

# Chemical and mineral composition of ectomycorrhizosphere soils of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) in the Ae horizon of a Luvisol

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

<sup>1</sup>Forestry Program, University of Northern British Columbia, Prince George, British Columbia, Canada V2N 4Z9, e-mail: arocenaj@unbc.ca; and <sup>2</sup>Department of Soil Science, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4. Received 28 May 1998, accepted 19 August 1998.

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. Differences in the properties of bulk forest and rhizosphere soils are often attributed to ectomycorrhizal association, or the symbiosis characterized by a fungal sheath surrounding the root (mantle) and intercellular root colonization (Hartig net). We compared the soil pH, total C, N, cation exchange capacity, and the contents of mica, chlorite, kaolinite, 2:1 expandable clays, feldspars and amorphous materials between two ectomycorrhizosphere soils (or soil environment in the vicinity of **ectomycorrhizae (ECM)**) and non-ectomycorrhizosphere soils to study the influence of ectomycorrhizae on chemical and mineralogical properties of soils. The two ectomycorrhizosphere soils were characterized by ectomycorrhizal colonization dominated by (1) *Piloderma* spp., and (2) *Mycelium radialis atrovirens* and **cottony yellow-brown (MRA-CYB)** types or where *Piloderma* spp. colonization was <2%. Our results showed that total C and N were higher in ectomycorrhizosphere than non-ectomycorrhizosphere soils, and the ectomycorrhizosphere soils dominated by *Piloderma* spp. had almost twice the total C and N as ectomycorrhizosphere soils with MRA-CYB. Soil pH was lower by half a pH unit in ectomycorrhizosphere soils compared to non-ectomycorrhizosphere soils. Cation exchange capacity as well as exchangeable Ca<sup>2+</sup>, Mg<sup>2+</sup> and K<sup>+</sup> were lower in non-ectomycorrhizosphere soil compared to ectomycorrhizosphere soils. We also found that cation exchange capacity, exchangeable Ca<sup>2+</sup>, Mg<sup>2+</sup> and K<sup>+</sup> values in soils dominated by *Piloderma* spp. were higher compared to ectomycorrhizosphere soils with insignificant *Piloderma* spp. Our results suggest that transformation rate of mica and chlorite to 2:1 expandable clays was predominant in ectomycorrhizosphere compared to non-ectomycorrhizosphere soils, likely as a result of high production of organic acids and direct extraction of K<sup>+</sup> and Mg<sup>2+</sup> by fungal hyphae. In ectomycorrhizosphere samples, it is suggested that K<sup>+</sup> and possibly amorphous Al<sub>2</sub>O<sub>3</sub> and Fe<sub>2</sub>O<sub>3</sub> could reconstitute the degraded mica and chlorite through the formation of hydroxy-interlayered 2:1 clays.

**Key words:** Ectomycorrhizosphere soils, subalpine fir, *Piloderma* spp.

Arocena, J. M., Glowa, K. R., Massicotte, H. B. et Lavkulich, L. 1999. **Composition chimique et minérale de l'ectomycorrhizosphère sous sapin subalpin (*Abies lasiocarpa* (Hook.) Nutt.) dans l'horizon Ae d'un luvisol.** Can. J. Soil Sci. **79**: 25–35. Les différences affectant les propriétés des sols forestiers et de leur rhizosphère sont souvent attribuées aux associations ectomycorhiziennes, c.-à-d. la symbiose créée par le manteau fongique entourant la racine et la colonisation intercellulaire de la racine (réseau de Hartig). Pour étudier l'influence des ectomycorhizes sur les propriétés chimiques et minéralogiques du sol, nous avons comparé le pH, les teneurs en C et en N totaux, la capacité d'échange cationique ainsi que les teneurs en mica, en chlorite, en kaolinite, en argiles gonflables 2:1, en feldspaths et en matières amorphes, de deux sols à ectomycorhizosphère (la zone de sol immédiatement au contact des ectomycorhizes) et de sols sans ectomycorhizosphère. Les deux ectomycorhizosphères étaient colonisées en dominance par 1) *Piloderma* spp. et 2) par *Mycelium radialis atrovirens* et par des champignons de type brun-jaune duveteux (MRA-CYB) avec moins de 2 % de *Piloderma* spp. Les concentrations de C et de N totaux étaient plus élevées dans les échantillons de sols avec ectomycorhizes que dans le sol sans ectomycorhizes et que le sol à *Piloderma* spp. contenait presque le double de C et de N totaux que le sol à MRA-CYB. Le pH du sol ectomycorhizien était de presque une unité plus bas que celui des échantillons non ectomycorhiziens. La capacité d'échange cationique ainsi que les concentrations de Ca<sup>2+</sup>, Mg<sup>2+</sup> et K<sup>+</sup> échangeables étaient plus basses dans le sol non ectomycorhizien. Par ailleurs, ces valeurs étaient plus élevées dans le sol à dominance de *Piloderma* spp. que dans celui ne contenant que très peu de ces espèces. Il semble que le taux de transformation du mica et de la chlorite en argile gonflable 2:1 était plus prononcé dans le sol à ectomycorhizosphère que dans le sol sans ectomycorhizosphère, vraisemblablement à cause de la forte production d'acides organiques et de l'exportation directe des ions K et Mg par les hyphes fongiques. Dans les prélèvements de sol entourant les ectomycorhizes, il semble que les ions K et éventuellement Al<sub>2</sub>O<sub>3</sub> et Fe<sub>2</sub>O<sub>3</sub> amorphes pourraient reconstituer le mica et la chlorite dégradés par la formation d'argile 2:1 à espaces interfoliaires hydroxylés.

