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Variability in the growth rates and foliage $\delta^{15}\text{N}$ values of black spruce trees across a slope gradient in the Alaskan Interior

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Title: Variability in the growth rates and foliage $\delta^{15}\text{N}$ values of black spruce trees across a slope gradient in the Alaskan Interior

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Abstract

Black spruce (*Picea mariana*) is a dominant species on north-facing slopes located on the permafrost in the Alaskan Interior, where tree growth rates vary significantly across slope gradients. To better understand the effects of nitrogen (N) and mycorrhizal associations on plant growth, we investigated the relationships between tree growth and isotope ratios of nitrogen ($\delta^{15}\text{N}$) in foliage and roots, because plant $\delta^{15}\text{N}$ values reflect tree dependencies on N uptake by mycorrhizae. We established two transects (one along a slope gradient, and a second on a north to east axis at a fixed elevation) and four plots on the slope gradient transect. We measured current shoot growth of saplings on transects and aboveground biomass (AGB) in the plots. We collected foliage and fine root samples and measured their $\delta^{15}\text{N}$ signatures and N concentrations. The AGB and shoot growth varied widely across the gradient: values were higher at high elevations and at east-northeast aspect. Foliage $\delta^{15}\text{N}$ values and N concentrations were positively correlated with tree growth, but growth rate was negatively correlated with the dependency on mycorrhizae for N uptake. Thus black spruce had a reduced dependency on mycorrhizae for N uptake on the upper slope, where conditions were N-rich. On the lower slope, where the soil active layer was shallow, black spruce growth was limited and elevated dependency on mycorrhizae for N uptake.

Keywords: ectomycorrhiza; ericoid mycorrhizal fungi; nitrogen isotope; nitrogen uptake; *Picea mariana*

37

38 **Introduction**

39 Carbon (C) stock accumulation and tree growth rates in boreal forests are generally limited
40 by nitrogen (N) availability (Shaver and Chapin 1980). Low temperatures, water logging, and high
41 soil organic matter content slow the rate of N mineralization (Klingensmith and van Cleve 1993),
42 thereby creating a major bottleneck in forest N fluxes (Van Cleve and Alexander 1981; Chapin 1983).
43 The reduced N supply does not meet the demands of plant growth because roots preferentially absorb
44 inorganic N from the soil (Kielland 1994). However, many forest plants are able to bypass the
45 mineralization step in decomposition by directly absorbing organic N, such as amino acids, through
46 ericoid mycorrhizal, ectomycorrhizal (EcM), or other mycorrhizal associations (Kielland 2001;
47 Lipson and Näsholm 2001; Kielland et al. 2006). Consequently, the mycorrhizal contribution in
48 boreal forests may account for a large proportion of N uptake by plants (Kielland 1994). Hobbie and
49 Hobbie (2006) calculated that *ca.* 61–86% of N was transferred by EcM fungi to host plants in
50 N-limited tundra environments. Although EcM associations increase N availability to plants, the
51 fungi make high C demands on their hosts (de Kroon and Visser 2003; Smith and Read 2008). Trees
52 usually reduce the C allocation to below-ground parts, such as the fine roots, in response to increased
53 soil N availability (Ågren and Franklin 2003; Franklin et al. 2009; Kenzo et al. 2015); the dependency
54 of N uptake on EcM also decreases with increasing soil N availability and plant productivity (Mayor
55 et al. 2015). However, the effects of environmental variability on mycorrhizal activities and their

relationships with tree growth patterns remain unclear.

Recent syntheses suggest that the stable N isotope ratios ($\delta^{15}\text{N}$) of plants and soils provide valuable information on soil-plant N cycling (Robinson 2001) and N uptake traits reflecting EcM associations. For example, large differences between the $\delta^{15}\text{N}$ values of foliage and the fine roots and soil are indicative of high N uptake dependency on EcM (Hobbie and Colpaert 2003; Mayor et al. 2012), because plant ^{15}N is depleted as N is transferred through fungi to the host plants (Craine et al. 2009; Högberg et al. 1996). In addition, differences in $\delta^{15}\text{N}$ values and N concentrations in terms of root size and root order reflect N availability and functional root traits including EcM associations (Ouimette et al. 2013). Comparisons of $\delta^{15}\text{N}$ signatures and N concentrations among the foliage and fine roots of various sizes yield promising insights into the associations between plants and EcM.

