Ectomycorrhizal functional diversity parallels fine root and leaf abundance with forest stand age

By

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Abstract

The abundance of fine roots and leaves in forests is predicted to peak during mid-succession and then decline. If fine roots decline more rapidly than leaves, reductions in fine roots could contribute disproportionately to stand decline. Ectomycorrhizal fungi (EcM), symbionts that facilitate nutrient acquisition of fine roots, may complement or parallel these shifts in root abundance. Two competing hypotheses frame the response of EcM functional diversity to stand age: a) the 'host-filter' hypothesis states that EcM fungi with emanating tissues (Distance mycorrhizas) increase with stand age, and b) the 'energy-limited' hypothesis states that carbon available for root symbionts decreases with stand age resulting in fewer Distance mycorrhizas. In the first hypothesis, EcM functional diversity complements root abundance, while in the second it parallels root abundance.

To test these competing hypotheses, I sampled fine roots to a depth of 90 cm below the soil surface and used allometric equations to estimate changes in root and leaf area index across a chronosequence of *Pinus banksiana* Lamb. stands ranging from 2–76 years average tree age. In addition to estimating changes in fine root and leaf area, I examined roots microscopically to track changes in the abundance of EcM functional types.

Both fine root and leaf area increased for the first 30–36 years and then plateaued, while the ratio of leaf to fine root area remained unchanged across the age gradient. Changes to fine root area with stand age depended on soil depth, with indications that old stands could be shifting a larger proportion of roots to deeper soil. This result is important because previous studies typically focused on upper soil horizons, thus, changes in root abundance below typical sample depths may have gone undetected. The abundance of Distance mycorrhizas did not increase in old stands, contrary to the host-filter hypothesis. Instead, the mean abundance of Distance

mycorrhizas paralleled changes to leaf area, a finding more in-line with the energy-limited hypothesis. Taken together, these results suggest that the soil exploration benefits of Distance mycorrhizas do not outweigh their cost in old forests, but that they too are constrained by reductions in productivity.

Preface

This thesis is an original work by Josh Wasyliw. No part of this thesis has been previously published.

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Abbreviations

DBH	Diameter at Breast Height
EcM	Ectomycorrhizal fungi
GLMM	Generalized Linear Mixed Model
LAI	Leaf Area Index
RAI	Root Area Index

Introduction

The fine roots of plants play a critical role in acquiring water and nutrients. Plants also invest heavily in fine roots, allocating them an estimated 33% of global annual net primary production (Jackson, Mooney, & Schulze, 1997). However, unlike leaves, fine roots are difficult to observe and measure in situ. Therefore, little is known about the role they play in changes of forest productivity, such as age-related growth decline (Ryan, Binkley, & Fownes, 1997). Some studies have found that ontogenetic changes that accompany plant aging reduce the biomass of or the allocation to roots relative to shoots (Gedroc, McConnaughay, & Coleman, 1996; Litton, Raich, & Ryan, 2007; McConnaughay & Coleman, 1999; Noulèkoun, Khamzina, Naab, & Lamers, 2017; Peichl & Arain, 2007; Samuelson et al., 2017; but see: Gower, McMurtrie, & Murty, 1996, and, Ryan, Binkley, Fownes, Giardina, & Senock, 2004). Also, studies that directly compare the abundance of fine roots and leaves in forests show that ratios of fine root area to leaf area (Schoonmaker, Lieffers, & Landhäusser, 2016), and fine root mass to leaf mass (Helmisaari, Makkonen, Kellomäki, Valtonen, & Mälkönen, 2002; Xiao et al., 2003) decline with increasing stand age. Such an imbalance could mean that older forests are limited by access to belowground resources. However, most studies on roots focus on the upper soil profile (≤ 30 cm deep, for boreal studies) (Pickles & Pither, 2014), where roots are thought to be most abundant (Jackson et al., 1996; Schenk & Jackson, 2002; Yuan & Chen, 2010). Relatively few roots deep in soil profiles can have a disproportionate impact on resource acquisition, and the ability of trees to cope with stress, such as seasonal drought (Binkley, 2015; Nepstad et al., 1994; Stone & Kalisz, 1991; Yang, Feng, Wang, Dai, & Fu, 2017). Therefore, if an increasing proportion of roots in old stands are located deeper and below the typical sampling depth, important patterns in belowground resource acquisition may be overlooked. Specifically, while root absorptive area

may decrease with stand age in the upper soil profile, fine root surface area may increase, undetected, at depth.

In addition to fine roots, most trees also rely on symbiotic mycorrhizal fungi to acquire belowground resources (Smith & Read, 2008). Ectomycorrhizal fungi (EcM) are dominant root symbionts in boreal forests that improve the nutrient acquisition of plants in exchange for carbohydrates. However, substantial variation in function exists within this guild and it remains unclear whether the functional diversity of the EcM community is affected by stand age. Recent studies show that functional characteristics are important drivers of EcM community structure (e.g. Moeller, Peay, & Fukami, 2014; Peay, Kennedy, & Bruns, 2011), and a common functional basis for classifying EcM is by external morphology, or 'exploration type' (Agerer, 2001, 2006). Some EcM exploration types produce abundant hyphae or transport structures that emanate from roots, while others produce little to no emanating hyphae (herein referred to as 'Distance mycorrhizas' and 'Contact mycorrhizas', respectively).