**Mots clés:** Ectomycorhizosphère, sapin subalpin, *Piloderma* spp.

Soil properties and biogeochemical processes in bulk forest soils are often different from those of the rhizosphere or the narrow soil environment affected by activities of living

roots (Foster et al. 1983; Curl and Truelove 1986). For instance, the weathering rate of micaceous mineral to 2:1 expandable type of clay is faster in rhizosphere compared to

non-rhizosphere soils (Hinsinger et al. 1991; Kodama et al. 1994). In soils under coniferous trees, however, most of these differences might be attributed to ectomycorrhizae established when ectomycorrhizal fungi form an intercellular Hartig net and a sheath of hyphae mantle around the root (Foster et al. 1983). These ubiquitous root-fungal associations modify many soil processes such as mineral weathering and nutrient uptake, especially in the ectomycorrhizosphere or the soil environment in the immediate vicinity of the ECM (Perry et al. 1987). Cromack, Jr et al. (1979) reported the concentration of up to 82 g calcium oxalate per square meter of soil in ECM system of *Hysterangium crassum* in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) while Snetselaar et al. (1990) found that the mycorrhizae of *Monotropa uniflora* accumulates calcium oxalate to avoid the potential calcium and oxalate toxicities. April and Keller (1990) observed the accumulation of amorphous oxides of Al and silica in the rhizosphere zone in forest soils of eastern United States. Hinsinger and Jaillard (1993) indicated that K concentration in soil solution from rhizosphere soils is higher than the non-rhizosphere soil of ryegrass, and Rygiewicz and Bledsoe (1984) and Rygiewicz et al. (1984) documented that ECM increased uptake of  $K^+$  and  $NH_4^+$  ions in coniferous trees. Ectomycorrhizae have been shown to increase cation uptake of plant roots through increased adsorbing surface area contributed by fungal mycelia (Sylvia 1998), chelation, or indirectly by influencing soil microbial activity (Grayston et al. 1996).

Berthelin (1983) also indicated that microbial weathering of soil minerals depends on the species of microorganisms. For example, ECM system of *H. crassum* in Douglas fir was reported to supply a high amount of oxalate to extract the Fe and Al from andesite (Cromack, Jr. et al. 1979). Leyval and Berthelin (1991) observed the transformation of phlogopite to vermiculite in rhizosphere of pine seedling inoculated with *Laccaria laccata*. Furthermore, the in vitro activities of two ectomycorrhizal fungi, *Paxillus involutus* and *Rhizopogon luteolus*, released the  $NH_4^+$  ions trapped in the interlayer space in vermiculite (Paris et al. 1994). Lapeyrie (1988) reported that *Paxillus involutus* could utilize bicarbonate ions from soil solution to synthesize oxalic acid. Recently, Jongmans et al. (1997) reported that in granitic rocks in Sweden, hyphae of *Suillus granulatus* and *Piloderma croceum* connect calcium feldspars and the tree root, thereby participating directly in tree nutrition.

The high diversity of fungal species involved in ECM and host specificity may impart various benefits, especially with regards to their ability to facilitate nutrient uptake from soil substrate to plants via mineral weathering and hyphal translocation. Few studies have addressed the biology of ECM in coniferous trees with respect to in situ role of mycorrhizae on mineral weathering in forest soils (Fitter and Garbaye 1994; Grayston et al. 1996; Molina et al. 1992; Courchesne and Gobran 1997).

In this study, we documented the different properties (chemical and mineralogical) of non-ectomycorrhizosphere soil and ectomycorrhizosphere soils of subalpine fir in the Ae horizon of a Brunisolic Gray Luvisol in the central interior of British Columbia. Specifically, we compared the pH,

total C and N, cation exchange properties, the contents of mica, chlorite, kaolinite, feldspars, 2:1 expanding clays and amorphous minerals between soils collected from non-ectomycorrhizosphere and two specific ectomycorrhizosphere soils.

## MATERIALS AND METHODS

### Description of the Study Area and Sample Collection

The study area was within the Sub-Boreal Spruce zone (Meidinger and Pojar 1991) and was located near the campus of the University of Northern British Columbia in Prince George, BC, Canada (53°54'N 123°49'W). The soil is a bisequum, a Brunisolic Gray Luvisol (Dawson 1989), developed on drumlinized basal till with 4-cm-thick forest floor and about 10-cm-thick Ae horizon. The annual precipitation ranges from 500 to 800 mm, and mean annual temperature is 3.3°C. Vegetation is dominated by hybrid white spruce (*Picea glauca* × *engelmannii*), subalpine fir (*Abies lasiocarpa*), and lodgepole pine (*Pinus contorta* var. *latifolia*) with minor amounts of Douglas fir (*Pseudotsuga menziesii*), trembling aspen (*Populus tremuloides*), cottonwood (*P. balsamifera* var. *trichocarpa*), and paper birch (*Betula papyrifera*). The understory vegetation is a mixture of shrubs such as thimbleberry (*Rubus parviflorus*), Prince's pine (*Chimaphila umbellata*), wildflowers (e.g., wild sarsaparilla (*Aralia nudicaulis*), bunchberry (*Cornus canadensis*), and round-leaf violet (*Viola orbiculata*)) as well as minor amounts of oak fern (*Gymnocarpium dryopteris*) and club-mosses (*Lycopodium complanatum* and *L. dendroideum*).

### Sample Collection

Soil samples were collected from June to August 1997 at the base of 16 subalpine fir trees with a 12 to 27 cm diameter at breast height. These samples were combined to obtain composite samples of non-ectomycorrhizosphere, ectomycorrhizosphere A and ectomycorrhizosphere B soils. **Ectomycorrhizosphere A (ECS-A)** was dominated by *Piloderma* spp., and **ectomycorrhizosphere B (ECS-B)** by MRA-CYB types, or where *Piloderma* spp. had approximately <2% colonization. We selected *Piloderma* spp. because it is commonly observed on subalpine fir and its yellow color was readily recognizable in the field. Morphological descriptions of *Piloderma* spp., MRA and CYB mycorrhizae are given in Table 1.

The humus layer was removed from a sampling point of approximately 2 m by 2 m at the base of each tree to expose the rooting zone in the LFH/Ae horizon boundary. Roots were carefully selected by tracing the rootlets to the main roots of the subalpine fir. After verification that the roots were linked to subalpine fir, roots with predominant *Piloderma* spp. colonization (ECS-A), and roots exhibiting colonization with other ECM (<2% *Piloderma* spp.) (ECS-B) were collected. **Non-ectomycorrhizosphere (N-ECM)** soil was collected where rootlets, rhizomorphs and extra radical mycelium were visually absent. Roots and soil were placed in plastic bags and stored at 4°C until processing. Care was taken during sampling to prevent ectomycorrhizosphere soil from falling off the rootlets.

