Ectomycorrhizal root tips in relation to site and stand characteristics in Norway spruce and Scots pine stands in boreal forests

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Summary Variations in ectomycorrhizal (EcM) short root tips of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) in 16 stands throughout Finland were studied, and their relationships with latitude, organic layer C:N ratio, temperature sum and foliage biomass were determined. There were no significant differences in EcM root tip frequency (number per milligram of fine roots) or root tip mass between tree species or between northern and southern sites. The EcM root tip number per unit area of the organic layer plus the 0–30 cm mineral soil layer varied between 0.8 and 2.4 million per m^2 for Norway spruce and between 0.7 and 2.9 million per m^2 for Scots pine, and it was higher in the northern Scots pine stands than in the southern Scots pine stands. Over 80% of the EcM root tips of both species were in the organic layer and the upper 0–10 cm mineral soil layer. We related EcM root tips to foliage mass because these two components are the most important functional units in boreal tree physiology. Both species, especially the Scots pine trees, had more EcM root tips in relation to foliage mass in northern Finland than in southern Finland. Scots pine trees had more EcM root tips in relation to foliage mass than Norway spruce in the same climatic region. The EcM root tip:foliage biomass ratio of Norway spruce was positively related to the C:N ratio in the organic layer, whereas that of Scots pine was negatively related to the temperature sum. The number of EcM root tips per milligram of fine root biomass was constant, implying that trees of both species increase nutrient uptake by increasing fine root production and hence their total number of EcM tips and the area of soil occupied by mycelia. Both tree species responded to nitrogen (N) deficiency by maintaining more EcM tips per foliage unit, and this may be related to a higher proportion of N uptake in an organic form.

Introduction

Boreal forests are characterized by a cold climate and by low rates of organic matter decomposition, resulting in low nitrogen (N) mineralization rates (Smolander et al. 2000), with organic forms of N dominating over NH_4^+ and NO_3^- in the soil. Although N availability is the main factor restricting the primary production of boreal forests (Tamm 1991), the actual availability of N varies according to site type and climate. Site type is closely related to soil pH and the availability of base cations (Tamminen 2000), with high values prevailing on fertile sites with a low organic layer C:N ratio. Differences in tree growth rates among sites of different geographical location but similar site type are mainly attributable to differences in growing season length and temperature (Mäkelä et al. 2008).

The two main coniferous tree species in the boreal forests of northern Europe are Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.). Although both species are able to grow on a wide range of site types, forest management has tended to restrict the species to sites where their timber production potential is the greatest. Norway spruce is primarily grown on fertile, moist site types, whereas Scots pine is grown on relatively infertile, coarser-textured soils. As a result of the long history of forest management, there is now a clear relationship between the distribution of these tree species and site type in northern Europe.

The vegetation in boreal forests has developed several mechanisms for increasing plant N uptake and conservation. Ectomycorrhizal (EcM) and ericoid fungi are the

dominating mycorrhizal associations in boreal coniferous forests. Read and Perez-Moreno (2003) hypothesized that selection has favored ectomycorrhizas and ericoids with a well-developed saprotrophic capacity in northern ecosystems, and a considerable proportion of the annual N and phosphorus requirements of boreal trees could be satisfied through the breakdown of organic polymers by the mycelia of the fungal symbiont. On a gradient from northern Sweden to central Europe, Taylor et al. (2000) reported that the characteristic fungal species of northern sites that can use organic N decreased in number as the availability of mineral N increased toward the south.

Estimating the role of EcM root tips or their external mycelia in nutrient and carbon (C) cycling requires quantification of their biomasses, surface areas, numbers and turnover rates (Fogel 1980). Variation in EcM root tip mass in three stands in Finland and Estonia was reported to range from 0.04 to 0.05 mg DW for Norway spruce and from 0.03 to 0.04 mg DW for Scots pine (Ostonen et al. 2007). However, this variation, or the variation in EcM tip frequency (number per unit mass of fine roots), has seldom been reported for ecological gradients (Godbold et al. 2003, Leuschner et al. 2004). In addition, there have been few attempts to relate EcM root tips to needle or leaf biomass, even though these two components are the most important functional units in boreal tree physiology: foliage for C transfer from the atmosphere to tree growth and other metabolic functions through photosynthesis, and ectomycorrhizas and their external mycelia for nutrient uptake and transfer from the soil to tree growth and metabolism.

As roots play a crucial role in N uptake, it has been suggested that root N concentration reflects the N supply in the same way as foliar N concentration (Adams et al. 1987). Although EcM root tips may be more sensitive indicators of soil nutrient availability than leaves, few studies have reported N concentrations of foliage, roots and EcM tips or mycelia from the same sites. Högberg et al. (1998) reported a gradient in fine root N concentration in Norway spruce from central Europe to northern Sweden, and lower N concentrations in non-EcM fine roots compared with EcM fine roots. Högberg et al. (1998) did not separate EcM tips from fine roots along the gradient, but reported higher N concentrations in EcM fungal sheaths in another study (Högberg et al. 1996) and compared the root N concentrations with those of foliage reported by Bauer et al. (1997) for the same sites.

Recently, empirical data have been used to develop regression equations for quantifying fine root biomass (Chen et al. 2004, Helmisaari et al. 2007), including EcM short root tips. Fine roots are generally quantified based on their diameter, the common upper diameter being 2 mm (Vogt et al. 1983). There has been criticism about using this root diameter as the criterion for classifying roots as nutrient uptake organs because roots with a diameter < 2 mm also include a large number of conducting roots that have little or no role in the absorption of nutrients and water (Pregitzer et al. 2002, Godbold et al. 2003). The majority of boreal coniferous fine roots are < 1 mm in diameter (Persson 1978, Ostonen et al. 2005), including short root tips that are mostly EcM, and are characterized by limited apical growth and high branching densities (Brundrett 1991). The mean diameter of Norway spruce and Scots pine short roots in Estonian and Finnish stands was 0.3–0.5 mm (Ostonen et al. 2007), with all of them being < 1 mm in diameter.