Exploration type is often suggested to influence EcM fungal species assembly (e.g. Castaño et al., 2018; Fernandez et al., 2017; Hobbie & Agerer, 2010; Moeller et al., 2014), but the underlying mechanisms remain unresolved, with two competing hypotheses emerging. External mycelium greatly increases soil volume available to roots, potentially improving water (Doddridge, Malibari, & Read, 1980; Pickles & Simard, 2016) and nutrient (Hobbie & Agerer, 2010; Moeller et al., 2014) acquisition of hosts. Therefore, Distance mycorrhizas may dominate over Contact mycorrhizas when foraging ability is prioritized by hosts (Bakker, Augusto, & Achat, 2006; Moeller et al., 2014; Nickel et al., 2018), such as situations where fine root surface area is low. I refer to this as the 'host-filter' hypothesis. Alternatively, the cost associated with production of emanating tissues may cause a decline in Distance mycorrhizas when carbon

fixation is reduced (Castaño et al., 2018; Fernandez et al., 2017; Saikkonen et al., 1999; Saravesi, Markkola, Rautio, Roitto, & Tuomi, 2008). I refer to this as the 'energy-limited' hypothesis. Importantly, age-related changes in fine root and leaf abundance (Brassard, Chen, & Bergeron, 2009; Ryan et al., 1997; Yuan & Chen, 2010) are likely to impact belowground resource acquisition and carbon fixation of trees and in turn this could influence exploration type abundance through one of these proposed mechanisms.

Here, I compare the resource acquisition strategy in a chronosequence of jack pine (*Pinus banksiana* Lamb.) stands. In contrast to most studies, I quantify changes in above and belowground resource acquisition structures simultaneously and measure roots deep in the soil profile. To discern between the competing hypotheses, the first objective of this study is to identify changes in surface area of fine roots and leaves with stand age; declines in leaf or root area suggest decreased ability to absorb resources. I predict that both leaf and root area will follow trends reported in other studies (Brassard et al., 2009; Ryan et al., 1997; Yuan & Chen, 2010), with a peak at mid succession followed by a decline or plateau. The second objective of this study is to characterize the functional composition of EcM communities across the age gradient. If fine root surface area follows the pattern predicted above, then under the host-filter hypothesis, older stands should rely more on EcM fungi to explore soils for resources than younger ones. Thus, an increase in Distance mycorrhizas should occur in older stands (e.g., Hagenbo et al. 2017). Alternatively, decreased leaf area (Ryan et al., 1997) and growth efficiency (Schoonmaker et al., 2016) in older stands may result in reductions of carbon allocated belowground to support EcM fungi (Litton et al., 2007). Therefore, according to the energy-limited hypothesis, Distance mycorrhizas should decrease with stand age (e.g. LeDuc, Lilleskov, Horton, & Rothstein, 2013; Rudawska, Wilgan, Janowski, Iwański, & Leski, 2018). This study

provides a comprehensive assessment of changes to resource acquisition with stand age and informs our understanding of the role of EcM fungi in age-related forest decline.

Methods

Site description

Stands dominated by *P. banksiana* in northeastern Alberta, Canada, south of the city of Fort McMurray, were selected for this study (Fig. 1, Table 1). Soils in these stands fall within the Brunisolic order (Soil Classification Working Group, 1998), with predominantly sandy to loamy soil textures (Table 2). Understory plant species *Arctostaphylos uva-ursi* (L.) Spreng., *Vaccinium myrtilloides* Michx., *Vaccinium vitis-idaea* L., *Rhododendron groenlandicum* (Oeder) Kron & Judd, *Rosa acicularis* Lindl. and *Cornus canadensis* L. were common to most stands. Other co-occurring overstory species included *Populus tremuloides* Michx. and *Picea mariana* (Mill.) Britton, Sterns & Poggenb. The study region is part of the Central Mixedwood Natural Subregion of the Alberta boreal forest characterized by long harsh winters and short warm summers (Beckingham & Archibald, 1996). Mean annual temperature of 1 °C and precipitation of 418.6 mm were recorded at a nearby climate station (56°39', 111°13') (Government of Canada, 2010).

To measure leaf, root and mycorrhizal attributes of forest stands, I established 12 plots across four age classes with three replicates. The age classes cover the typical life cycle of a boreal forest: 'Seedling' (2—5 years), 'Sapling' (12—16 years), 'Mid age' (30—36 years), and 'Old growth' (65—76 years). To estimate the age of each stand, I used increment cores or cross sections from 15 trees per plot (Appendix 1, S1), except for the Seedling plots where age was approximated from fire history. In June 2018, I established a 400 m^2 (20 m x 20 m) plot within

each of the 12 stands. I positioned each plot at least 15 m from any vegetation boundary or disturbance to limit edge effects. In the oldest age-class, all trees within the plot were measured for diameter at breast height (DBH; 1.37 m) and height. The same measurements were made for Sapling and Mid age plots, but for trees occurring in 5 m radius subplots due to higher tree density. Seedling measurements are detailed in the 'Root area and leaf area indices' section.

Root area and leaf area indices

I measured the surface area of fine roots as a proxy for belowground resource acquisition ability. Soil cores (7.62 cm diameter) were taken at five locations per plot, in six 15 cm depth increments to a total depth of 90 cm (12 plots × 5 locations × 6 increments = 360 'samples'; total volume of soil sampled was 0.25 m^3). Each 15 cm soil core was removed individually and the depth of the core hole measured to ensure consistency. The forest floor layer was included in upper 0–15 cm soil sample, as this layer was typically very thin (≤ 5 cm). Soil coring locations were separated by at least 12 m, to reduce the autocorrelation in EcM fungal community composition (Lilleskov, Bruns, Horton, Taylor, & Grogan, 2004). Samples were immediately put on ice and stored at -20 °C until processing. Thawed samples were separately passed through a series of sieves from 2 mm to 600 µm and roots were removed in four successive 10-minute time intervals. The washed, living roots were classified into 'pine' and 'non-pine' based on morphology and colour; this classification was verified using molecular techniques. Pine roots from each sample and time increment were placed in water-filled plastic trays and scanned on a flatbed scanner at 800 dpi (Epson Perfection V600 Photo). WinRHIZO image analysis software (Regent Instruments Inc., Quebec) was then used to determine the surface area of fine (< 2 mm diameter) and coarse roots (≥ 2 mm diameter). Fine root surface area was then scaled per unit

ground area (root area index; RAI). Once the EcM tally was complete (see 'Shifts in ectomycorrhizal exploration types'), pine roots were dried at 65 °C for 96 hours and weighed for biomass. Non-pine roots were lyophilized at -45 °C for 72 hours in a freeze-drier (Labconco, Kansas City, MO, USA), and weighed in preparation for identification using molecular techniques.