**Table 1. Morphological descriptions of the dominant ectomycorrhizae of subalpine fir in Ae horizon of Luvisol**

Ectomycorrhizae	Branching pattern, surface texture, lustre, color	Mantle characteristics, inner mantle (IM)-outer mantle (OM)	Emanating hyphae (EH), mycelial strand (MS)
<i>Piloderma</i> spp.	Irregular systems, coarsely felty, matte, bright yellow	OM felt prosenchyma, finely verrucose, hyphae 2.5–3 µm wide, septate; IM net prosenchyma, hyphae 1.5–2.5 µm wide; mantle 10–40 µm thick	EH abundant, 3 µm wide, bright yellow, verrucose, septate, H-shaped anastomoses with septa, no clamps; MS numerous, strand hyphae as per EH
<i>Mycelium radicans atrovirens</i> (MRA)	Single, finely grainy to slightly felty, shiny, black/dark brown, root apex sometimes hyaline	OM net prosenchyma with typically inflated outer hyphal cells, hyphae 1–5 µm wide; IM net synenchyma, hyphae 2.5–3 µm wide; mantle 10–15 µm thick	EH common, 2–3 µm wide, finely verrucose, septate, brown/black, no clamp; MS not observed
Cottony, cream yellow-brown (CYB)	Single to monopodial pinnate, felty to cottony, matte, rarely reflective, cream brown to golden yellow	OM net prosenchyma, hyphae 1–4 µm wide; IM net synenchyma, hyphae 2–3 µm wide; presence of tortuous hyphae with elbow-like projections or swellings, common in OM, rare in IM; mantle 5–25 µm thick	EH common, straight, 1–3 µm wide, septate; MS abundant, loosely formed and undifferentiated, with hyphae 2.5–3.5 µm wide, septate, no clamp, no elbow-like projections observed on MS hyphae
<i>Cenococcum geophilum</i> Fr.	Single to monopodial pinnate (rare), finely to coarsely grainy, shiny, black.	OM net synenchyma, hyphae 4–7 µm wide, with distinct “stellar” pattern; IM net synenchyma, hyphae 1–4 µm; mantle 5–25 µm thick	EH common, straight, thick walls, 3–6µm wide, septate, smooth, dark brown-black; MS not observed
Cottony-white	Single, cottony, reflective, white	OM felt prosenchyma, hyphae 2–7 µm wide, with distinct globular ornamentation; IM net synenchyma to non-interlocking irregular synenchyma, hyphae 3–6 µm.	EH common, 3–5 µm wide, occasional clamps, medium sized globular ornamentation on hyphae and clamps; MS common, loosely formed, undifferentiated to slightly differentiated, strand hyphae as per EH.
<i>Russula</i> -like	Single, finely grainy or felty to cottony, mostly shiny, light tan brown	OM felt prosenchyma to regular synenchyma, cells 8–25 µm wide; IM net synenchyma, hyphae 2–3 µm wide, septate; mantle 8–15 µm thick; no laticifers observed	EH rare to common, 2–4 µm wide, tortuous, clamps frequent; MS not observed
<i>Lactarius</i> -like 1	Single to monopodial pinnate, smooth to finely grainy, shiny, light to dark brown	OM net synenchyma, hyphae 1–3 µm wide, with numerous swollen cells that stain purple-red in toluidine blue; IM net synenchyma, hyphae 2–3 µm wide, rare laticifer-like cells, 6–10 µm wide; mantle 10–15 µm thick	EH rare to common, 3–4 µm wide with small verrucose ornamentation and thick walls 0.5–1µm, intense purple-red reaction with toluidine blue, clamps common; MS not observed

Ectomycorrhizosphere soil was collected from the <3-mm-thick layer of soil that adhered to the rootlets by agitating the rootlets on a 2-mm sieve until most of the soil from the roots was collected. The rootlets were then washed with de-ionized water and the soil washed from the rootlets was collected and referred to as **ectomycorrhizoplane (ECP)** soil. Six composite samples for each of ECS-A and ECS-B were analyzed for mycorrhizal colonization and three composite samples for physical, chemical and mineralogical composition. Ectomycorrhizoplane samples from ECS-A soil were designated as ECP-A and samples from ECS-B were designated as ECP-B. In this study, ECS-A and ECS-B as a group of ectomycorrhizosphere soils are referred to as ECS soils while ECP-A and ECP-B as a group of ectomyc-

orrhizoplane soils are referred to as ECP soils. The ECS and ECP soils were air dried for subsequent analysis. Because only a small amount of ECP soil was collected, the analyses conducted on these samples were limited to chemical estimates of clay mineralogy. The rootlets from ECS soils were stored at 4°C until mycorrhizal assessment.

**Mycorrhizal Colonization**

Ectomycorrhizosphere A and ECS-B root samples were assessed for ECM diversity and abundance and described according to guidelines outlined by Goodman et al. (1996), Ingleby et al. (1990) and Agerer (1987–1996) using bright field microscopy. Roots were placed in a glass dish (~30 cm × 20 cm × 5 cm) and cut into short pieces to randomize root

**Table 2. Mean abundance (%) of ectomycorrhizae present in the ectomycorrhizosphere A (ECS-A) and B (ECS-B) samples for sub-alpine fir ( $n = 6$ )**

Ectomycorrhizae	ECS-A	ECS-B
<i>Piloderma</i> spp.	66 (3.3) <sup>z</sup>	1.6 (2.3)
<i>Tomentella</i> -like type 1	0.69 (1.12)	0.22 (0.54)
<i>Tomentella</i> -like type 2	— <sup>y</sup>	1.4 (3.5)
<i>Cenococcum geophilum</i> Fr.	17 (7)	17 (13)
<i>Amphinema</i> -like type 1	—	1.10 (2.4)
<i>Amphinema</i> -like type 2	0.13 (0.33)	1.33 (3.3)
<i>Suillus</i> -like	—	0.43 (0.88)
Cottony, white	1.7 (2.9)	2.3 (5.5)
MRA	2.2 (1.4)	32 (28)
<i>Lactarius</i> -like type 1	5.8 (2.6)	8.9 (12)
<i>Lactarius</i> -like type 2	—	0.85 (2.1)
Cottony-cream yellow brown	3.5 (5.4)	24 (21)
<i>Inocybe</i> -like	—	0.28 (0.69)
<i>Russula</i> -like	2.7 (4.1)	5.1 (3.0)
Smooth, tan-brown	—	0.40 (0.98)
Light gray	—	0.86 (2.10)
E-strain	—	1.2 (1.5)
Brown-white	0.03 (0.07)	—
Woolly, white type	0.17 (0.41)	1.8 (2.9)
Number of root tips counted per sample	994 (97)	1193 (204)

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

<sup>y</sup>Represents absence of ectomycorrhizae.

tips. Selected mycorrhizal tips were described according to branching pattern, surface texture, luster, color, mantle characteristics, emanating hyphae and other morphological properties to identify ECM morphotypes. Some were stained with toluidine blue to enhance contrast. Root tips with wrinkled, brown/decayed cells were categorized as dead. Damaged or broken root tips were not counted. Ectomycorrhizal abundance was estimated until either 500th live root tips or 1000th dead root tips. The proportion of colonization by each ECM morphotype was calculated. The Sorensen similarity index was calculated following the method suggested by Legendre and Legendre (1983). An index of 1 indicates a total conformity, while zero indicates total dissimilarity. Permanent slide mounts, photographs and frozen samples were kept for each ECM type.