Black spruce (*Picea mariana*) stands cover >40% of the Alaskan Interior landscape (van Cleve et al. 1983), where they are distributed across diverse environments. The stands are particularly dominant on north-facing slopes and bottomlands with underlying permafrost (Vioreck et al. 1983). The growth of black spruce varies widely and is weakly correlated with soil C and N in these Alaskan regions (Hollingsworth 2008). Black spruce has EcM fungal symbionts, and is able to take up organic forms of N from the soil through them (Ruess et al. 2003). Black spruce stands on permafrost reportedly allocate extremely large resources to fine root (<2 mm diameter) biomass and mycorrhizae compared to other forest ecosystems (Steele et al. 1997; Ruess et al. 2003; Noguchi et al. 2012). This elevated C allocation to belowground organs may increase tree dependency on dissolved organic N

(DON) uptake by EcM symbionts in N-limited environments (Mayor et al. 2012). Mayor et al. (2012) found that foliage $\delta^{15}\text{N}$ values varied in different stands in Alaska within the range -4.5 to -11.0‰ . The authors also pointed out that low stand biomasses may be related to low $\delta^{15}\text{N}$ values, which are indicative of high N uptake dependency on EcM under N-limited conditions. Aboveground biomass (AGB), belowground biomass (BGB) (Noguchi et al. 2012, 2016), and soil N status (Heilman 1966; Jones and Kielland 2002; Vogel et al. 2005) in black spruce stands also vary significantly at micro-scales on the same slope. Although climatic conditions such as rainfall may be similar across slope gradients, the active soil layer depth above the permafrost is very variable (Rohrs-Richey and Mulder 2007; Noguchi et al. 2012). This variability leads to changes in soil N availability, and may thus affect the rates of plant N uptake and growth because a thick, warm active soil layer facilitates N mineralization and the absorption of inorganic ions released into the soil water (Jones and Kielland 2002). We postulated that variability in the growth and AGB of black spruce within a single slope might reflect dependency on EcM for N uptake, and the soil N conditions (e.g., the C:N ratio). We hypothesized that black spruce stands with higher AGBs and higher growth rates might be located at sites that were N-rich and that the trees might exhibit lower EcM-dependency in terms of N uptake, whereas those with lower AGBs and growth rates would be found at N-limited sites and exhibit greater EcM-dependency. We also explored whether size was dependent on differences in the $\delta^{15}\text{N}$ signatures of fine roots, and whether such differences were relevant to the growth rate and extent of EcM colonization of black spruce under natural N-limited conditions. With these objectives, we

measured the growth rates, and the foliage and root $\delta^{15}\text{N}$ signatures, of black spruce trees on different slopes. We set up two transects: one deployed across the gradient from the bottom of a slope upward to the ridge, and a second running on a north to east axis along the contour at one elevation (Fig. 1). We also established four plots on the transect running across the elevation gradient. We measured AGB, the active soil layer depth, the soil C:N, foliage $\delta^{15}\text{N}$ values, and the N concentrations of adult black spruce trees. We also compared changes in shoot growth, and the $\delta^{15}\text{N}$ values of foliage and fine roots of different sizes of saplings distributed along the two transects.

Materials and Methods

Study sites

Our study was conducted on a north-facing slope in the Caribou Poker Creek Research Watershed (CPCRW) of the University of Alaska Fairbanks (CPCRW; 65°14'N, 147°49'W). The mean annual temperature and mean annual precipitation were -2.5°C and 400 mm, respectively (Petrone et al. 2006). The slope was dominated by black spruce with occasional several broad-leaved woody plants, such as species of *Ledum* and *Betula* spp. Broad-leaved trees such as species of *Alnus* and *Populus* were found on the ridge and east-facing slope. Stand age was similar across the slope (*ca.* 90 years; Noguchi et al. 2016).

Plot survey and sampling

To determine AGB variation on the slope and the associated variation in nitrogen status, we set up four plots (N0–N3; 10 × 10 m) from the top to bottom of the gradient (Fig. 1). Soil profile surveys and penetration tests were conducted to measure the active layer depth during the growing season. The diameters and heights of all trees in the plots were measured in 2009. AGB and BGB (mainly coarse roots, <5mm in diameter) were calculated using allometric equations for black spruce (Noguchi et al. 2012). The N status of black spruce stands was evaluated by measuring the foliage $\delta^{15}\text{N}$ values and N concentrations. Foliage samples were collected from the tops of the crowns (collected from *ca.* three mature individuals and saplings on each occasion). We also collected soil samples from O and A horizons in N0, N1, N2, and N3 plots (14 samples in total).