Pregitzer et al. (2002) suggested classifying fine roots on the basis of their branching order, with root tips being classified as first-order roots (Guo et al. 2004). Pregitzer et al. (2002) and Guo et al. (2004) reported that fine root diameter decreased with decreasing root order, *Pinus palustris* Mill. first-order roots having a mean diameter of 0.35 mm (Guo et al. 2004). Bauhaus and Messier (1999) reported only small differences (mean values of 0.24–0.34 mm) in the diameters of the three first root orders of *Abies balsamea* (L.) Mill. and *Picea glauca* (Moench) Voss. For nutrient uptake and C cost studies, separating roots < 1 mm in diameter and their first-order EcM short root tips from other fine roots could be functionally the most meaningful classification of the fine root system in EcM boreal trees.

Helmisaari et al. (2007) found clear relationships between fine root (diameter < 2 mm) biomass and fine root N concentration, as well as site and stand factors. For the same sites studied by Helmisaari et al. (2007), we have deepened the analysis to include only roots < 1 mm in diameter and their EcM root tips. We hypothesized that similar relationships as for fine roots exist between the number or biomass of EcM root tips and site and stand factors, if EcM root tip biomass and EcM root tip number per unit biomass of fine roots are related to the biomasses of the fine roots from which they branch. If this hypothesis is correct, then the use of fine root biomasses in ecological models (e.g., Mäkelä et al. 2008) for mycorrhizal trees in boreal forests to account for nutrient uptake capacity would be justified. Establishment of these relationships would also be a major step in the large-scale quantification of EcM root tips for determining their quantitative roles in nutrient and C budgets.

We studied the relationships between the two major functional components of boreal trees, the EcM short roots and the needles, in eight stands each of Norway spruce and Scots pine in Finland along a latitude (climatic and site fertility) gradient. Our specific aims were: (1) to quantify the variation in Norway spruce and Scots pine EcM root tip frequency and biomass, as well as N concentrations at different sites along a latitude gradient; (2) to assess how EcM root tip amounts and their relationship to foliage are related to tree species, site fertility and climate and (3) to test whether the N concentrations of EcM root tips are more sensitive to site fertility and climate than are foliar N concentrations, and whether this pattern differs between Norway spruce and Scots pine.

Materials and methods

Experimental stands

We studied a latitude gradient in Finland consisting of eight Norway spruce (*P. abies*) stands and eight Scots pine (*P. sylvestris*) stands. The stands belong to the intensive monitoring network of the EU/Forest Focus and UN-ECE/ICP forests Level II monitoring programs. The latitude gradient is also a climatic and site fertility gradient, with all the factors, namely temperature, precipitation and growing season length decreasing toward the north (Lindroos et al. 2008, Salemaa et al. 2008).

All the stands were mature: 10 of the stands were between 55 and 90 years old, and six stands were between 120 and 200 years old (Helmisaari et al. 2007). Some of the stands in northern Finland were older than the southern stands because trees grow more slowly and reach maturity considerably later in the north. The stands in southern Finland were located in the humid continental region and those in northern Finland in the subarctic climatic region. The long-term (1971–2000) mean annual effective temperature sum (threshold + 5 °C) varied between 662 and 1460 d.d. (degree days), and mean annual precipitation varied between 428 and 647 mm (Table 1). The length of the growing season was 169–177 days in southern Finland and 106–136 days in northern Finland (Lindroos et al. 2008). Mean annual total N deposition in the open was low, varying between 0.8 and 3.9 kg N ha^{-1} during 2001–2004 (Lindroos et al. 2007). We considered the boreal stands between latitudes 60° and 64° N to be in southern Finland, and those to the north of 64° N in northern Finland (Helmisaari et al. 2007). This division was based on latitudinal changes in growth conditions, and it was more distinct for Norway spruce than for Scots pine.

Each stand contained three 30 × 30 m subplots, with a surrounding buffer zone. Stand measurements were made between the growing seasons of 1999 and 2000 on all subplots in each stand. In each subplot, tree species, diameter, tree height and crown length were measured on all trees with a diameter at breast height of at least 4.5 cm. This allowed the accurate determination of the individual basal area, as well as respective stand-level characteristics (Helmisaari et al. 2007). Foliage biomass estimates for individual trees were calculated with tree species, diameter, height and crown length as input variables in the functions of Marklund (1987, 1988).

Site types varied from xeric to herb-rich heaths. The organic layer varied in thickness between 5 and 10 cm in the Norway spruce stands, and between 2 and 5 cm in the Scots pine stands. The organic layer in the stands was, on average, about 2 cm thicker in southern Finland than in northern Finland (Helmisaari et al. 2007). The C:N ratio of the organic layer at the Norway spruce sites varied between 27 and 31 in southern Finland and between 44 and 47 in northern Finland, whereas it was between 33 and 45 at all the Scots pine sites, except on one site where it was 53 (Table 1). The Norway spruce sites were located on till soils and the Scots pine sites on sorted glaciofluvial soils. For detailed information about the sites and stands, see Derome et al. (2007) and Helmisaari et al. (2007).