### Physical and Chemical Analyses

The particle size analysis was conducted on air-dried soil samples (<2 mm in diameter), subjected twice for 3 min to ultrasonic dispersion using Braunsonic<sup>TM</sup> probe operated at 400 W of power. The treated samples were suspended in a 2-L beaker after wet-sieving to separate the sand (50–2000  $\mu\text{m}$ ) from the clay (<2  $\mu\text{m}$ ) and silt (2–50  $\mu\text{m}$ ) 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 suspension of 1:1 soil:water ratio using Orion<sup>TM</sup> pH meter. The **cation exchange capacity (CEC)** and exchangeable cations in the soils were determined using  $\text{NH}_4\text{OAc}$  buffered at pH 7.0 (Kalra and Maynard 1991). The amounts of exchangeable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$  and  $\text{Na}^+$  were determined from the  $\text{NH}_4\text{OAc}$ -extract using **Inductively**

**Table 3. Mean values for physical and chemical properties of two ectomycorrhizosphere (ECS-A and ECS-B) and non-ectomycorrhizosphere (N-ECM) soils ( $n = 3$ )**

Soil Properties	ECS-A	ECS-B	N-ECM
Particle size dist. ( $\text{g kg}^{-1}$ )			
Sand*	680a (40) <sup>z</sup>	600b (40)	590b (10)
Silt	270 (50)	340 (40)	350 (8)
Clay	50 (4)	50 (2)	50 (4)
pH <sup>y</sup>	4.6 (0.25)	4.9 (0.26)	5.2 (0.60)
Total carbon*** ( $\text{g kg}^{-1}$ soil)	39a (5.1)	22b (2.9)	13c (3.0)
Total nitrogen*** ( $\text{g kg}^{-1}$ soil)	1.6a (0.2)	1.0b (0.1)	0.7c (0.03)
C/N ratio***	24a (0.11)	22b (0.58)	20c (0.36)
CEC*** ( $\text{cmol}_c \text{ kg}^{-1}$ soil)	10a (0.40)	6.8b (0.54)	4.9c (0.35)
Ex. cations ( $\text{cmol}_c \text{ kg}^{-1}$ soil)			
Ca**	8.1a (0.37)	5.6b (1.1)	4.2b (0.37)
Mg**	1.1a (0.05)	0.74b	0.64b (0.05)
K**	0.32a (0.01)	0.25b (0.02)	0.15c (0.01)
Na	0.07 (0.01)	0.07 (0.001)	0.05 (0.01)
Base saturation (%)	94 (8)	96 (11)	>100 (6.4)

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

<sup>y</sup> $n = 6$ .

a–c Across each row, means followed by the same letter are not significantly different.

\*, \*\*, \*\*\*  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively.

**Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).** Three replicates of the samples 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 from the Canadian Certified Reference Materials Project identified as SO-2 and SO-3 were used as internal 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 Philips 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 **ethylene glycol (EG)** and **glycerol (GLY)**, while the K-saturated sample was heated at 300 and 550°C. The 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, (4) smectite — 1.7 nm reflection in Ca-GLY and Ca-EG treatments, and (5) vermiculite — 1.7 nm reflection in Ca-EG treatment and absence of 1.7 nm reflection in Ca-GLY treatment. 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  $\text{kg}^{-1}$  muscovite and 89.7 g Mg  $\text{kg}^{-1}$  chlorite based on the chemical composition  $\text{K}(\text{Si}_3\text{Al})\text{Al}_2\text{O}_{10}(\text{OH})_2$  for muscovite