Transect sampling and sample treatment

The two transects were set up in July 2014. Line 1 was deployed across the gradient from the bottom of a slope upward to the ridge, and Line 2 run on a north-to-east axis along the contour at one elevation (Fig. 1). Line 1 covers the four plots (N0–N3) described above; they intersected at the 320-m contour. We collected black spruce sapling (30–70 cm tall) shoots from 92 individuals (Line 1 = 72, Line 2 = 20) at 20-m intervals horizontally based on GPS (60CSz, Garmin, Kansas, USA) along the transect lines. Shoots were collected from the tops of the crowns. Current shoot growth (cm) was measured on three branches of each tree. Leaves were oven-dried at 50°C for 1 week, and then ground to a fine powder with a ball mill (MM200, Retsch, Haan, Germany). Ericoid mycorrhizal fungi in

boreal forests also strongly discriminate against ^{15}N (Hobbie and Högberg 2012). Thus, we analyzed the foliage $\delta^{15}\text{N}$ values of the evergreen shrub *Ledum palustre* (Ericaceae) with ericoid mycorrhizal symbiosis, which grew on the forest floor, along Line 1 (Rohrs-Richey and Mulder 2007). Leaves of *L. palustre* from the elevation transect were also taken at 40-m intervals ($n = 19$), which was at the same position (i.e., grown within a 1-m radius) as the sampled black spruce saplings.

To estimate the contribution of EcM to N uptake, we also collected fine roots of black spruce from alternating individuals growing along the transect on the slope gradient (Line 1; 36 samples). We observed the hyphal status of EcM samples with a stereomicroscope, and categorized them into the following classes: 4, almost all root tips had hyphal colonization; 3, $\frac{3}{4}$ of root tips colonized; 2, $\frac{1}{2}$ of root tips colonized; and 1, $<\frac{1}{2}$ of root tips colonized. Roots were also divided into three diameter size classes: <0.5 mm, 0.5–1 mm, and 1–2 mm. We measured $\delta^{15}\text{N}$ values in each of these and in a few hyphal clumps that we dissected.

Measurement of stable N isotope composition and N concentration

We determined the stable N isotope composition of soils, leaves, and roots using conventional methodology (elemental analyzer /isotope ratio mass spectrometry; EA/IRMS, Thermo Finnigan, Delta V Advantage interfaced with a FlashEA1112 HT, Thermo Fisher Scientific, Waltham, Massachusetts, USA). N isotope ratios are presented here in the following notation: $\delta^{15}\text{N} = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$ (‰), where R_{sample} is the $^{15}\text{N}/^{14}\text{N}$ ratio of the sample, and R_{standard} is the ratio in

atmospheric N₂. All of the samples were measured in duplicate, and the averages were used to reduce experimental errors (Tanaka-Oda et al. 2016). Total leaf and root N concentrations were determined with a NC analyzer (Sumigraph NC-900, Sumika Chemical Analysis Service, Tokyo, Japan).

Results

Relationship between AGB and foliage $\delta^{15}\text{N}$ values across the slope gradient

The AGB of black spruce trees varied 7-fold among slope positions: 12.5 Mg ha⁻¹ on lower slopes, to 85.7 Mg ha⁻¹ on upper slopes (Table 1). Mean diameter at breast height (DBH) was higher on the upper slope plot than at lower elevation. The active layer depth in the soil was significantly deeper in the upper slope plot; in both N0 and N1 the depth was >100 cm; in plot N3, the depth was about 60 cm. The depth of the soil organic layer increased with decreasing elevation, whereas the mineral soil N concentration was higher on the upper than the lower slope (Table 1). We performed multiple regression analysis using the soil $\delta^{15}\text{N}$ value as the dependent variable, and the soil C:N ratio and the plot as independent variables. The soil $\delta^{15}\text{N}$ values were significantly (negatively) correlated with the organic soil C:N ratio, but not with the plot (Table 2).

The $\delta^{15}\text{N}$ values and N concentrations of foliage collected from mature trees and saplings of black spruce increased with plot AGB (Fig. 2AB). The foliage $\delta^{15}\text{N}$ values and N concentrations ranged from $-11.9 \pm 0.1\text{‰}$ to $-3.7 \pm 0.3\text{‰}$ and $0.6 \pm 0.0\%$ to $1.1 \pm 0.1\%$, respectively, in the lower and upper slope plots. The foliage $\delta^{15}\text{N}$ values were positively correlated with foliage N concentrations

(Fig. 2C). These relationships were similar for both mature and sapling trees.