To identify bulk samples of roots classified as 'non-pine,' the lyophilized roots from each core were pulverized using a TissueLyser II (Qiagen Inc, Hilden, Germany), and DNA was then extracted with 2% hexadecyltrimethylammonium bromide (Roe, Rice, Bromilow, Cooke, & Sperling, 2010) followed by cleaning with 5% hexadecyltrimethylammonium bromide (Griffiths, Whiteley, O'Donnell, & Bailey, 2000). The *trn*T- *trn*L intergenic spacer, the *trn*L intron, and the *trn*L- *trn*F intergenic spacer were targeted and amplified with primers (A2: FAM, C: VIC, E: NED) developed by Taberlet et al. (1991) and modified by Cronn et al. (2002). DNA fragment lengths were obtained following Metzler, La Flèche, & Karst (2019). Resolved fragment lengths were compared to those developed by Metzler, La Flèche, & Karst (2019) to assign root identities to species. To confirm the identity of roots classified as 'pine,' DNA was extracted from 30 root tips using Extract-N-Amp solution (Sigma-Aldritch, Inc.). Again, the *trn*T- *trn*L, *trn*L intron and *trn*L- *trn*F regions were targeted with primers specified above. DNA was amplified, amplicon fragment lengths were obtained and species were identified following Metzler, La Flèche, & Karst (2019).

As a proxy for carbon fixation ability, I calculated the leaf area index (LAI) of each plot. To do so, I first estimated specific leaf area for each of the three oldest age classes as follows: 30 needles were collected throughout the canopy of two trees per plot and frozen until further processing. Thawed needles were scanned on a flatbed scanner at 400 dpi and the projected leaf

area measured using WinSEEDLE software (Regent Instruments Inc., Quebec). These needles were then oven dried at 65 °C for 72 hours and weighed. The ratio of leaf area to dry mass was calculated and averaged by age class as an estimate of specific leaf area. Next, to estimate the dry leaf mass of each tree as a function of DBH, I used an existing equation developed through the destructive harvesting of 77 *P. banksiana* trees (Hegyi, 1972). The leaf mass estimate was scaled by specific leaf area to determine leaf area per tree. Finally, LAI was calculated as the leaf area per unit ground surface area and estimates were checked for accuracy by comparing the leaf area results from the Hegyi equation (1972) with those obtained through destructive harvesting (Appendix 1, section S2; Fig. S1).

I also estimated LAI for Seedling plots, but with a direct approach. I measured the heights of a minimum of 108 seedlings within a subplot (6.2 – 39.1 m^2). Since one of the seedling plots was slightly older, the trees were larger and less dense, requiring a larger subplot to encompass a similar number of trees. I harvested 20 seedlings from each subplot and immediately froze them until further processing. Specific leaf area was determined for 10 needles per harvested seedling as specified above and averaged for this age class. The remainder of the needles from each seedling were dried at 65 °C for 72 hours and weighed. A regression equation was developed for this age class to predict full seedling leaf mass from height (Table S1). Leaf mass for the remainder of the seedlings in each subplot was estimated using this regression, leaf area was determined using the specific leaf area relationship, and LAI calculated.

Stand aboveground biomass growth

To estimate stand productivity, I determined five-year biomass increment for jack pine trees in each plot. I used increment cores or cross sections to estimate the 2012 DBH of 10–15

trees from each plot in the Sapling, Mid age and Old growth age classes. I then developed regressions for each age class, to predict 2012 DBH for the remaining trees as a function of 2017/2018 DBH (Table S3). I was then able to estimate plot biomass of aboveground tree components for the measurement year (here called 2017/2018, since this was the outermost tree ring) and 2012, using equations developed by Lambert, Ung & Raulier (2005). The difference between 2017/2018 and 2012 biomass was divided by five and scaled to per-hectare values for an estimate of five-year plot biomass increment. Additionally, aboveground biomass estimates were used to calculate growth efficiency, or production per unit leaf area, for each plot (Waring, 1983).

Shifts in ectomycorrhizal exploration types

Pine roots from each soil sample were examined with a dissecting microscope to quantify changes in the abundance of EcM exploration types (Stemi 305 cam, Zeiss, Oberkochen, Germany). If the root tips present in a soil core were too numerous, a random subsample of 100 was selected for classification. In all, 13,935 root tips were examined for mycorrhizal characteristics. Colonized, intact root tips were classified into simplified 'exploration types' based on Agerer (2001). EcM root tips were classified as 'Distance' if they had emanating hyphae or rhizomorphs, or 'Contact' if lacking in these structures (Appendix 1, section S3; Fig. S2). This approach allowed me to group putatively functionally similar EcM, which might otherwise be separated within the traditional 'exploration type' framework; similar approaches have been used in other studies (Hupperts, Karst, Pritsch, & Landhäusser, 2017; López-García et al., 2018; Nickel et al., 2018). I also classified pine roots as either damaged/deteriorated or uncolonized when they did not fall into the previous categories. I used WinRHIZO software to

estimate the total number of root tips present in each sample by analyzing the root scan files with high debris removal and root detection parameters. Each image file was also manually edited to remove as many misidentified root tips as possible. I estimated the total number of Distance and Contact mycorrhizas by multiplying the number of root tips identified through image analysis by the proportion of each exploration type identified in the subsample.