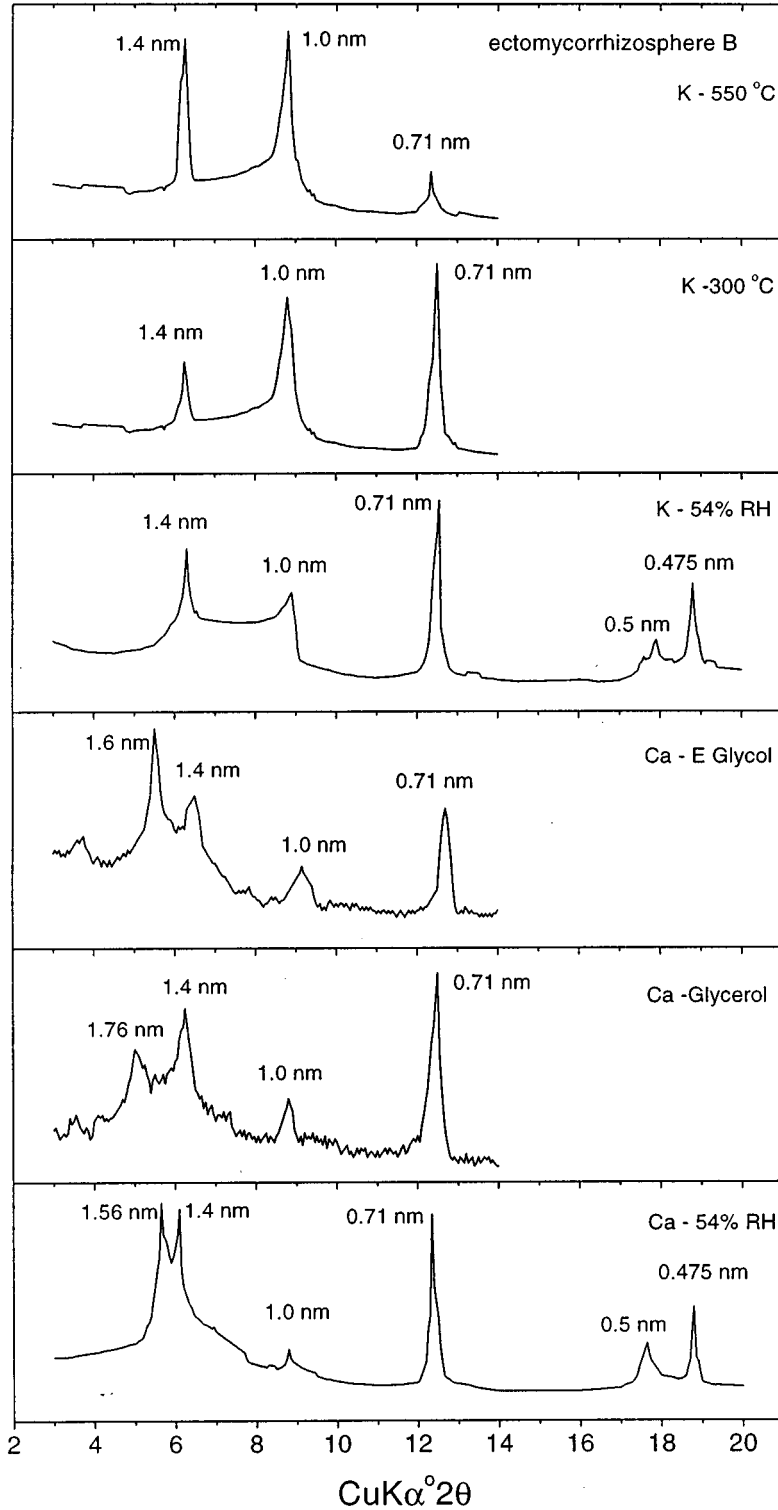


Fig. 1. X-ray diffraction patterns of the clay fraction from ECS-B sample subjected to seven pre-treatments.

(Fanning et al. 1989), and  $(Mg, Fe, Al)_6(SiAl)_4O_{10}(OH)_8$  for chlorite (JCPDS Card # 7-78). The amount of 2:1 expandable clays (vermiculite and smectite) was estimated from  $CEC_{Ca}$  and  $CEC_K$  of the clay fraction following the guidelines of Alexiades and Jackson (1965).  $CEC_{Ca}$  was the CEC of the clay measured by the amount of Ca replaced by

$MgCl_2$  while  $CEC_K$  was determined from the amount of  $K^+$  replaced by  $NH_4Cl$  after overnight heating of the K-saturated clay at  $110^\circ C$ . Kaolinite content was estimated from a modified procedure of Warren and Dudas (1992) by subtracting the integrated area under the 0.712 nm peak in the K-550°C sample from the integrated area of the same reflect-

**Table 4.** Mean contents (g kg<sup>-1</sup>) of selected phyllosilicates and oxides in clay fraction, and feldspars in sand fraction in N-ECM, ECS and ECP soils (n = 3)

Minerals	N-ECM	ECS-A	ECS-B	ECP-A	ECP-B
<i>Clay</i>					
Mica***	220a (0.7) <sup>z</sup>	175b (4.6)	190b (17)	190b (5.9)	175b (7.4)
Chlorite**	100a (6.2)	90b (7.3)	100a (1.8)	85b (7.3)	75c (2.6)
Kaolinite	275 (119)	135 (49)	219 (122)	ND <sup>y</sup>	ND
Amor. Al <sub>2</sub> O <sub>3</sub> **	7.4a (1.6)	8.6a (4.9)	6.4a (1.3)	2.0b (0.79)	2.6b (0.78)
Amor. Fe <sub>2</sub> O <sub>3</sub> ***	2.4a (0.29)	3.9b (1.7)	3.1b (0.38)	0.89c (0.15)	0.44c (0.21)
Amor. SiO <sub>2</sub>	0.53 (0.02)	0.62 (0.23)	0.57 (0.01)	0.60 (0.12)	0.41 (0.03)
FeOOH <sup>x</sup>	20 (3.7)	20 (3.1)	25 (3.2)	20 (0.82)	20 (0.52)
Vt+Sm**	160a (14)	200a (24)	185b (24)	135c (19)	140c (4.4)
(Vt+Sm)/Mi***	0.73a (0.06)	1.1b (0.13)	0.97b (0.14)	0.72a (0.08)	0.81a (0.04)
(Vt+Sm)/Ch**	1.6a (0.04)	2.2b (0.28)	1.9a (0.23)	1.9a (0.20)	1.6a (0.08)
<i>Sand</i>					
Albite, NaAlSi <sub>3</sub> O <sub>8</sub>	212 (32)	193 (3.7)	195 (3.4)	ND	ND
Anorthite, CaAl <sub>2</sub> Si <sub>2</sub> O <sub>8</sub>	112 (34)	103 (11)	95 (9.7)	ND	ND
Orthoclase, KAlSi <sub>3</sub> O <sub>8</sub>	92 (8.8)	84 (7.5)	88 (6.1)	ND	ND

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

<sup>y</sup>ND = not determined.

<sup>x</sup>Vt = vermiculite, Sm = smectite, Mi = mica; Ch = chlorite; FeOOH (goethite) — assumed as the dominant crystalline Fe oxide.

a–c Across each row, means followed by the same letter are not significantly different

\*\*, \*\*\*Significant at P < 0.01 and P < 0.001, respectively.

tion in the Ca-saturated sample scanned at ambient conditions. Sand fraction was prepared for XRD analysis using the random mount method. 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. Identification of the minerals in the sand fraction was based on the following criteria: (1) quartz — 0.426, 0.333, 0.246, 0.228 and 0.182 nm reflections, (2) feldspars — 0.645, 0.404–0.420, 0.315–0.325 nm reflections, (3) amphiboles — 0.826, 0.324 and 0.304 nm reflections. We estimated the amounts of feldspars such as albite, anorthite and orthoclase from total contents of K, Na, and Ca in the sand fraction, respectively. Mineralogical analyses were conducted in three replicates from each ectomycorrhizosphere and non-ectomycorrhizosphere soils.

### Statistical Analysis

We analyzed the data by ANOVA using Statistica<sup>TM</sup> (version 5). 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.