Fine root and EcM root tip sampling and analysis

Fine root samples for biomass and nutrient determinations were taken between July 20 and August 12, 1998 from the 16 stands (eight each of Norway spruce and Scots pine). Twelve soil cores were taken in each stand with a soil corer, as described by Helmisaari et al. (2007). The cores were divided into sections comprising the organic layer and the 0–10, 10–20 and 20–30 cm mineral soil layers. Roots were separated from the soil by washing and were sorted into living and dead roots, into different species and further into diameter classes. Roots < 2 mm in diameter were classified as fine roots (Persson 1983), and the living roots were further sorted into roots with a diameter < 1 mm. Thus, if not otherwise specified, fine roots in this study are roots with a diameter < 1 mm. For each sorted sample of living roots, 10% was used for counting the number of EcM tips on short roots with the aid of a microscope and was separately weighed. If there were EcM root tip clusters (as occurred in Scots pine), each tip was counted.

Fine root samples for determining the mean biomass and N concentrations of EcM root tips were sampled in August 2007 in five stands of each species. Eight soil core samples were taken in each stand, separately from the organic layer and the mineral soil (0–20 cm) layer. The number of short root tips in each sample ranged from 43 to 218. We calculated the EcM short root tip mass (mg) as the dry mass of all the short roots in a sample divided by the number of root tips in the sample. Specific EcM short root tip length (SRL, m g^{-1}) was determined as described by Ostonen et al. (1999).

To calculate mean biomass of an EcM root tip and the SRL for a stand, we weighted the root tip biomasses and SRL in each soil layer with the total number of EcM root tips in the same layer. Further, we used the mean EcM root tip biomass, and their number per fine root (diameter < 1 mm) mass unit, and the biomass of fine roots per m^2 to quantify EcM root tip biomasses per m^2. To determine the root tip C:N ratio, total C and N concentrations in the EcM root tips were determined with a CHN analyzer (Perkin Elmer 6400 Series II).

Soil and foliage sampling and analysis

Soil organic layer samples for determining C and N concentrations were taken from 20 systematically located points on the plot. The samples were bulked to give four composite samples per layer as described by Derome et al. (2001). The samples were dried at 40 °C and were milled to pass a 1-mm sieve. Total C and N concentrations were analyzed with a CHN-600 analyzer (Leco).

Table 1. Location, climate and site and stand characteristics. Temperature sum and precipitation are for the period 1971–2000. Temperature sum equals the sum of the differences between daily mean temperatures and the threshold of $+5$ °C. Stand characteristics in spring 2000 are given for the main tree species (*P. abies* and *P. sylvestris*) and are described in detail in Helmisaari et al. (2007). Foliage, fine root and EcM root tip biomasses are expressed on the basis of dry mass per unit ground area. Fine root biomass refers to roots <1 mm in diameter and EcM tips are their short root tips.

Stand	Latitude (N)	Longitude (E)	Annual mean temperature sum (°C)	Annual mean precipitation (mm)	Organic layer C/N	Stem number (ha^{-1})	Basal area (m^2 ha^{-1})	Foliage biomass (g m^{-2})	Fine root biomass (g m^{-2})	EcM tip biomass (g m^{-2})
Norway spruce										
Tammela	60°38′	23°48′	1262	641	30.6	544	22.7	1059	138.9	68.6
Evo	61°14′	25°04′	1212	614	27.4	769	32.5	973	101.7	–
Punkaharju	61°48′	29°19′	1304	610	28.0	374	28.3	1228	101.8	–
Juupajoki	61°51′	24°18′	1142	632	28.4	770	30.3	1272	108.6	56.9
Uusikaarlepyy	63°33′	22°29′	1142	511	27.0	944	34.7	1409	97.8	58.1
Oulanka	66°18′	29°30′	776	568	46.7	1112	11.8	515	95.2	–
Kivalo	66°20′	26°38′	832	561	43.9	1644	21.3	1202	214.4	107.8
Pallasjärvi	67°60′	24°14′	687	495	46.7	1096	13.0	898	166.6	87.7
Scots pine										
Tammela	60°37′	23°50′	1285	642	32.8	581	21.2	369	93.1	49.8
Miehikkälä	60°42′	27°50′	1361	641	40.9	407	16.6	263	96.3	30.2
Punkaharju	61°46′	29°20′	1294	609	42.2	948	29.3	413	119.2	–
Juupajoki	61°52′	24°13′	1166	633	36.1	378	17.9	260	98.9	54.2
Lieksa	63°09′	30°42′	1070	644	52.7	377	22.2	216	94.5	–
Ylikiiminki	64°58′	26°23′	1033	538	43.7	548	12.5	281	228.7	111.3
Kivalo	66°21′	26°44′	891	559	44.7	1748	21.3	474	258.4	132.0
Sevettijärvi	69°35′	28°54′	662	428	42.2	355	13.3	303	175.3	–

Branches were collected from the upper third of predominant or dominant trees ($n = 10$) on each study plot during October–November 1997. The branches were transported to the laboratory and stored in a freezer (–18 °C) during the period between sampling and pre-treatment. In the pre-treatment procedure, the branches were cut to separate shoot sections bearing different needle age classes. Shoots with the same needle age class from each tree were pooled and subsequently treated as a separate sample. The shoots were dried at 60 °C for 10 days and the needles were then removed from the shoots. Dried needles were milled with an ultracentrifugal mill (Retsch type Zm 1, mesh size 1 mm). The N concentrations of the current-year needles of 10 trees per plot were determined with a CHN-600 analyzer (Leco) and averaged for each plot (Merilä and Derome 2008).

Statistical methods

Normality of the variables was checked by the Lilliefors and Shapiro–Wilk's tests, and the homogeneity of the group variances was checked by the F and Levene tests. As a rule, the two-sided t test was used to determine the statistical significance of differences between group means. Simple regression models were used to estimate the relationships. In all cases, a level of statistical significance of $\alpha = 0.05$ was accepted.