Variation in sapling shoot growth and foliage $\delta^{15}\text{N}$ values among slope locations

The growth of current year shoots on saplings varied among slope locations (0.8–3.6 cm yr⁻¹). Current shoot growth rate increased from the bottom of the slope to the ridge, although several individuals on the ridge grew slowly (Fig. 3A). Foliage N concentration increased slightly with elevation (Fig. 3A). Foliage $\delta^{15}\text{N}$ values also increased with elevation (Fig. 3A), but were variable and tracked the changing pattern along the north to east axis at right angles to the slope. Current shoot growth was reduced at locations with northern and eastern aspects and elevated in locations with east–northeast aspects (Fig. 3B). Foliage N concentrations varied in a manner similar to the $\delta^{15}\text{N}$ values (Fig. 3B). Shoot growth rate was positively correlated with both foliage N concentration (Fig. 4A) and the $\delta^{15}\text{N}$ value (Fig. 4B). The foliage $\delta^{15}\text{N}$ values were significantly and positively correlated with foliage N concentrations (Fig. 4C). We also measured the foliage N concentrations and $\delta^{15}\text{N}$ values of the shrub *L. palustre*, which is associated with ericoid mycorrhizal fungi, along the gradient transect. Both of the parameters increased with elevation, tracking patterns similar to those in black spruce. The foliage $\delta^{15}\text{N}$ values of *L. palustre* were also positively correlated with foliage N concentration (Fig. 5).

$\delta^{15}\text{N}$ variation in fine roots

The root $\delta^{15}\text{N}$ values and N concentrations varied significantly among the root diameter classes,

189 decreasing with increasing diameter; the isotopic signature values and N concentrations were $-6.1 \pm$
 190 0.3‰ , $-8.0 \pm 0.3\text{‰}$, and $-9.7 \pm 0.4\text{‰}$ and $1.00 \pm 0.02\%$, $0.84 \pm 0.03\%$, and $0.64 \pm 0.42\%$ in the three
 191 root diameter classes of <0.5 mm, $0.5\text{--}1$ mm, and $1\text{--}2$ mm, respectively (Fig. 6, ANOVA, $P < 0.01$, n
 192 $= 34$). We performed multiple regression analysis using the fine root $\delta^{15}\text{N}$ value as the dependent
 193 variable and the fine root diameter and N concentration as the independent variables (Table 3). The
 194 root $\delta^{15}\text{N}$ value correlated negatively with root diameter, but not significantly with root N
 195 concentration. We also performed multiple regression analysis on the difference in $\delta^{15}\text{N}$ value
 196 between small and large fine roots [$\delta^{15}\text{N}_{\text{small} - \text{large}}$; we subtracted the value for large fine roots
 197 ($1\text{--}2\text{-mm}$ diameter) from that of small ($<0.05\text{-mm}$ diameter) fine roots] (the dependent variable) and
 198 current shoot growth rate, EcM hyphal status, foliage N concentration, the ratio of small-to-total fine
 199 root biomass (biomass small/total), and the N concentration ratio between small and large fine roots
 200 ($\%N_{\text{small/large}}$) (independent variables). The root $\delta^{15}\text{N}_{\text{small} - \text{large}}$ value correlated negatively with
 201 current shoot growth, but positively with the $\%N_{\text{small/large}}$ fine root ratio (Table 4).

202 The average slope elevation differed significantly by EcM hyphal status. Trees with
 203 high-level EcM colonization (e.g., of high EcM hyphal status; grade 3 or 4) were common in lower
 204 slope positions (status 1; $448 \pm 2 \text{ m}^a$, 2; $420 \pm 6 \text{ m}^a$, 3; $359 \pm 5 \text{ m}^b$, 4; $298 \pm 6 \text{ m}^c$, ANOVA, $n = 34$; the
 205 different superscripts indicate significant differences based on Tukey's HSD test; $P < 0.0001$). The
 206 average $\delta^{15}\text{N}$ value of hyphae was higher (-4.4‰) than that of small fine roots ($-6.1 \pm 0.3\text{‰}$). The
 207 $\delta^{15}\text{N}$ values of the smallest fine roots ($<0.5\text{-mm}$ diameter) did not change along the slope, but the

values of roots 1–2 mm in diameter were lower at low elevations; however, several individual roots in this larger diameter class had low $\delta^{15}\text{N}$ values at high elevations (data not shown).