Once counted, two root tips of each exploration type per sample, were selected for DNA extraction and identification. Fungal DNA was extracted using Extract-N-Amp solution (Sigma-Aldritch, Inc.) and the ITS regions of the fungal rDNA was amplified using the ITS1-F and ITS4 primer pair (Gardes & Bruns, 1993; White, Bruns, Lee, & Taylor, 1990) at the Molecular Biology Service Unit of the University of Alberta. Geneious software version 2019.1.1 (Biomatters, Auckland, New Zealand) was used to edit and align forward and reverse sequences for successfully amplified samples. BLAST searches of GenBank and UNITE databases were then conducted to assign names based on closest match. Species names were assigned to sequences over 400 bp long, when pairwise identity exceeded 99% and samples were assigned to genera only when it ranged from 97–99% (Table S4).

Data analysis

For analyses on data representing belowground tree variables, including RAI, EcM count and EcM proportion, I combined cores into two soil depth increments. The upper two soil cores at each location were pooled as the 'Upper' increment (0—30 cm), and the lower four were pooled as the 'Lower' increment (30—90 cm). This binning strategy was selected since 30 cm, equivalent to the Upper increment in my study, is a conventional sampling depth for belowground studies in the boreal (Pickles & Pither, 2014) and generally considered to contain

the majority of fine roots in that biome (Jackson et al., 1996; Schenk & Jackson, 2002; Yuan & Chen, 2010). The 30—90 cm soil depth, or Lower increment here, represents deep roots that may be missed in conventional research designs. Sampling to this depth, should capture a large proportion of roots, encompassing the extrapolated 95% rooting depth of boreal forest ecosystems (Schenk & Jackson, 2002).

Root area index, leaf area index and stand biomass growth

Root area index (RAI) of pine was compared across stand age classes and the two soil depth increments using a linear mixed effect model, including soil core within site as a nested random effect. Before fitting the model, the response variable was transformed to better satisfy model assumptions using Tukey's Ladder of Power as implemented with the transformTukey() function in the rcompanion package, version 2.3.7 (Mangiafico, 2019). Additionally, to compare changes in vertical root distribution between stand age classes and to remove any effect of binning soil depth increments, I used the following equation, developed by Gale & Grigal (1987):

$$Y = 1 - \beta^D$$

Cumulative root fraction (Y) was measured for each 15 cm increment (D), down to 90 cm. These values were used to calculate β values for each site. The β values describe vertical root distribution, with higher values representing a deeper root distribution and lower values representing a shallower distribution. The β values were transformed using Tukey's Ladder of Power as described above and compared by stand age class with a linear model.

Variables for which one value was obtained at the plot level (LAI, LAI to RAI ratio, leaf area to biomass ratio, root area to biomass ratio, aboveground biomass increment, and growth efficiency) were compared among age classes using linear models.

Shifts in ectomycorrhizal exploration types

I compared shifts in the abundance of exploration types with stand age and soil depth in two ways. First, I compared the estimated numbers of root tips bearing Distance mycorrhizas, per cm^2 of fine root surface, across stand ages and soil depths with a generalized linear mixed model (GLMM), using the glmmTMB (version 0.2.3) package in R (Brooks et al., 2017). I used a GLMM model because count data violates the assumptions of general linear models (St-Pierre, Shikon, & Schneider, 2018). Stand age and soil depth were included as fixed effects along with an age × depth interaction term. Soil core nested within site was included as a random effects term. I specified a negative binomial error distribution and a log link in the model to account for overdispersion. I also included a zero-inflation term in the model to account for the two processes that generate zero counts: (1) when no roots were found in the core and, (2) when roots were present in the core, but no Distance mycorrhizas were observed.

The second analysis to detect shifts in exploration types compares the prevalence of root colonization by different EcM exploration types. Specifically, the relative abundance of EcM root tips colonized by Distance rather than Contact mycorrhizas (hereafter referred to as: 'proportion of Distance mycorrhizas') was compared by stand age class and soil depth using a GLMM with a beta-binomial probability distribution and a logit link. I used the glmmTMB package in R to fit this model (Brooks et al., 2017). Stand age and soil depth were included as fixed effects along with an age × depth interaction term. Soil core nested within site was included as a random effects term. I selected a GLMM model because proportion data is bounded and therefore violates the assumptions of general linear models. Further, the data displayed overdispersion, which can cause biased model outputs in unadjusted logistic regression. Therefore, I used a beta-binomial error structure which can account for this extra

variation (Harrison, 2015). Before running the regression, the seedling sites were removed, because of the low number of cores from these sites that contained roots. Similarly, any cores in the older sites that contained no pine roots were also removed from the analysis.

Additionally, to test for relationships between LAI, representing potential carbon supply, and EcM exploration types, separate GLMM models were formulated to assess the fixed effect of LAI on (a) number and (b) proportion of Distance mycorrhizas. Finally, I used a GLMM model to determine if EcM functional response was influenced by root density as proposed by Peay, Kennedy & Bruns (2011). I included soil core nested within site as a random effect term in each model.

Model selection and adequacy

All statistical analyses were performed in R version 1.1.463 (R Core Team, 2019) and results were considered significant when $P < 0.1$. When significant results were obtained, I conducted post-hoc Tukey tests using the emmeans (version 1.3.4), or effects (version 4.1), packages (Fox, 2003; Fox & Weisberg, 2019; Lenth, Singmann, Love, Buerkner, & Herve, 2019).