Results

EcM root tips and site and stand characteristics

Ectomycorrhizal short root tip frequency (number per milligram of fine roots of diameter < 1 mm) varied between 8 and 15 mg^{-1} for Norway spruce and between 7 and 12 mg^{-1} for Scots pine, with no significant difference between the species or between the northern and southern sites (Figure 1A). There was a tendency for a decreasing number of EcM root tips with increasing depth of the mineral soil but, because of the large variation, the differences between the soil layers were generally not significant (data not shown).

The biomass of an average EcM root tip varied between 0.044 and 0.074 mg for Norway spruce and between 0.044 and 0.047 mg for Scots pine (Figure 1B). Although Norway spruce tips tended to have a higher mean biomass than Scots pine tips, the differences were not significant between species or between southern and northern sites. Specific root length of an EcM short root tip averaged 45 ± 6 m g^{-1} for Norway spruce and 50 ± 7 m g^{-1} for Scots pine ($n = 5$ for both species), and did not differ significantly between species or between stands in southern (46 ± 6 m g^{-1}, $n = 6$) and northern (50 ± 8 m g^{-1}, $n = 4$) Finland.

Mean fine root (diameter < 1 mm) biomass per unit ground area was similar in the Scots pine (146 ± 67 g m^{-2}, $n = 8$) and Norway spruce (128 ± 43 g m^{-2}, $n = 8$) stands, but higher ($P < 0.05$) in the northern stands (190 ± 57 g m^{-2}, $n = 6$) than in the southern stands
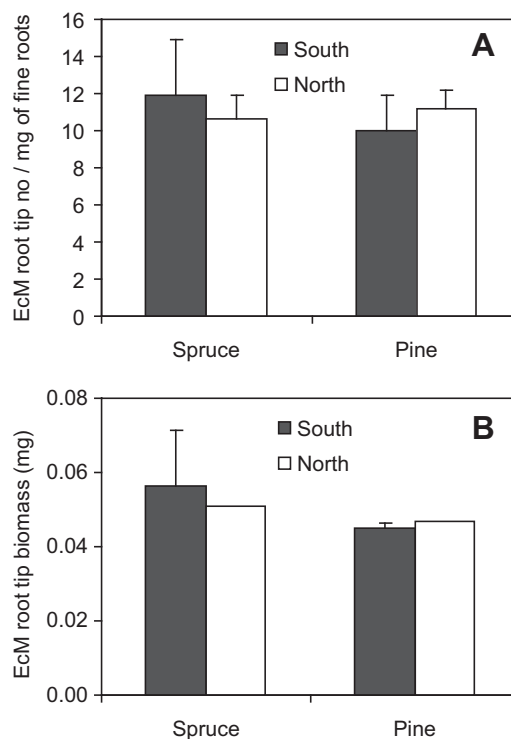

Figure 1. (A) Ectomycorrhizal root tip number per milligram of fine root biomass (diameter < 1 mm) and (B) Ectomycorrhizal root tip biomass (mg). Samples for determining EcM root tip numbers were collected in August 1998 from the organic layer plus the upper 30-cm mineral soil layer in eight *P. abies* stands and eight *P. sylvestris* stands (five stands of each tree species in southern Finland and three in northern Finland). Samples for determining EcM root tip biomasses were collected in August 2007 from the organic layer plus mineral soil (0–20 cm) in five Norway spruce stands and five Scots pine stands (three stands of each tree species in southern Finland and two in northern Finland). Values are means and the bars denote standard deviations between stands. See Table 1 for description of the stands.

(105 ± 14 g m^{-2}, $n = 10$) (Table 1). The mean proportion of Norway spruce and Scots pine EcM root tip biomass of the total fine root (diameter < 1 mm) biomass in the organic layer plus the 0–30 cm mineral soil layer was $50 \pm 7\%$, and this did not vary significantly between species ($n = 5$ for both Norway spruce and Scots pine) or between the northern ($n = 6$) and southern ($n = 4$) stands.

The distribution of EcM root tips with soil depth naturally followed that of the fine root biomass. The majority, $88 \pm 7\%$, of the Norway spruce EcM root tips and $80 \pm 7\%$ of the Scots pine EcM root tips were in the organic layer plus the upper 0–10 cm mineral soil layer (Figure 2A and B), and $86 \pm 8\%$ of the Norway spruce fine roots and $77 \pm 5\%$ of the Scots pine fine roots were in the same layers. Norway spruce had more EcM root tips in the organic layer ($63 \pm 16\%$) than in the upper 0–10 cm mineral soil ($25 \pm 10\%$), whereas Scots pine had more EcM tips in the upper mineral soil ($44 \pm 10\%$) than in the organic layer ($36 \pm 11\%$).
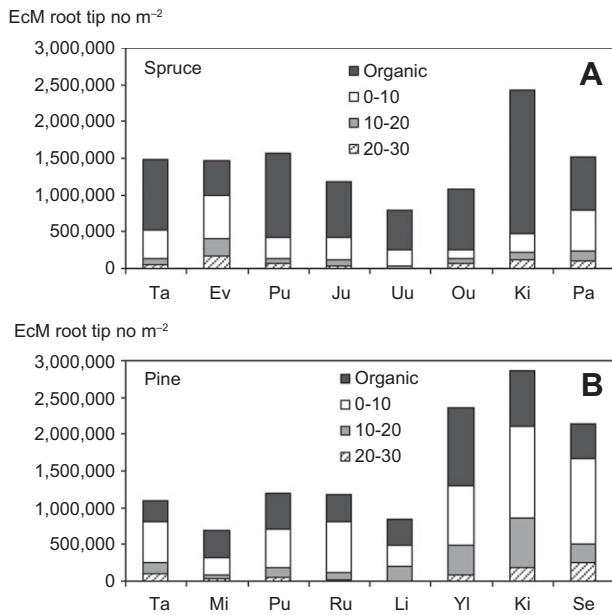
Figure 2. Ectomycorrhizal root tip number per unit area of the organic layer or mineral soil (0–10, 10–20 and 20–30 cm) layer in (A) *Picea abies* and (B) *Pinus sylvestris* stands. Samples for determining EcM root tip numbers were collected in August 1998 from the organic layer plus upper 30-cm mineral soil layer in eight *P. abies* stands and eight *P. sylvestris* stands (five stands of each tree species in southern Finland and three in northern Finland). Stands on the *x*-axis are in ascending order of latitude (Table 1), and the last three stands in both panels are located in northern Finland.
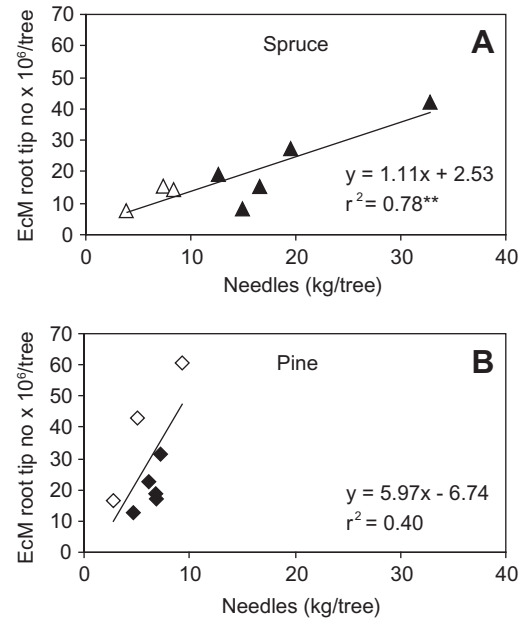