Discussion

Variation in spruce growth and N uptake traits among slope positions

The micro-scale variation in foliage $\delta^{15}\text{N}$ and AGB values among elevations on a northeast-facing slope were relatively large and comparable to macro-scale variation over all of the black spruce stands across Alaska (Mayor et al. 2012). Differences in permafrost conditions (e.g., the depth of the active soil layer) within the same slope may explain the observed variations in AGB value, shoot growth, extent of EcM colonization, and N status, of both adult black spruce and black spruce saplings. Higher soil temperatures on the upper slope may have promoted organic decomposition and mineralization (Heilman 1966; Binkley et al. 1994), thereby reducing the thickness of the soil organic layer and the soil C:N ratio and elevating the soil N concentration (Table 1, 2). The fact that the soil $\delta^{15}\text{N}$ value and C:N ratio were negatively correlated also indicated that high-level N mineralization was associated with a high soil $\delta^{15}\text{N}$ (e.g., on the upper slope), whereas low-level N mineralization was associated with a low soil $\delta^{15}\text{N}$ (e.g., on the lower slope). Under these circumstances, the black spruce roots were able to reduce their dependency on mycorrhizal N uptake, as indicated by the increments in foliage $\delta^{15}\text{N}$ values (Hobbie et al. 2000; Mayor et al. 2012) and the frequent occurrence of individual trees with low levels of root EcM colonization. A high leaf N concentration may also increase

227 photosynthetic capacity (Macdonald and Lieffers 1990), thereby favoring tree growth at upper, as
228 compared to lower, slope positions. In contrast, the AGB values, foliage $\delta^{15}\text{N}$ signatures and N
229 concentrations on the lower slope were significantly lower than those at higher elevation. Mayor et al.
230 (2012) reported that black spruce stands with low foliage $\delta^{15}\text{N}$ values and N concentrations growing
231 on soils with high C:N ratios and low N availabilities rely more on EcM fungi for the uptake of N,
232 particularly DON. The shallow active layer depth, low soil temperature, and low soil N
233 concentrations at the lower slope positions may increase the limitations imposed on plant N uptake,
234 making the black spruce more dependent on EcM fungi for uptake of N. The mycorrhizal
235 relationships exhibited by many plant species are most evident in N-limited environments (Van Cleve
236 and Alexander 1981). Indeed, the root $\delta^{15}\text{N}$ of saplings increased significantly with decreasing root
237 diameter, reflecting the increased levels of ^{15}N -rich EcM fungi on smaller roots; this was most
238 apparent in lower slope positions where the permafrost table was high (Noguchi et al. 2016). Similar
239 $\delta^{15}\text{N}$ patterns have been reported in the small fine roots of *Larix* species that host EcM fungi
240 (Ouimette et al. 2013). Moreover, the black spruce may exhibit a higher level of EcM colonization at
241 lower rather than upper slope positions; the EcM hyphal status of the small fine roots of saplings was
242 more often positive at lower slope positions. Although EcM association promotes N uptake by plants,
243 trees may need to allocate up to 20% of their net photosynthetic production to the mycorrhizal
244 symbionts to achieve this uptake (Hobbie and Hobbie 2008). Moreover, black spruce growing on a
245 high permafrost table (i.e., with a thin active soil layer) under N-limited conditions have large

allocations of resources to below-ground components, particularly to 'small' (diameter <0.5 mm) fine roots (Noguchi et al. 2016). Similarly, Ingestad and Ågren (1988, 1991) found that trees exhibiting low foliage N concentrations when they grew under N-limited conditions allocated more biomass to below- than above-ground components. The lower foliage N concentration of black spruce growing on lower slopes may also indicate that, similarly, more biomass is allocated to the below-ground components. This large carbon allocation to below-ground components, especially the fine roots, and to EcM symbionts, may also restrict AGB production on lower slopes with high permafrost tables and N-limited conditions.

Slope aspect also influenced shoot growth and the foliage $\delta^{15}\text{N}$ values of the black spruce saplings. Higher sapling growth rate at the east–northeast-facing location may have been due to the thicker active soil layer and elevated inorganic N concentration in the soil at that position (Valentine et al. 2006). However, the growth rate was reduced on the east-facing slope, which had lost its permafrost; the soil was N-rich at this location (Valentine et al. 2006). This reduction in growth rate on the east-facing slope may have resulted from interspecific competitive pressure exerted by broad-leaved trees, such as aspen. Indeed, the dominance of black spruce was significantly reduced on this slope, in agreement with other studies (Viereck et al. 1983; Oechel and Lawrence 1985).