## RESULTS

### Ectomycorrhizal Colonization

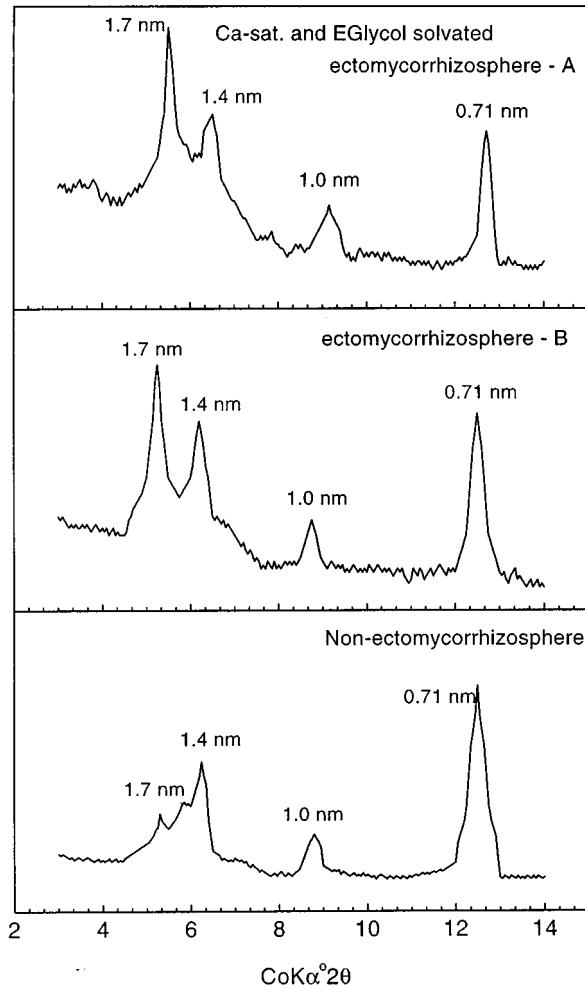
Based on detailed morphological analysis, we described 11 distinct ECM morphotypes in ECS-A samples and 18 ECM morphotypes in ECS-B samples. Ectomycorrhizal colonization was <5 % in N-ECM soils, reaffirming our designation of non-mycorrhizosphere. The calculated Sorensen similarity index was 0.5 between ECS-A and ECS-B samples. The

descriptions for the dominant ECM morphotypes are given in Table 1.

In ECS-A samples, 66% of the ECM were *Piloderma* spp., 17% *Cenococcum geophilum* and 17% representing a combination of nine other morphotypes (Table 2). In ECS-B samples, three ECM types comprised 75% of all ECM: MRA was the most abundant (32%) followed by a “cottony, cream yellow-brown” (CYB) morphotype (24%), and *Cenococcum geophilum* (17%). *Piloderma* spp. was present at 1.6%. Other morphotypes, such as a *Russula*-like type and a *Lactarius*-like were present at 5% and 9%, respectively. Thirteen other ECM morphotypes comprised <10% of ECM in ECS-B samples (Table 2).

### Physical and Chemical Properties

Soil samples contained 5% clay while the silt fraction content ranged from 270 to 350 g kg<sup>-1</sup> (Table 3). The amounts of clay and silt fractions did not vary significantly between the N-ECM, ECS-A and ECS-B samples. However, the content of sand fraction varied among the samples at 59% for N-ECM samples, 60% for ECS-B samples, and 68% for ECS-A samples. The pH in rhizosphere soils was lower than N-ECM soils. Soil pH ranged from pH 4.6 in ECS-A to pH 5.2 in N-ECM soil. The contents of total C and N were lowest in N-ECM, highest in ECS-A soils, and varied significantly between all soils (Table 3). The C/N ratio also varied between samples and ranged from 20 in N-ECM soils to 24 in ECS-A soils. The CEC in ECS-A samples was 10 cmol<sub>c</sub> kg<sup>-1</sup> soil and was equivalent to twice the CEC in N-ECM samples. The CEC in ECS-A samples was also significantly higher than ECS-B samples. The amount of exchangeable cations followed the order: exchangeable Ca<sup>2+</sup> > Mg<sup>2+</sup> > K<sup>+</sup> > Na<sup>+</sup>. The amounts of exchangeable Ca<sup>2+</sup>, Mg<sup>2+</sup>, and K<sup>+</sup> in ECS-A samples were significantly higher than in ECS-B and N-ECM samples. The base saturation for all samples



**Fig. 2.** X-ray diffraction patterns of Ca-saturated and Ethylene glycol 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.

was above 94% and was not significantly different between N-ECM, ECS-A and ECS-B soil samples.

### Mineral Composition

Based on XRD analysis, we identified the following phyllosilicates in the clay fraction of all samples: mica, chlorite, 2:1 expandable clays, kaolinite and quartz (Fig. 1). The XRD peaks at 1.0 and 0.5 nm indicated that muscovite was the species of mica in the samples. The amount of mica was significantly higher in N-ECM compared to ECM and ECP samples (Table 4). Chlorite content of ECS-B and N-ECM was significantly higher than ECP soils. The sum of 2:1 expandable clays (vermiculite + smectite) was higher in ECS soils compared to ECP and N-ECM soils. The ratio of the sum of 2:1 expandable clays over mica was significantly lower in N-ECM samples compared to ECS-A and ECS-B samples (Table 4). The ratio of the sum of 2:1 expandable clays over chlorite was lower in N-ECM soils compared to ECS and ECP samples. These trends were also evident in

the relative intensities of 1.7, 1.4, and 1.0 nm reflections in the XRD patterns of ethylene glycol solvated clay samples (Fig. 2). Generally, kaolinite was higher in the N-ECM samples compared to ECS samples. The amounts of amorphous  $\text{Fe}_2\text{O}_3$  and  $\text{Al}_2\text{O}_3$  were lowest in the ECP samples while the amount of amorphous  $\text{SiO}_2$  was similar for all samples.

In the sand fraction, the following minerals were identified based on the results of XRD analysis: quartz, feldspars, amphiboles, and traces of mica and chlorite (Fig. 3). Quartz was the dominant mineral in all the samples and albite, anorthite and orthoclase were higher in N-ECM samples compared to ECP and ECS samples. Among the different species of feldspars, albite was consistently the most abundant followed by anorthite and orthoclase (Table 4).