Figure 3. Relationship between number of EcM root tips per average tree and foliage biomass per average tree, in (A) *Picea abies* and (B) *Pinus sylvestris* stands. Open symbols denote stands in northern Finland and filled symbols denote stands in southern Finland. For each species, $n = 8$ stands, five in southern Finland and three in northern Finland, see Table 1 for description of the stands. Samples for determining EcM root tip numbers were collected in August 1998 from the organic layer plus the upper 30 cm mineral soil layer. Asterisks indicate the significance of the P values: *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$.

The EcM root tip number per unit area of the organic layer plus the 0–30 cm mineral soil layers varied between 790,000 m^{-2} and 2,430,000 m^{-2} for Norway spruce and between 687,000 m^{-2} and 2,866,000 m^{-2} for Scots pine (Figure 2A and B). The number of EcM root tips per m^2 was higher in the northern Scots pine stands than in the southern Scots pine stands (Figure 2B), whereas only one northern Norway spruce stand (Kivalo) had a higher number of EcM tips per m^2 than the Norway spruce stands in southern Finland (Figure 2A). However, because the Kivalo stand has a high number of trees per ha (Table 1), the EcM root tip number per tree in this stand was similar to that in the northern Norway spruce stands (Figure 3A).

As the number of trees varied per stand, we also compared the parameters between stands based on foliage mass and the number of EcM root tips per average tree in each stand. The EcM root tips per average tree and foliage biomass per average tree correlated positively for Norway spruce (Figure 3A), but weakly for Scots pine (Figure 3B, $P = 0.09$). Southern and northern Scots pine sites were clearly separated but without significant correlations because the number of sites was small (Figure 3B). The Norway spruce trees (Figure 3A), and especially the Scots pine (Figure 3B) trees, had more EcM root tips in relation to foliage mass in northern Finland than in southern

Finland. The Scots pine trees also had more EcM root tips in relation to their foliage mass than the Norway spruce trees. For both species, EcM root tip number per average tree correlated with the basal area per tree (northern Finland, $y = 1879.4x − 6.97$, $r^2 = 0.97$, $P < 0.001$, $n = 6$; and southern Finland, $y = 624.5x − 6.74$, $r^2 = 0.69$, $P < 0.01$, $n = 10$).

Because mean EcM root tip mass did not vary significantly between species or between the southern and northern stands (Figure 1B), and the total EcM root tip biomass per tree or per unit ground area was in most cases related to the respective total tip number, the EcM root tip:foliage biomass ratio was significantly higher in Scots pine than in Norway spruce, and significantly increased from south to north (Figure 4). The variation in foliage biomass contributed strongly to the differences between tree species – foliage biomass was several times higher in Norway spruce than in Scots pine (Table 1). The higher foliage biomass in the south also contributed to the differences between south and north especially for Norway spruce, and for Scots pine the EcM numbers (Figure 2B) also made a contribution.

An individual tree in northern Finland had twice the number of EcM tips in relation to its foliage biomass than an individual tree in southern Finland. Furthermore, an individual Scots pine tree had over three times more EcM
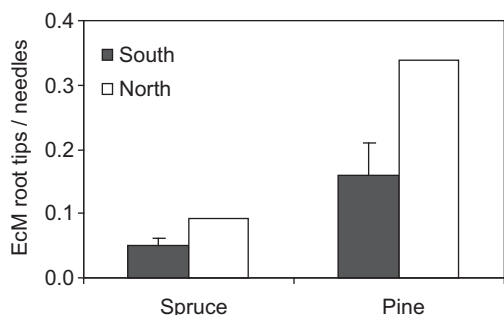
Figure 4. Ectomycorrhizal root tip:foliage biomass ratios for *P. abies* and *P. sylvestris* stands in southern and in northern Finland. Values are mean and bars denote standard deviations between stands; *n* = 3 stands of each tree species in southern Finland and two in northern Finland, see Table 1 for description of the stands. Ectomycorrhizal root tip samples were collected in August 2007 from the organic layer plus mineral soil (0–20 cm).
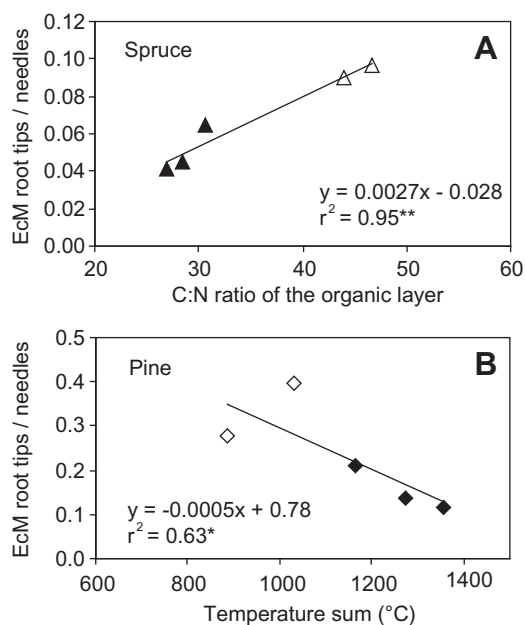