To ensure model adequacy for linear models and linear mixed effect models, plots of fitted versus residuals were assessed to ensure equal variance and Q-Q plots were generated to check for violations of normality. When these assumptions were violated, models were fit using the gls() or lme() functions in the nlme package in R, version 3.1-137 (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2019). This package allows for the incorporation of variance structures in linear models, which helped ensure conformance with model assumptions. For GLMM models, a minimum of 5000 data sets were generated for each observation, from the fitted model

using the simulateResiduals() function in the DHARMa package in R, version 0.2.4 (Hartig, 2019). From these simulated datasets, empirical cumulative density functions and scaled residuals were produced. Subsequently, scaled residual versus fitted plots and Q-Q plots were assessed for uniformity.

Results

Root area and leaf area indices

Older age classes had a greater fraction of pine roots occurring deeper in the soil profile than did Seedling plots ($F(3,8) = 31$, $P < 0.001$) (Fig. 2). No pine roots were observed deeper than 60 cm in Seedling plots, but they were found up to 90 cm deep in all other age categories. Of root tips classified as 'pine,' 25 of 30 could be assigned a species based on amplicon fragment lengths. In total, 92% of resolved 'pine' samples were identified as *Pinus banksiana*. Additionally, in the amplicon fragment analysis of the bulk 'non-pine' samples, 242 of 320 produced species matches. Jack pine was not detected in 84% of the resolved 'non-pine' samples. Other than jack pine, roots frequently identified in soil cores included *Vaccinium myrtilloides* (velvetleaf blueberry) and *Carex concinna* (low northern sedge).

The effect of stand age on RAI differed by soil depth increment (age × depth interaction, Wald $\chi^2(3) = 18$, $P < 0.001$). In the Upper 0–30 cm soil increment, average RAI increased until Mid age by 2 m^2m^{-2}, before leveling off (Fig. 3); mean values in Mid age and Old growth classes were similar (2.1 ± 0.3 and 2.1 ± 0.4 m^2m^{-2}, respectively). Conversely, in the Lower 30–90 cm soil increment, average RAI increased more gradually from Seedling to Mid age class, by 0.35 m^2m^{-2}. Again, RAI was not significantly different between Mid and Old growth age classes (Fig. 3). Leaf area index also varied significantly between age classes ($F(3,8) = 48$, $P < 0.001$). Leaf

area index increased from Seedling to Mid age (0.04 ± 0.01 and 1.8 ± 0.2 m^2m^{-2}, respectively) (Fig. 4), and mean LAI declined non-significantly from Mid to Old growth age classes (t(4) = 0.9, $P = 0.8$). Although stand-level LAI trended downward following Mid age and RAI did not, the ratio of LAI:RAI did not significantly vary with stand age class (F (3,8) = 0.05, $P > 0.9$) (Table 3).

Stand aboveground biomass growth

Annual stand biomass growth from 2012–2017/2018 was similar between Sapling and Mid age (2310 ± 333 vs. 2780 ± 296 kg ha^{-1} $year^{-1}$, respectively), but there was a decline from Mid to Old growth age classes (t(6) = 2.6, $P = 0.09$, Old growth: 1530 ± 375 kg ha^{-1} $year^{-1}$). Stand biomass growth efficiency was reduced by 50% between Sapling and Mid age (1.57 ± 0.05 vs. 0.78 ± 0.01 kg y^{-1} m^{-2} leaf area; t (2.29) = 15, $P = 0.005$), and further reduced by 36% between Mid age and Old growth (0.78 ± 0.01 vs. 0.50 ± 0.04 kg y^{-1} m^{-2} leaf area; t (2.46) = 6.4, $P = 0.03$). Additionally, the surface area of leaves (F (2,6) = 129, $P < 0.001$) and fine roots (F (2,6) = 3.7, $P = 0.09$) both declined relative to aboveground biomass, from Sapling to Old growth age plots (Table 3).

Shifts in ectomycorrhizal exploration types

Across the chronosequence, I categorized most pine root tips as Contact mycorrhizas. Contact mycorrhizas were formed by fungal species including *Russula decolorans* and *Wilcoxina rehmii* (Table S4). Distance mycorrhizas included fungal species *Suillus luteus* and *Cenococcum geophilum* (Table S4). In samples selected for DNA sequencing, 51% were successfully amplified. In total, 106 sequences were identified to genus or species level, four of which are not

considered mycorrhizal, and 12 sequences were identified to higher taxonomic levels. Of the samples identified to EcM fungal species, only 43 were previously assigned an 'exploration type' at that level of resolution. For 68% of these samples (29/43), my designation aligned with that of the literature (Table S4). However, I also observed intraspecific variation in emanating tissue in *Cadophora finlandica, Russula decolorans*, *Russula delica* and *Wilcoxina rehmii*, which together made up 70% of this species-level dataset.

The effect of stand age on the number of Distance mycorrhizas, differed by soil depth increment (age × depth interaction, Wald χ^2 (3) = 11, P = 0.01). In the Upper soil increment, the number of Distance mycorrhizas was lowest in the Seedling age class (0.4 ± 0.3 no. cm^{-2} of fine root), increased until Mid age (5.3 ± 1.0 no. cm^{-2} of fine root), and remained constant thereafter (Fig. 5). In the Lower depth increment, the number of Distance mycorrhizas was low in Seedling and Sapling age classes compared to Mid age (Sapling vs. Mid age, t(108) = 3.3, P = 0.008) and Old growth age classes (Sapling vs. Old age, t(108) = 2.9, P = 0.02) (Fig. 5). Leaf area index did not vary with the number of Distance mycorrhizas (Wald χ^2 (1) = 2, P = 0.2).

