter in ECS soils. As organic matter (measured as total C) increases, the CEC also increases due to the negative charge associated with its functional groups. The difference in CEC between ECS-A and ECS-B soils, although not significant, could be attributed to higher C content and pH values of ECS-B. Higher amounts of 2:1 type clays in ECS compared to N-ECM soils could also contribute to higher CEC values.

### Mineral Composition of ECS and N-ECM Soils

In ECS soils, the relatively lower amounts of mica and chlorite, as well as results from X-ray diffraction analysis (Fig. 2), may indicate higher rates of mineral weathering in ECS compared to N-ECM soils. These findings are consistent with those of Arocena et al. (1999) where amounts of mica and chlorite in ECS samples were significantly lower compared to N-ECM soils for subalpine fir, and they corroborate results from other studies (April and Keller 1990; Hinsinger et al. 1991; Kodama et al. 1994; Courchesne and Gobran 1997). In these four studies cited, bulk and rhizosphere materials were compared and soil minerals other than mica and chlorite (e.g., amphibole) were affected by increased weathering. Higher amounts of 2:1 expandable clays and exchangeable  $\text{K}^+$  in ECS-A compared to ECS-B and N-ECM soils may indicate faster formation of 2:1 expandable clays in soils influenced by *Piloderma* compared to other fungi.

Earlier reports on high rates of mica and chlorite weathering are mostly with respect to the rhizosphere of agricultural crops but similar weathering mechanisms may also be operating in the rhizosphere of trees. In the rhizosphere zones, both physical and chemical processes increase rates of mineral weathering. The pressures exerted by growing roots and associated fungal hyphae could mechanically alter minerals by causing realignment, bending, and fracturing (April and Keller 1990). In addition, Robert and Berthelin (1986) showed micrographs of hyphae probing between mica flakes to extract essential  $\text{K}^+$ . In many studies, root-induced release of interlayer  $\text{K}^+$  was the main reason for the high rates of chemical weathering in rhizosphere zones (Hinsinger et al. 1991; Hinsinger et al. 1992; Hinsinger and Jaillard 1993; Kodama et al. 1994). The formation of vermiculite (a weathering product of mica) is usually caused by the release of  $\text{K}^+$  from mica (Fanning et al. 1989) and can take place within a few days (Hinsinger et al. 1992). Primarily, whenever the uptake of  $\text{K}^+$  by plant roots was greater than the release of  $\text{K}^+$  from the mica, the release of  $\text{K}^+$  from mica was enhanced (Arocena et al. 1999). The formation of vermiculite from phlogopite (a mica species) in the rhizosphere was observed when the  $\text{K}^+$  concentration fell below  $80 \mu\text{mol dm}^{-3}$  (Hinsinger and Jaillard 1993). In the present study, the significantly higher exchangeable  $\text{K}^+$  in ECS compared to N-ECM samples could have originated from the breakdown of muscovite and its subsequent adsorption to the exchange sites of newly formed 2:1 expanding clays. The complete dissolution of the mica lattice could explain another pathway of mica weathering in the rhizosphere (Hinsinger and Jaillard 1993).

Although kaolinite is predicted to be more soluble than chlorite based on thermodynamic equilibria (Rai and Kittrick 1989), our results seem to indicate the opposite. Deviations from thermodynamic prediction are not uncommon because those predictions do not consider the kinetics of the reactions and/or the thermodynamic data may not be reliable (Rai and Kittrick 1989). Other factors that may have contributed to the observed deviations from predicted equilibria include the presence of organic buffers, and amorphous phases, composition and crystallinity of the minerals (Brandt et al. 2003).

Higher acidity in ECS compared to N-ECM soils may mean higher dissolution rates for minerals because organic acids are known to accelerate weathering. One pH unit lower in ECS samples compared to N-ECM soils can increase the rate of mineral dissolution by a factor of three (Drever and Stillings 1997). Higher levels of organic acids have been reported in rhizosphere soils compared to bulk soil (Grierson 1992; Griffiths et al. 1994). In addition, concentrations of organic acids may be high in the microenvironments around fungal hyphae (Drever and Vance 1994; Drever and Stillings 1997). Griffiths et al. (1994) suggested that *Gautieria monticola*, an hypogeous ectomycorrhizal fungi, which produces large amounts of oxalic acid, might weather soil minerals. Both citric and oxalic acid are involved in the dissolution of feldspars (Manley and Evans 1986). Organic acids (e.g., oxalic, citric), produced by ECM in rhizosphere soils, could initiate the removal of the hydroxide sheet from chlorite (Arocena et al. 1999). The removal of  $Mg^{2+}$  from hydroxide sheet is also a possible pathway in the formation of 2:1 clay from the weathering of chlorite. Oxalate produced by ECM is an active agent of mineral weathering because of its high complexing capability (Cromack Jr. et al. 1979; Robert and Berthelin 1986; Lapeyrie 1988). In a related study, we have found that verrucose and angular ornamentations in *Piloderma* ECM mostly appear to be crystals of Ca-oxalate complexes (Arocena et al. 2001).

### SUMMARY AND CONCLUSION

In summary, study results show that soil properties such as total C, total N, pH, CEC, and exchangeable cations are different in ECS compared to N-ECM soils. Total C and N were higher in ECS than in N-ECM soils whereas soil pH was lower, possibly due to higher microbial activity in ECS soils. The CEC as well as exchangeable  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $K^+$ , and  $Na^+$  were also higher in ECS soils. Elevated CEC in ECS soils most likely resulted from higher total organic matter that has increased pH dependent charge, as well as from the high content of 2:1 expanding clays. No significant differences were found in the chemical composition between *Piloderma*-dominated soils and soils dominated by other (<1% *Piloderma*) ECM fungi.

The trends in the amounts of mica, chlorite, kaolinite, and 2:1 expandable clays in ECS compared to N-ECM soils support earlier findings (e.g., Hinsinger et al. 1992; Kodama et al. 1994; Arocena et al. 1999). Kaolinite seems to weather faster than mica and chlorite in ECS compared to N-ECM soils. The ratios of vermiculite plus smectite to mica and chlorite indicate that weathering of mica and chlorite may have occurred in ECS soils, especially in ECS-A where vermiculite and smectite were higher compared to ECS-B and N-ECM. Possible differences in the biological activities between some ECM morphotypes on hybrid spruce may be influencing these soil processes. Although the study focused on Gray Luvisol soils and hybrid spruce in mature forest stands, the results suggest that the presence of ectomycorrhizal fungi can enhance the weathering of minerals in the ECS of these root systems.

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