The trends across elevations of a species associated with ericoid mycorrhizal fungi were similar to those of black spruce symbiotic with EcM. The mycorrhizae of ericoid mycorrhizal plants have good proteolytic and litter-degrading capabilities, which confer adaptive advantages to host

265 plants growing on organically rich soils with low rates of N cycling (Hobbie and Högberg 2012).
266 Ericoid mycorrhizae also strongly discriminate against ^{15}N (Craine et al. 2009; Kenzo et al. 2016).
267 The foliage $\delta^{15}\text{N}$ values and N concentrations of ericaceous *L. palustre* plants growing on the same
268 slope were strongly correlated. Thus, on the lower slope, where soils were N-limited, both ericoid
269 mycorrhizal and EcM mycorrhizal plants were highly dependent on associated fungi for uptake N
270 from the soil.

271

272 **Assessing the mycorrhizal dependence of N uptake**

273 Differences in $\delta^{15}\text{N}$ values between root diameter classes and foliage may be indicative of black
274 spruce dependency on N uptake by EcM fungi. EcM fungi preferentially transfer ^{14}N to host plants
275 and therefore become somewhat ^{15}N -enriched relative to the N source and the host trees (Hobbie et al.
276 2000; Hobbie and Colpaert 2003). Thus, large differences in $\delta^{15}\text{N}$ values between the hypha and plant
277 body (foliage and roots) usually indicate elevated dependency on N uptake through EcM mycorrhiza
278 (Högberg et al. 1996). Additionally, a positive relationship between foliage N concentration and the
279 $\delta^{15}\text{N}$ value may indicate variation in both EcM dependence and N availability among slope positions
280 in which N availability differs. Many non-N-fixing plant species, including spruce, exhibited similar
281 positive relationships when compared across a gradient of N availability; the extent of dependence on
282 mycorrhizal fungi for N uptake also varied (Hobbie et al. 2000; Craine et al. 2009). Hobbie et al.
283 (2000) also suggested that correlations observed among different species across a gradient of N

availability may be linked to their relative allocations of below-ground carbon to mycorrhizal fungi.

Recent studies have revealed functional differences between the fine root diameters or root orders in several plant species associated with EcM (Guo et al. 2008; Ouimette et al. 2013). EcM association only occurs in narrow root sections, such as the root tips and small fine roots (diameter <0.5 mm), which are able to absorb nutrients; larger fine roots are usually less associated with EcM (Guo et al. 2008). The negative relationship between fine root diameter and the $\delta^{15}\text{N}$ value in the black spruce (Table 3) reflect enhancement of the level of ^{15}N -enriched EcM fungi on smaller fine roots, especially those of a diameter less than 0.5 mm. The relatively constant $\delta^{15}\text{N}$ values in the hyphae across slope positions and environmental conditions (Mayor et al. 2012) matched the constant $\delta^{15}\text{N}$ values in the smallest class of fine roots (<0.5 mm), most of which contained hyphae that we collected across the slope gradient (Ouimette et al. 2013). In contrast, the $\delta^{15}\text{N}$ value of larger fine roots (1–2 mm diameter) varied among slope positions and followed the trends in foliage $\delta^{15}\text{N}$ values, which were lowest at lower slope positions. Thus, the differences in $\delta^{15}\text{N}$ values between smaller and larger fine roots ($\delta^{15}\text{N}_{\text{small} - \text{large}}$ values) may also reflect the extent of the dependency of black spruce N absorption on EcM. The negative relationship between the root $\delta^{15}\text{N}_{\text{small} - \text{large}}$ value and the current shoot growth rate (Table 4) reflects growth limitation caused by high-level dependency on N absorption through the EcM fungi, which consume a large amount of the carbon resource (Mayor et al. 2012). Although saplings of high-level EcM colonization status were located on lower slopes with low soil C:N ratios, multiple regression showed that EcM status was not significantly associated with

the difference in $\delta^{15}\text{N}$ values between smaller and larger fine roots (Table 4). A limitation of our work is that we visually estimated the extent of EcM colonization of fine root biomass samples. This may not accurately reflect the actual EcM biomass (Nilsson et al. 2005), perhaps explaining the lack of observed significance.