Figure 5. Relationship between the EcM root tip:foliage biomass ratio and (A) C:N ratio of the organic layer in *P. abies* stands, and (B) temperature sum in *P. sylvestris* stands (*n* = 5 stands for each tree species, see Table 1 for description of stands). Open symbols denote stands in northern Finland and filled symbols denote stands in southern Finland. Asterisks indicate the significance of the *P* values: *P < 0.05; **P < 0.01 and ***P < 0.001.

Figure 6. Relationship between stand mean N concentration of (A) ectomycorrhizal root tips, *n* = 10 stands and (B) needles (*n* = 16 stands, see Table 1 for description of the stands), and the C:N ratio in the organic layer. Triangles denote the *P. abies* stands and squares denote the *P. sylvestris* stands. Open symbols denote stands in northern Finland and filled symbols denote stands in southern Finland. Asterisks indicate the significance of the *P* values: *P < 0.05; **P < 0.01 and ***P < 0.001.

P = 0.07, *n* = 5) than to the C:N ratio. Both the organic layer C:N ratio (especially in the Norway spruce stands) and the temperature sum followed a gradient from southern to northern Finland (Table 1). The correlation between the EcM root tip:foliage biomass ratio and the C:N ratio of the organic layer in the Norway spruce stands (Figure 5A) was related not only to differences between the southern and the northern stands, but also to differences within the southern stands (P < 0.05). However, more data from more sites are needed to determine if these relationships also exist within a narrower range of site and climate factors.

N concentrations in the EcM tips, fine roots and foliage

Nitrogen concentrations in EcM root tips and needles did not significantly differ between species (Figure 6A and B). Based on both species and the 10 sites shown in Figure 6A, mean N concentrations were significantly higher (P < 0.001) in EcM root tips (mean 1.30 ± 0.24%) and needles (mean 1.15 ± 0.11%) than in fine roots (mean 0.81 ± 0.17%, diameter < 2 mm, Helmisaari et al. (2007)). The range of N concentrations was greater in EcM root tips than in needles.Nitrogen concentrations in EcM root tips (Figure 6A) and fine roots (diameter < 2 mm, Helmisaari et al. (2007)) of both tree species were significantly related to the C:N ratio of the organic soil layer. When the needle N concentrations of Norway spruce and Scots

root tips in relation to foliage biomass than an individual Norway spruce tree in the same climatic region. Moreover, the EcM root tip:foliage biomass ratio of Norway spruce was positively related to the C:N ratio in the organic layer and that of Scots pine was negatively related to the temperature sum (Figure 5A and B). The EcM root tip:foliage biomass ratio of Norway spruce was also related to the temperature sum, but less significantly (r^2 = 0.71,
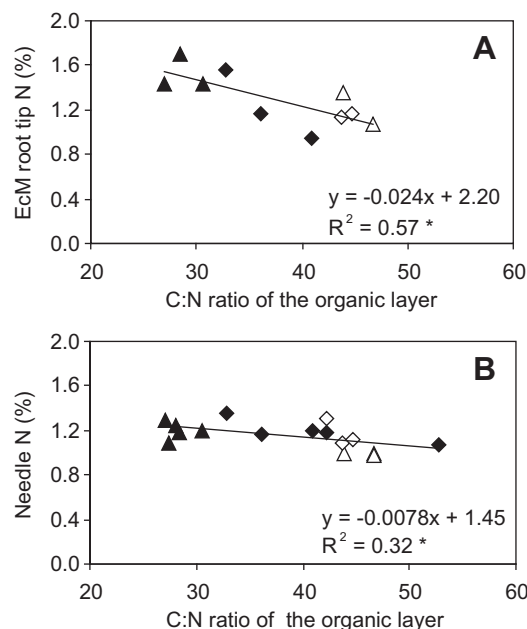
pine stands were treated separately, the relationships with the C:N ratio of the organic soil layer (Figure 6B) improved (Norway spruce: $y = -0.012x + 1.53$, $r^2 = 0.77$, $P < 0.01$; Scots pine: $y = -0.012x + 1.70$, $r^2 = 0.52$, $P < 0.05$; $n = 8$ for both species). In the organic soil layer, the fine root C:N ratio varied between 45 and 81 (with one exception, Lieksa, C:N ratio of 103), and the C:N ratio of EcM root tips varied between 26 and 45. The EcM root tip C:N ratio was strongly correlated with the C:N ratio in the organic soil layer ($y = 0.689x + 10.50$, $r^2 = 0.63$, $P < 0.01$, $n = 10$). Mean C concentration of the EcM tips was $45.8 \pm 1.2\%$ (data not shown).