The global model for the proportion of Distance relative to Contact mycorrhizas did not indicate an interaction of stand age and soil depth (Wald χ^2 (2) = 4.0, P = 0.13), but pairwise Tukey tests showed otherwise (Table S15). The proportion of Distance mycorrhizas did not differ across stand age class in the Upper soil depth increment (Table S15, Fig. 6). In contrast, in the Lower increment, the proportion of Distance mycorrhizas was significantly lower in Sapling than that in the Mid age class (t(75) = 2.4, P = 0.04). The proportion of Distance mycorrhizas was not related to root density (cm^2 cm^{-3} soil) (Wald χ^2 (1) = 1.1, P = 0.3) or LAI (Wald χ^2 (1) = 1.2, P = 0.3).

Discussion

Age and soil depth related changes to leaf and root area

The first objective of this study was to assess changes to leaf and fine root surface area across an age sequence of jack pine forests. Both variables increased in early stand development, peaked in Mid age, and then plateaued (Fig. 3, Fig. 4). These results suggest that the pattern of age-related changes predicted in leaf (Ryan et al., 1997) and root (Brassard et al., 2009; Yuan & Chen, 2010) biomass also apply to LAI and RAI.

Additional studies have suggested that ontogenetic changes during the course of plant development lead to decreases in the abundance of fine roots relative to leaves (Helmisaari et al., 2002; Schoonmaker et al., 2016; Xiao et al., 2003). Such a decrease in RAI:LAI ratio could result in a relative shortage of belowground resource supply and underlie age-related growth decline. Contrary to previous studies (Helmisaari et al., 2002; Schoonmaker et al., 2016; Xiao et al., 2003), my results show that there was no statistically significant change in leaf to fine root ratio with stand age (Table 3). Therefore, I found no evidence to suggest that an imbalance between above and belowground resource acquisition systems occurs with stand development and there is no indication that access to either pool of resources contributed disproportionately to stand decline. Since LAI and RAI change in tandem in this study, my findings support an isometric relationship between above and belowground acquisition systems (G. Chen, Hobbie, Reich, Yang, & Robinson, 2019).

Differences between my findings and those of previous studies could result from differences in age distribution of stands (Helmisaari et al., 2002; Schoonmaker et al., 2016) or root classification methodology (Xiao et al., 2003). Studies by Schoonmaker et al. (2016) and Helmisaari et al. (2002) included forest stands with an average tree age of 100 years or greater.

Therefore, those authors may have documented changes to leaf:root ratios that occur at a later stage of development than assessed herein. The age of the forest stand measured by Xiao et al. (2003), however, was similar to the 'Old growth' age class included here, but in that study roots were not separated by plant species. Therefore, it is unclear if there was a trend in leaf to root abundance in the focal species. Otherwise, the estimates of stand LAI obtained in the current study are within the range observed in *P. banksiana* (Table S2). Fine root surface area values were not compared to results of other studies, because they represent the quantity of roots extracted in a fixed time increment, not total stand fine root surface area.

Interactive effects of stand age and soil depth on RAI

Importantly, in the current study, stand age was not the only factor influencing fine root abundance. Rather, the effects of stand age on RAI were dependent on soil depth (Fig. 3). In the upper 0–30 cm, a rapid increase in RAI until Mid age is followed by an abrupt plateau between Mid and Old age, while, in the lower 30–90 cm, RAI maintains a gradual, though not statistically significant increase. These observed trends from Mid to Old growth could indicate: a) the beginning of an increase in root allocation to deep soil horizons or b) a time-lag between roots in upper and lower soil. Results of the cumulative root fraction suggest that increases in stand age class correspond to a larger proportion of fine roots allocated to deeper soil (Fig. 2). Similarly, some previous studies show evidence of increases in density of deep roots in older stands (Billings et al., 2018; Sun, Dong, Mao, & Li, 2015; Varik, Aosaar, Ostonen, Lõhmus, & Uri, 2013, but see Bakker, Turpault, Huet, & Nys, 2008). Therefore, these findings combined with those of previous work suggest that trees in older stands change their acquisition strategy to rely more heavily on deep soil resources.

Shifts in ectomycorrhizal exploration types

The second objective of this study was to characterize shifts in the functional composition of EcM fungal communities with forest stand age. My aim was to weigh support for two competing hypotheses regarding the relationship between forest stand age and relative abundance of EcM exploration types. Specifically, the host-filter hypothesis states that reductions in fine root area at old age, will result in a higher reliance on EcM fungi by plant hosts, represented by an increase in Distance mycorrhizas. Alternatively, the energy-limited hypothesis suggests that the abundance of EcM exploration types is regulated by host carbon supply. The abundance of Distance mycorrhizas should therefore decline in old age along with leaf area. My results are more consistent with the energy-limited than the host-filter hypothesis.

In opposition to the host-filter hypothesis, the abundance of EcM root tips with emanating structures, namely Distance mycorrhizas, displayed an increasing trend from Seedling to Mid age class (30–36 years average tree age) and subsequently plateaued (Fig. 5, Fig. 6). Thus, the Old growth age class with a decreasing fine root to biomass ratio (Table 3), also had a similar number and proportion of Distance mycorrhizas to the Mid age class. These results conflict with the host-filter hypothesis, because reductions in fine root area relative to tree size did not correspond to an increase in Distance mycorrhizas. In fact, no significant relationship was found between root density and Distance mycorrhizas (contrary to Peay, Kennedy, & Bruns, 2011). My results are more consistent with the energy-limited hypothesis as the abundance of Distance mycorrhizas paralleled LAI across the age gradient (Fig. 4, Fig. 5, Fig. 6). Leaf area index measures the leaf surface area available for photosynthesis, therefore, it is one of the factors controlling carbon availability and production (Ryan et al., 1997). While relationships between LAI and (a) number of Distance mycorrhizas, and (b) proportion of Distance

mycorrhizas were not statistically significant, I found indirect support for this hypothesis. Mean estimates of LAI and biomass growth were highest in the Mid age class, which suggests that these stands have the highest carbon supply and may explain the higher abundance of 'costly' Distance mycorrhizas (Castaño et al., 2018; Defrenne et al., 2019; Fernandez et al., 2017; Fransson, 2012). Conversely, mean LAI and biomass growth decreased in the Old growth age class, suggesting that these stands may have less carbon available for mycorrhizal symbionts resulting in a lower abundance of Distance mycorrhizas.