Conclusions

The foliage and fine root $\delta^{15}\text{N}$ values, growth rates, and AGB of black spruce changed significantly on a slope gradient, reflecting the permafrost conditions, N availability, and the extent of N uptake dependence on EcM. N-rich conditions with thick active soil layers on upper slopes were associated with high foliage $\delta^{15}\text{N}$ values, foliage N concentrations, and growth rates. In the N-limited conditions on the lower slope, where the active soil layer was shallower, colonization by EcM and dependence of EcM for N uptake were elevated, and black spruce growth and AGB were reduced. The variation in $\delta^{15}\text{N}$ values with fine root diameters also indicates the dependency on EcM fungi for N absorption and functional differences between the fine root diameters. Future climate change or anthropogenic N deposition may markedly increase N mineralization and alter the N and C cycles in N-limited ecosystems, particularly boreal forests (Hyvönen et al. 2007). Our findings may contribute to accurate assessments of the influence of future climate change on the black spruce forests of Alaska.

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Figure and Table Legends

Fig. 1. Locations of the study site and the two transect lines. Circular symbols indicate sampled individuals. *N0–N3*, sample plots (10 × 10 m). *CPCRW*, Caribou Poker Creek Research Watershed. The figure was created using the Digital Elevation Model (DEM) of *CPCRW* (Chapin and Hollingsworth, 2010).

Fig. 2. Linear relationships among foliage $\delta^{15}\text{N}$ values, aboveground biomass (AGB) and N concentrations (%) in black spruce. *N0–N3*: The sample plots. Closed and open symbols refer to mature and sapling trees, respectively. Values are means $\pm$ standard error (SE).

Fig. 3. Current shoot growth and foliage $\delta^{15}\text{N}$ values at different elevations (A) and aspects (B) in black spruce. Plotted lines track moving averages. N, NE, and E refer to aspect.

Fig. 4. Relationship between foliage $\delta^{15}\text{N}$ value, current shoot growth and foliage N concentration in black spruce.

Fig. 5. Foliage N concentrations (A) and $\delta^{15}\text{N}$ values (B) at different elevations, and the relationship between N concentration and the foliage $\delta^{15}\text{N}$ value (C) in *Ledum palustre*.

Fig. 6. The relationships between the root $\delta^{15}\text{N}$ value of each black spruce root diameter class and N concentration. The results of multiple regression analysis on the root diameter, root $\delta^{15}\text{N}$ and root N concentration were shown in Table 3.

Table 1 Stand conditions of the study plots. Values for DBHs, heights, and active layer depths, are means $\pm$ SEs. Different superscripted lower case letters attached to these values indicate significantly different pairwise differences (Tukey’s HSD multiple comparisons tests; $P < 0.05$). N0–N3, see Figure 1 for plot locations on slope; DBH, diameter at breast height; AGB, aboveground biomass; BGB, belowground biomass.

Table 2 Results of multiple regression analysis on soil $\delta^{15}\text{N}$ value as the dependent variable and the soil C:N ratio and plot as the independent variables.

Table 3 Results of multiple regression analysis on the root $\delta^{15}\text{N}$ value as the dependent variable and root diameter and root N concentration as the independent variables.

Table 4. Results of multiple regression analysis on the difference in root $\delta^{15}\text{N}_{\text{small} - \text{large}}$ value [$\delta^{15}\text{N}$ values of large (1–2-mm diameter) fine roots subtracted from that of small (<0.05-mm diameter) fine roots] as the dependent variable and current shoot growth rate, EcM hyphal status, foliage N concentration, the biomass ratio of small fine roots to total fine roots, and the N concentration ratio of small fine roots to large fine roots, as independent variables.

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Fig. 1. Locations of the study site and two transect lines. Circular symbols indicate sampled individuals. *N0–N3*, sample plots (10 × 10 m). *CPCRW*, Caribou Poker Creek Research Watershed. The figure was created using the Digital Elevation Model (DEM) of *CPCRW* (Chapin and Hollingsworth, 2010).
79x49mm (300 x 300 DPI)

Fig. 2. Linear relationships among foliage $\delta^{15}\text{N}$ values, aboveground biomass (AGB) and N concentrations (%) in black spruce. N0–N3: The sample plots. Closed and open symbols refer to mature and sapling trees, respectively. Values are means $\pm$ standard error (SE).
55x17mm (300 x 300 DPI)