Discussion

All the short roots at the Norway spruce and the Scots pine study sites were EcM. In agreement with this study, Taylor et al. (2000) reported an absence of quantitative changes in the extent of mycorrhizal formation along a transect of four Norway spruce sites running from northern Sweden to central Europe. Taylor et al. (2000) also found that more than 90% of all the root tips in the organic layer were mycorrhizal irrespective of their position along the transect or the amount of N deposition. Børja and Nielsen (2008) reported that 55–91% of Norway spruce roots in Norway were mycorrhizal.

Minirhizotron studies at the Kivalo site showed that EcM short root tips are formed on young, unsuberized, elongating fine roots (Helmisaari et al. unpublished data). Our finding of similar EcM root tip frequencies (per unit mass of fine roots) in Scots pine and Norway spruce, and in the southern and the northern sites, implies that the mechanism for increasing nutrient uptake in these species is by increasing the production of fine roots, and hence the total number of EcM root tips, rather than by increasing the frequency of EcM tips on the roots. In accordance with our results, Leuschner et al. (2004) reported similar frequencies (numbers per unit mass of fine roots) of beech short roots in acidic and in basic soils. However, fertilization has been reported to affect the frequencies of root tips. Ahlström et al. (1988) showed that the mean number of root tips per unit length of fine root was higher in the control plot than in the solid and in the liquid fertilized plots, because of a higher occurrence of mycorrhizal aggregates.

As the frequency and mass of EcM root tips did not vary between the tree species or between the northern and the southern sites, the proportions of fine roots were also stable. Ectomycorrhizal short root tips accounted for $28 \pm 6\%$ of the biomass of fine roots with a diameter < 2 mm (Helmisaari et al. 2007) and $50 \pm 7\%$ of the biomass of fine roots with a diameter < 1 mm. According to Ostonen et al. (2005), in a fertile stand in Estonia, EcM tips formed 39% of the biomass of fine roots < 1 mm in diameter, which is slightly lower than our value. Our results are also supported by those of Persson (1978), who investi-

gated various fine root parameters in *P. sylvestris*, including mass, length, surface area, volume and number of root tips. Variations in all these parameters were closely correlated, because the mean diameter of roots and the branching pattern from one sampling occasion to the next varied slightly relative to root length.

The proportion of fine roots with a diameter < 1 mm out of the total biomass of fine roots with a diameter < 2 mm (Helmisaari et al. 2007) was $55 \pm 11\%$ in the organic layer plus the 0–30 cm mineral soil layer, and did not differ significantly between the species or between the northern and southern stands. Ostonen et al. (2005) and Børja et al. (2008) reported comparable percentages (41–55% and 66%) in Norway spruce stands in Norway and Estonia, respectively.

We found stable relationships between the biomass of the most active part of the fine root system (the EcM root tips with their mycelia), fine roots on which these short root tips grow, and the coarser, mostly transporting fine roots within the Norway spruce and the Scots pine stands studied. Withington et al. (2006) concluded that the finest roots are not discrete entities like leaves, but represent the terminal end of a network. From the perspective of nutrient uptake capacity, the empirical existence of these quantitative network relationships is important, because most ecological models (e.g., Mäkelä et al. 2008) using root parameters consider fine roots as a single group.

The mean EcM root tip biomasses were determined on samples taken in August 2007, whereas the fine root samples for root biomass determination, and EcM numbers per root mass unit, were taken in August 1998. In previous studies, the maximum fine root biomass (Makkonen and Helmisaari 1998) was reached in August for Scots pine (Makkonen and Helmisaari 1998) and for Norway spruce (Ostonen et al. 2005). Both 1998 and 2007 had mean June–August temperatures similar to the long-term mean (1971–2000). In 1998, however, precipitation in the summer months was 1.3–2 times higher than the average (Finnish Meteorological Institute 1998), whereas the precipitation in June–August 2007 was close to the long-term average. Even if the differences in precipitation caused some variation in the EcM tip mass between years, there were no regional abnormalities, for example, drought periods, that could have influenced our latitude gradient.

At the stand level, there were no differences in EcM root tip number associated with tree species. In the organic layer, our range for Norway spruce EcM root tip number was 482,000–1,960,000 tips m^{-2}, which is higher than 303,000 tips m^{-2} reported by Børja and Nielsen (2008) for Norway spruce in central south-east Norway. Our EcM root tip number of 687,000–2,866,000 m^{-2} for Scots pine is comparable to the 1,768,000 per m^2 in a mature Scots pine stand in Sweden reported by Ahlström et al. (1988). In agreement with our results, Bobkova (1987) found no differences between tree species in EcM tip biomass values that varied in taiga forests from 17 to 33 g m^{-2}

and from 10 to 35 g m^{-2} for Norway spruce and Scots pine stands, respectively. Our EcM root tip biomass results (57–108 g m^{-2} for Norway spruce and 30–132 g m^{-2} for Scots pine) are, however, higher than the values reported by Bobkova (1987).

Processes related to fine root biomass, such as resource allocation and nutrient and water uptake, should be investigated on a tree basis (Helmisaari et al. 2007). A varying number of trees per unit area may make it more difficult to compare stand-level variables, such as root or foliage biomass, between species or sites. When we expressed EcM root tip biomass per unit biomass of foliage, there was a clear latitudinal trend both for Norway spruce and for Scots pine, characterized by increasing values from southern to northern Finland. However, for Scots pine there were also latitude-related differences at the stand level: EcM root tips per unit ground area were twice as high in the northern Scots pine stands as in the southern Scots pine stands.