In addition to the results of the current research, other studies have provided evidence for a link between host carbon supply and EcM functional diversity. For example, temperature related changes that increase or decrease host photosynthetic rate have been shown to coincide with increased (Defrenne et al., 2019) and decreased (Fernandez et al., 2017) abundance of Distance EcM, respectively. Similarly, drought stress (Castaño et al., 2018) and host defoliation (Saikkonen et al., 1999; Saravesi et al., 2008) were found to result in the loss of Distance EcM in favor of Contact EcM, but increased atmospheric CO_2 concentration resulted in the opposite pattern (Godbold & Berntson, 1997).

Comparison to other studies addressing EcM functional diversity with stand age

Other studies have also addressed the relationship between stand age and relative abundance of EcM exploration types and some have reported no trend in exploration type abundance (Hagenbo, Kyaschenko, Clemmensen, Lindahl, & Fransson, 2018) or a decrease of 'long-distance' exploration types with increasing stand age (LeDuc, Lilleskov, Horton, & Rothstein, 2013; Rudawska, Wilgan, Janowski, Iwański, & Leski, 2018). For instance, a study by Hagenbo et al. (2018), found no trend in exploration type abundance with increasing stand age.

However, the use of ingrowth bags in the study was also shown to bias against several genera known to produce emanating tissues (Agerer, 2006; Tedersoo & Smith, 2013). In contrast, LeDuc et al. (2013), found that Distance EcM were most abundant in a 5-year-old jack pine stand but, this trend was driven by one site in their un-replicated chronosequence. The lack of a trend in the remaining sites makes it difficult to rule out this site as an outlier. Finally, the study by Rudwaska et al. (2018) found a successional shift in dominance from 'long-distance' exploration types to 'contact' types in a *Pinus sylvestris* chronosequence. This shift was primarily driven by the replacement of *Suillaceae* by *Russulaceae* species, a pattern that also appears in my subsample of identified EcM root tips (Table S4). A key difference is that *Russula delica*, an abundant species in my data set, produces emanating tissues and was therefore frequently classified as a Distance mycorrhiza, possibly explaining some of the discrepancy between studies. This trend also shows that other functional traits, such as enzyme activity of *Russula* species (J. Chen et al., 2019; Kyaschenko, Clemmensen, Hagenbo, Karltun, & Lindahl, 2017; Lilleskov, Hobbie, & Horton, 2011), might play a role in EcM succession.

Interactive effects of stand age and soil depth on Distance mycorrhizas

For both measures of EcM exploration type abundance, trends across stand age differed by soil depth. Specifically, the increase with stand age in number of Distance mycorrhizas observed in the Upper soil increment, lagged in time in the Lower soil increment (Fig. 5), and while the proportion of Distance relative to Contact mycorrhizas did not change with stand age in the upper soil increment, deeper in the soil profile it was lower in the Sapling age class (Fig. 6). These results may signify that the benefits provided by emanating EcM tissues do not supersede the cost of finding new roots in Sapling sites – a point in stand development when

deep roots are scarce (Fig. 3), and leaf area is low (Fig. 4). These findings highlight the importance of sampling depth in uncovering changes in functional composition of the ectomycorrhizal community, a conclusion identified in other studies (Baier, Ingenhaag, Blaschke, Göttlein, & Agerer, 2006; Genney, Anderson, & Alexander, 2006; Santalahti, Sun, Jumpponen, Pennanen, & Heinonsalo, 2016)

Limitations to the study

In contrast to many studies that rely on DNA sequences alone to assign exploration type (eg. Clemmensen et al., 2015; Hagenbo et al., 2018; LeDuc et al., 2013; Moeller et al., 2014), I used visual assessment to classify EcM root tips. This methodology helps to avoid problems with intraspecific and intrageneric variation in EcM morphology (Agerer, 2006; López-García et al., 2018; Tedersoo & Smith, 2013). For example, four genera (from DNA sequencing) and four species (from visual examination) were categorized as both Contact and Distance type in the current study, demonstrating intrageneric and intraspecific variation in this trait, respectively (Table S4). However, in processing roots, some emanating hyphae may have detached from root tips, which may have resulted in a bias towards Contact mycorrhizas. I also used a conservative approach to the classification of root tips, where roots were only classified as Distance if they were confirmed to produce emanating tissues. Therefore, most disagreements between the current study and the literature (12/14) occurred when I observed no emanating fungal tissues for EcM species described in the literature as Distance type (Table S4). Thus, the abundance of Distance mycorrhizas may be underestimated in my study.

The results of the DNA analysis indicate that not all roots were correctly identified to plant species. However, it is rare for authors to report their success in the identification of roots,

or to confirm it with DNA methods. Therefore, it is not possible to compare identification success with that of other studies. This could be problematic, for roots which lack distinctive traits. In this study, it was more common for 'pine' DNA to be detected in 'non-pine' bulk samples, which could be the result of: a) pine roots without distinctive pine traits being classified as 'non-pine' or b) fragments of pine roots being included in the bulk samples. In the opposite case, spruce species may have been rarely (8%) classified as pine, when their mycorrhizal short root tips had a similar appearance to those of pine.