## DISCUSSION

### Ectomycorrhizal Colonization and Tree Nutrition

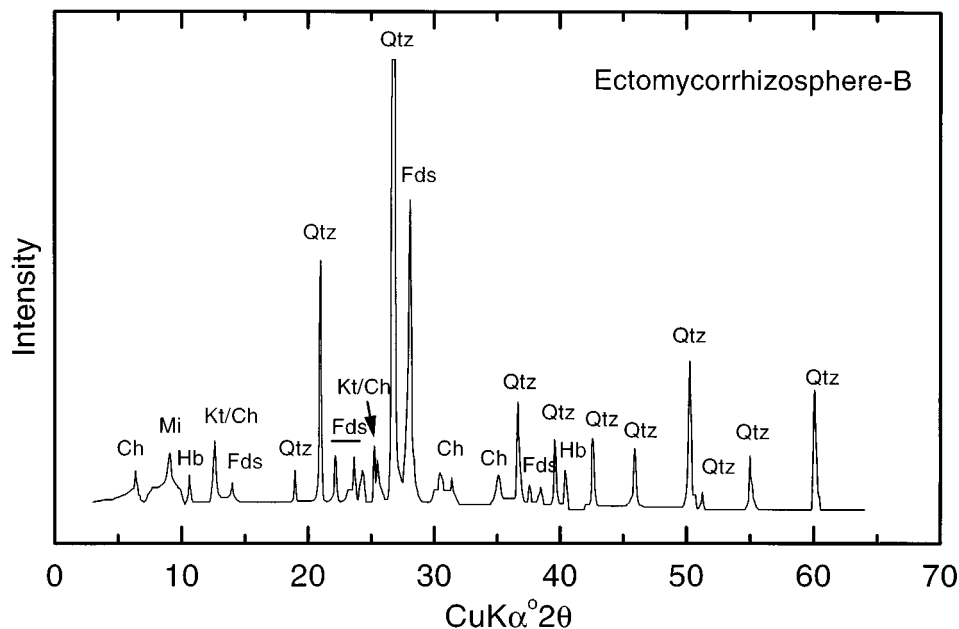
In this study, *Piloderma* spp. represented an important component of the ectomycorrhizae sampled, and was located in the LFH/Ae boundary although it was present in the woody debris in the study site. This is contrary to an earlier report that growth of *Piloderma* spp. was confined to organic materials (Goodman et al. 1996). *Piloderma fallax* has also been reported to be abundant in several 90 to 440-yr-old Douglas fir stands on Vancouver Island, in decaying wood, sometimes in fragmented litter, but not in the mineral soil (Goodman et al. 1996). Jongmans et al. (1997) recently reported *Piloderma croceum* to be involved in granite weathering in Sweden.

*Cenococcum geophilum* was moderately abundant in our samples, located in the LFH/Ae boundary. This corroborates well with *C. geophilum* being a dominant component in 23- to 180-yr-old *Abies amabilis* forests in the Pacific Northwest (Vogt et al. 1981). *Cenococcum* is distributed worldwide (Trappe 1964) and reports in British Columbia indicate it exists between the forest floor and the mineral soil (Harniman and Durall 1996).

The *Russula* and *Lactarius*-like morphotypes, although not common, were consistently present and were similar to morphotypes described for *Abies lasiocarpa* in the Rocky Mountains of Alberta (Kernaghan et al. 1997). The 18 ECM morphotypes described illustrated the diversity of ectomycorrhizal fungi interacting with *A. lasiocarpa* roots in this localized area. Although this study was not designed to measure fungal diversity, it suggests a variety of fungal symbionts may be influencing mineralization of forest soil nutrient, and water acquisition for tree growth.

### Chemical Properties of Ectomycorrhizosphere and Non-ectomycorrhizosphere Soils

The lower pH in ECS soils compared to N-ECM soils is consistent with earlier reports. Berthelin (1983) attributed the low pH in the mycorrhizosphere zone to the activities of ECM such as oxidation of inorganics (e.g., sulphur) and the production of organic acids (e.g., oxalic, carbonic, citric, acetic). Low pH could also result from a high rate of  $\text{NH}_4^+$  uptake by plants (Marschner et al. 1987) as roots exude  $\text{H}^+$  into the rhizosphere soil to counteract the depleted positive



**Fig. 3.** X-ray diffraction patterns of the sand fraction from ECS-B sample showing the presence of quartz (Qtz), chlorite (Ch), kaolinite (Kt), hornblende (Hb), and feldspars (Fds).

charge arising from the uptake of  $\text{NH}_4^+$ . The presence of ECM has been reported to enhance the adsorption of  $\text{NH}_4^+$  and  $\text{K}^+$  in coniferous trees (Rygiewicz and Bledsoe 1984; Rygiewicz et al. 1984). In Douglas fir stands, Cromack, Jr. et al. (1979) reported that pH 4.9 in soils colonized by *Hysterangium crassum* was significantly lower than pH 6.1 measured in uncolonized soil.

The higher amounts of total C and N in ECS soils compared to N-ECM soils can be attributed to the presence of roots and the ectomycorrhizal fungi associated with them. The fungi (and other organisms) in the rhizosphere were significant sinks for carbon assimilated by the host plant, and upon death contributed their biomass to the high contents of total C and N in ECS soils. For example, up to 30% of total C assimilated by *Pinus sylvestris* can be used by *Suillus luteus* (Söderström and Read 1987) while Douglas fir could reportedly allocate up to 73% of assimilated C to ECM (Fogel and Hunt 1983). Grayston et al. (1996) indicated that *Pinus sylvestris* ECM soils contained more root exudates than non-ectomycorrhizosphere soils.

The relative contents of total N between ECS and N-ECM soils paralleled total C as N is also a necessary component of microbial biomass. Higher total C and N in ECS-A compared to ECS-B might be associated with high biomass per unit of root weight in the ECS-A samples. Although there was a significant difference in the C:N ratio between soil samples, all C:N ratios were close to the threshold of 20:1 indicating higher mineralization rates compared to fresh organic matter with C:N ratio between 40:1 and 100:1 (Myrold 1998).

The higher CEC in ECS soils compared to N-ECM soils might be attributed to the high amount of organic matter because clay contents in N-ECM and ECS samples were similar. The higher amount of 2:1 type of clays in ECS soils compared to N-ECM soils could also contribute to the high

CEC. The differences in CEC between ECS-A and ECS-B soils may also be explained by the differences in organic matter content due to different degrees of ECM colonization. Higher CEC in ECS-A than ECS-B samples imply an increase in the capability of ECS-A soil to store essential elements. For instance, ECS-A soils held an additional 0.64 g of  $\text{Ca}^{2+}$   $\text{kg}^{-1}$  soil compared to ECS-B samples and may be the preferred environment for *Piloderma* species.