Our results indicate that, in a colder climate with a shorter growing season, trees may have to maintain more short roots active in nutrient uptake per unit biomass of foliage. It has been assumed that the cost of root formation and maintenance is proportional to root mass (Eissenstat and Yanai 1997, Pregitzer et al. 2002, Ostonen et al. 2007). Consistent with the model presented by Mäkelä et al. (2008), low N availability caused by either site infertility or a short growing season (Jarvis and Linder 2000) seems to enhance allocation to belowground biomass as an optimization measure, whereas the fraction of assimilates allocated to the production of foliage is unaffected by N availability (Mäkelä et al. 2008). Differences in foliage biomass between species, however, strongly affected their EcM root tip:foliage biomass ratios. A lower N uptake owing to light limitation (Mäkelä et al. 2008) in the southern, fertile Norway spruce stands may be one factor explaining why there were less EcM root tips (and external mycelia) for N uptake per unit biomass of foliage in these stands.

It is also possible that the form of available soil N plays a role in the fate of C allocated belowground. On the same sites as we studied, Mustajärvi et al. (2008) reported that 80% of the percolation water N flux was dissolved organic N. The capacity of EcM fungi to produce the enzymes involved in the degradation of organic matter and to mobilize organic forms of N is well documented (Näsholm et al. 1998, Read and Perez-Moreno 2003, Lindahl et al. 2005). Because mineral N availability is especially low in the north (Read 1991), the uptake of organic N may impose less demands for C use in N assimilation (Wallander 1995, Wallanda and Kottke 1998) but requires a larger root and mycelial network, and subsequently C allocation to the production and maintenance of the network.

Our results clearly demonstrated that, in the north, the harsh climate and the high soil C:N ratio are closely related. The increased need for high EcM root tip:foliage biomass ratios in the north may be related not only to poor fertility, as indicated by the C:N ratio, but also to the considerably shorter growing season, as indicated by the temperature sum. Temperature sum had a strong relationship with the EcM root tip:foliage biomass ratio for Scots pine, probably because, in the Scots pine stands, the variation in climate along the latitude gradient was larger than the variation in the C:N ratio. The EcM root tip number per average tree correlated with tree basal area after we had stratified the data for southern and northern stands. Thus, a northern Norway spruce or Scots pine tree had three times more EcM root tips in relation to basal area than a southern tree, further demonstrating the need to increase nutrient uptake, especially of N, in northern stands by producing more fine roots, and thereby increasing the area of soil occupied by EcM mycelia.

Our knowledge of the biomass of mycorrhizal mycelium in the field is still limited (Lindahl et al. 2002). We did not investigate EcM mycelia in this study but, similar to our results on EcM tips, the results of Wallander et al. (2001) and Nilsson et al. (2005) suggest that EcM mycelial biomass and production are strongly coupled to tree fine root biomass, and that the seasonal maximal growth of EcM mycelia coincides with that of fine roots (Wallander et al. 2001). Nilsson et al. (2005) studied short natural nutrient gradients and reported that the total fungal biomass was highest in soils with the lowest nutrient availability and tree productivity. Nilsson and Wallander (2003) reported a negative influence of N on growth of external EM mycelia in an N-fertilization study. Korkama et al. (2007) found a significant positive correlation between visually estimated mycelial biomass and root tip density, as well as fine root biomass and density in a Norway spruce field trial.

In the context of nutrient uptake, mycorrhizal fungi may be considered an extension of the nutrient-absorbing surface of roots (Tuomi et al. 2001). The results of several studies (Wallander et al. 2001, Nilsson and Wallander 2003, Nilsson et al. 2005, Korkama et al. 2007) support the hypothesis that there is a clear quantitative relationship between fine roots, EcM tips and their mycelia, and that EcM tips and mycelia respond in a similar manner to variation in site nutrient availability. On the basis of the data published by Hobbie and Colpaert (2003), Alberton et al. (2007) calculated that fungi contributed 18% to the EcM root mass of Scots pine seedlings at high N availability and 21% at low N availability. In their study, hyphal length was significantly affected by fungal species (Alberton et al. 2007), indicating that changes in the EcM fungal community may be related to hyphal extension and to an increased ability for nutrient uptake (Lilleskov et al. 2002).

In agreement with our results, the N concentrations of the EcM root tips of Norway spruce and Scots pine in taiga forests were similar and varied between 0.7% and 1.9% (Bobkova, 1987). Our EcM root tip N concentrations were also comparable to those in first- and second-order roots (1.1–1.6%) of longleaf pine reported by Guo et al. (2004). Similar to our findings, Högberg et al. (1998) reported a

gradient in Norway spruce fine root N concentrations from 2.1% in central Europe to 1.1% in northern Sweden, these values being comparable to the range of foliage N concentrations (1.5–0.7%) on the same sites (Bauer et al. 1997). In our study, needle N concentration had a more significant relationship with the organic layer C:N ratio in Norway spruce than in Scots pine. Similar, but even more significant relationships were reported by Merilä and Derome (2008) based on a large dataset.

Mean EcM root tip N concentrations were determined on samples taken in August 2007, whereas the foliage samples were taken in October–November 1997. Needle nutrient concentrations in the autumn vary only slightly between years (Merilä 2007). Therefore, the different sampling year for foliage and for EcM root tips did not influence the comparison of N concentrations in these organs.

Tree tissue N concentrations are related to soil N availability, but also reflect the pattern of C allocation. Nitrogen concentration had a narrower range in foliage than in EcM root tips, indicating that, under N-deficiency conditions, needle N concentrations are regulated by adjusting the EcM root tip:foliage ratio according to N availability. According to the model of Mäkelä et al. (2008), it is optimal to maintain canopy N concentrations at a value that is independent of an increase in N availability: the greater the availability of N, the lower the fine root density required to supply the required canopy N. We conclude that both Norway spruce and Scots pine respond to N deficiency resulting from low fertility or a short growing season, or both, by maintaining more EcM root tips per unit biomass of foliage, and this may be associated with an increased proportion of N uptake in an organic form.

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