A) Elevation

B) Aspect

Fig. 3. Current shoot growth and foliage $\delta^{15}\text{N}$ values at different elevations (A) and aspects (B) in black spruce. Plotted lines track moving averages. N, NE, and E refer to aspect.
121x83mm (300 x 300 DPI)

Fig. 4. Relationship between foliage $\delta^{15}\text{N}$ value, current shoot growth and foliage N concentration in black spruce.
54x16mm (300 x 300 DPI)

Fig. 5. Foliage N concentrations (A) and $\delta^{15}\text{N}$ values (B) at different elevations, and the relationship between N concentration and the foliage $\delta^{15}\text{N}$ value (C) in *Ledum palustre*.
48x13mm (300 x 300 DPI)

Fig. 6. The relationships between the root $\delta^{15}\text{N}$ value of each black spruce root diameter class and N concentration. The results of multiple regression analysis on the root diameter, root $\delta^{15}\text{N}$ and root N concentration were shown in Table 3.
77x69mm (300 x 300 DPI)

Table 1 Stand conditions of the study plots. Values for DBHs, heights, and active layer depths, are means $\pm$ SEs. Different superscripted lower case letters attached to these values indicate significantly different pairwise differences (Tukey’s HSD multiple comparisons tests; $P < 0.05$). N0–N3, see Figure 1 for plot locations on slope; DBH, diameter at breast height; AGB, aboveground biomass; BGB, belowground biomass.

Plot	N3	N2	N1	N0
Position	Lower slope	Middle slope	Upper slope	Ridge
Forest type	Black spruce	Black spruce	Black spruce	Black spruce mixed with broad leaf trees
Altitude (m)	260	330	360	460
Active layer depth (cm)	73.7 $\pm$ 6.6 ^b (n = 3)	62.7 $\pm$ 4.5 ^b (n = 3)	113.0 $\pm$ 10.1 ^a (n = 3)	120.7 $\pm$ 3.3 ^a (n = 3)
Organic layer depth (cm)	26	18	13	7
Soil N at A horizon (g kg ⁻¹)	3.1	3.6	4.6	5.5
Stand density (trees ha ⁻¹)	5000	8100	3800	3600
DBH (cm)	3.1 $\pm$ 0.2 ^b	4.3 $\pm$ 0.2 ^{ab}	7.9 $\pm$ 0.8 ^{ab}	8.5 $\pm$ 0.6 ^a
Height (m)	3.0 $\pm$ 0.2 ^c	4.5 $\pm$ 0.2 ^{bc}	7.1 $\pm$ 0.6 ^{ab}	9.4 $\pm$ 0.5 ^a
AGB (Mg ha ⁻¹)	12.5	42.8	85.7	78.9
BGB (Mg ha ⁻¹)	5.0	18.4	43.0	38.5

TANAKA-ODA et al Table2

Table 2 Results of multiple regression analysis on soil $\delta^{15}\text{N}$ value as the dependent variable and the soil C:N ratio and plot as the independent variables.

	Soil $\delta^{15}\text{N}$ (‰)
Soil C:N	-0.13***
Plot	0.75

Draft

Table 3 Results of multiple regression analysis on the root $\delta^{15}\text{N}$ value as the dependent variable and root diameter and root N concentration as the independent variables.

	Root $\delta^{15}\text{N}$
Root diameter	-3.02***
Root N concentration	1.66

The values are standardized regression coefficients. Asterisks represent significant differences where *** is $P < 0.001$.

Draft

TANAKA-ODA et al Table 4

Table 4 Results of multiple regression analysis on the difference in root $\delta^{15}\text{N}_{\text{small} - \text{large}}$ value [$\delta^{15}\text{N}$ values of large (1–2-mm diameter) fine roots subtracted from that of small (<0.05-mm diameter) fine roots] as the dependent variable and current shoot growth rate, EcM hyphal status, foliage N concentration, the biomass ratio of small fine roots to total fine roots, and the N concentration ratio of small fine roots to large fine roots, as independent variables.

	Root $\delta^{15}\text{N}_{\text{small} - \text{large}}$
Current shoot growth	-1.67*
EcM hyphal status	-0.60
Foliage %N	-2.46
Biomass ratio of small to total fine roots (biomass small/total)	4.18
N concentration ratio of small to large fine roots (%N small/large)	3.52**

The values are standardized regression coefficients. Asterisks represent significant differences where * is $P < 0.05$ and ** is $P < 0.01$.