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

In the clay fraction, the significantly higher amounts of mica and chlorite in N-ECM soils compared to ECS-A and ECP soils indicated a differential rate of mineral weathering between ECS, ECP and N-ECM soils. This is consistent with earlier studies (e.g., April and Keller 1990; Hinsinger et al. 1991; Kodama et al. 1994; Courchesne and Gobran 1997). Transformation of mica and chlorite to 2:1 expandable clays was indicated by the higher ratios of the sum of 2:1 expandable clays over mica, and chlorite in ECS soils compared to N-ECM and ECP soils. The high rate of mica and chlorite weathering to 2:1 expandable clays was also suggested by the higher contents of exchangeable  $\text{K}^+$  and  $\text{Mg}^{2+}$  in ECS compared to N-ECM soils. The high amount of exchangeable  $\text{K}^+$  could originate from the breakdown of muscovite, while  $\text{Mg}^{2+}$  could come from the weathering of chlorite. Although early reports on high rates of mica and chlorite weathering were mostly for the rhizosphere of agricultural crops, the weathering mechanisms may also be operative in the rhizosphere of trees. Faster rates of mineral weathering in the rhizosphere result from both physical and chemical processes. Mechanical alterations of the minerals could include realignment, bending and fracturing of minerals due to pressure exerted by growing roots and associated hyphae and rhizomorph on soil minerals (April and Keller

1990). Robert and Berthelin (1986) showed micrographs of hyphae probing between mica flakes to extract  $K^+$ . Generally, the high rate of chemical weathering in the rhizosphere was attributed to root-induced release of interlayer  $K^+$  (Hinsinger et al. 1991; Hinsinger et al. 1992; Hinsinger and Jaillard 1993; Kodama et al. 1994). The release of  $K^+$  from mica often resulted in the formation of vermiculite (Fanning et al. 1989) and could take place within a few days (Hinsinger et al. 1992). Primarily, the release of  $K^+$  from mica was enhanced whenever the uptake of  $K^+$  by plant roots was greater than the release of  $K^+$  from the mica. Hinsinger and Jaillard (1993) detected the transformation of phlogopite (a mica species) to vermiculite when the  $K^+$  concentration in the rhizosphere fell below  $80 \mu\text{mol dm}^{-3}$ . It also indicates that complete dissolution of the mica lattice could be another pathway of mica weathering in the rhizosphere (Hinsinger and Jaillard 1993).

The low content of 2:1 expandable clays in ECP compared to ECS soils suggests another pathway in the weathering of mica. April and Keller (1990) proposed that high levels of  $K^+$  in rhizoplane soils could result from the reconstitution of degraded mica through potassium fixation. Potassium fixation occurs when the activity of  $K^+$  exceeds the critical level during periods of high transpiration rate or the presence of high amounts of Al and other cations that inhibit  $K^+$  uptake by plants (Cumming et al. 1985). The high amounts of Al and Fe can be incorporated in the interlayer of 2:1 clays to form hydroxy-interlayered 2:1 clay (Farmer et al. 1985). This may explain why a significantly lower amount of amorphous  $\text{Al}_2\text{O}_3$  and  $\text{Fe}_2\text{O}_3$  was observed in ECP compared to ECS and N-ECM soils. The formation of vermiculite and smectite from the removal of hydroxide sheet in chlorite is commonly observed in soil environments (Barnhisel and Bertsch 1989). Organic acids (e.g., oxalic, citric) produced by ECM in rhizosphere soils could initiate the removal of the hydroxide sheet from chlorite. Direct removal of  $\text{Mg}^{2+}$  from hydroxide sheet by the action of fungal hyphae such as those from *Piloderma* is also a possible pathway in the weathering of chlorite. Oxalate produced in ECM is an active agent of mineral weathering because of its high complexing capability (Robert and Berthelin 1986; Lapeyrie 1988). For example, ECM system of *Hysterangium crassum* in Douglas-fir produced high amounts of oxalate to extract the Fe and Al from andesite (Cromack, Jr. et al. 1979). In our study, verrucose and globular ornamentations in ECM appear to be crystals of metal-oxalate complexes.

Weathering of minerals in the sand fraction was evident by the lower amounts of feldspars in ECS samples compared to N-ECM samples. The high exchangeable  $\text{Ca}^{2+}$  in ECS samples might have originated from the weathering of feldspars, particularly anorthite in the sand fraction.

## CONCLUSION

This study suggests that soil properties such as total C, total N, pH, cation exchange capacity, exchangeable cations, contents of mica, chlorite, and 2:1 expandable clays are different in ectomycorrhizosphere soils (ECS) compared to non-ectomycorrhizosphere soils (N-ECM). In addition,

some differences exist in soils dominated by *Piloderma* spp. compared to MRA-CYB-dominated ECS soils. Total C and N were higher in ECS soils than N-ECM soils, and the ECS soils dominated by *Piloderma* spp. had almost twice the total C and N compared to ECS soils dominated by MRA-CYB types. Soil pH was lower by half a pH unit in ECS compared to N-ECM and may be indicative of the higher microbial activity in ECS. Cation exchange capacity as well as exchangeable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  were lower in N-ECM compared to ECS. We also found that CEC, exchangeable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  values in soils dominated by *Piloderma* spp. were higher compared to ECS dominated by MRA-CYB types, suggesting differences in biological activities of the various ECM morphotypes in subalpine fir. This requires further research as our experimental design did not allow us to investigate the sources of the differences in CEC and exchangeable cations between the two ectomycorrhizospheres. Future studies will address the efficiency of each individual ECM morphotypes in extracting specific exchangeable cations or their preferred mineral. This has significant implications for tree nutrition as soils with high CEC and high amounts of exchangeable cations are considered fertile soils, able to supply trees with essential nutrients.

In support of earlier studies, we concluded that the weathering rate of mica and chlorite to 2:1 expandable clays was more rapid in ECS compared to N-ECM. The primary process was probably root-induced removal of interlayer  $K^+$  in the case of mica, and the removal of  $\text{Mg}^{2+}$  from the hydroxide sheet in chlorite. The process may have been enhanced by organic acids produced by the ECM. We did not observe any differences in the rate of mica and chlorite weathering between the two ECM morphotypes tested. Information from ectomycorrhizoplane samples suggested that  $K^+$  and maybe amorphous  $\text{Al}_2\text{O}_3$  and  $\text{Fe}_2\text{O}_3$  could reconstitute degraded mica and chlorite through the formation of hydroxy-interlayered 2:1 clays. The higher CEC, exchangeable cations, and rate of mica and chlorite (and feldspars) weathering in ectomycorrhizosphere soils compared to non-ectomycorrhizosphere soils were indications of the essential functions of ECM in nutrient cycling in these subalpine fir ecosystems.

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