Also, I did not measure soil nutrients in this study, but forest aging may correspond to changes in soil nutrient availability, which in turn can affect fine root abundance (Gedroc et al., 1996; Shipley & Meziane, 2002). Nitrogen (N), for example, is often limiting to plant growth in boreal forests. However, studies report inconsistent age-related changes to nitrogen availability in jack pine stands. LeDuc & Rothstein (2013) reported low total nitrogen in the first 18 years of a jack pine chronosequence in Michigan, followed by stabilization at higher concentrations for the remaining 42 years. Conversely, Hu et al. (2014) found a slight decrease of inorganic N availability in the mineral soil but not the forest floor of a northeastern Alberta jack pine chronosequence. In general, however, increases in C:N ratio (Gower et al., 1996; Hume, Chen, Taylor, Kayahara, & Man, 2016) and immobilization of N in detritus (Fisk, Zak, & Crow, 2002; Gower et al., 1996; Grier, Vogt, Keyes, & Edmonds, 1981) are expected to occur with stand age. This transition to prevalence of organic over inorganic sources of N could make nitrogen less available to trees. Growth studies suggest that jack pine increases fine root biomass in response to reduced N concentration and decreases fine root biomass in response to increased N concentration (Pokharel, Kwak, & Chang, 2017; Tan & Hogan, 1997, 1998). However, the results from my study do not indicate increased fine root abundance in old stands, when nutrients

are expected to be tied-up in organic forms. Therefore, under these assumed abiotic conditions, ontogeny still appears to have a more important influence on fine root surface area.

Conclusion

Results of this study show that changes in leaf and fine root area follow similar age-related patterns in stands of *P. banksiana*, with increases until Mid succession, followed by a plateau. I found no evidence for a change in the ratio of LAI:RAI at any point in stand development, but there were indications that older stands may be transitioning a larger proportion of fine roots to deeper soil. Thus, deep roots may play a more important role in resource acquisition for old forest stands.

Contrary to the host-filter hypothesis, at the Old growth stage the soil exploration benefits of Distance mycorrhizas do not appear to outweigh their cost to the host tree as they do not increase in abundance. Rather, the abundance of Distance mycorrhizas parallels that of fine roots and leaves across the age gradient. The results of this study appear more consistent with the energy-limitation hypothesis and suggest that EcM functional diversity is constrained by stand productivity. However, the abundance of Distance mycorrhizas was not statistically reduced from Mid to Old growth stands and this project could serve as a preliminary survey of the relationship between stand age and EcM functional diversity. I propose that future studies on this topic include forests at later stages of development to provide insight into whether the patterns observed in this study are sustained in older forests.

Tables

Table 1. Characteristics of the 12 study plots, located in *Pinus banksiana* stands in northeastern Alberta, Canada.

Age Class	Mean age (years)	Latitude	Longitude	Basal area (m^2 ha^{-1})	Mean DBH* (cm)	Percent Pine**	Density (stems ha^{-1})
Seedling	2	56.333	-110.956	-	-	70	169861
Seedling	2	56.391	-111.031	-	-	92	208668
Seedling	5	56.266	-111.470	-	-	93	27867
Sapling	12	55.867	-112.155	4.4	3.9	100	3451
Sapling	12	55.672	-111.209	7.2	3.8	100	5880
Sapling	16	56.224	-111.696	6.9	4.4	98	4474
Mid age	30	55.863	-110.815	15.0	6.2	98	4474
Mid age	31	55.832	-110.850	20.9	7.5	99	4091
Mid age	36	56.275	-111.580	33.3	9.3	75	5113
Old growth	65	56.145	-110.878	22.1	20.5	90	950
Old growth	72	55.866	-110.821	20.5	25.1	88	725
Old growth	76	55.990	-110.9054	30.0	24.1	100	700

* Mean DBH for jack pine trees at each site

** For seedling sites, percent pine was calculated as proportion of total stems, for other age categories, it was calculated as proportion of basal area

Table 2. Soil texture data, by soil increment (Upper = 0–30 cm; Lower = 30–90 cm) for study plots located *Pinus banksiana* stands in northeastern Alberta, Canada. Soil texture samples were collected at one location, near the center of each plot.

Age class	**Mean age (years)**	**Soil depth increment**	**Clay % (< 2 µm)**	**Silt % (2–50 µm)**	**Sand % (> 50 µm)**
Seedling	2	Upper	3	18	79
		Lower	3	8	89
Seedling	2	Upper	9	46	45
		Lower	24	38	38
Seedling	5	Upper	9	61	31
		Lower	17	35	48
Sapling	12	Upper	23	20	57
		Lower	26	23	51
Sapling	12	Upper	3	19	78
		Lower	5	19	76
Sapling	16	Upper	10	27	63
		Lower	24	21	56
Mid age	30	Upper	3	4	93
		Lower	4	0	96
Mid age	31	Upper	4	3	93
		Lower	3	6	91
Mid age	36	Upper	2	11	87
		Lower	7	14	79
Old growth	65	Upper	3	6	91
		Lower	1	2	97
Old growth	72	Upper	2	4	95
		Lower	3	1	96
Old growth	76	Upper	8	42	50
		Lower	5	9	86

Table 3. Leaf area index (LAI), fine root area index (RAI), stand aboveground biomass, and their ratios by age class of *Pinus banksiana* stands in northeastern Alberta, Canada. Values are raw averages (± SE) by stand age (n = 3).

Age class	**Mean age (years)**	**LAI:RAI**	**Leaf area: Stand biomass (m^2 kg^{-1})**	**Fine root area: Stand biomass (m^2 kg^{-1})**
Seedling	2-5	0.68 (0.23)[a]	-	-
Sapling	12-16	0.71 (0.15)[a]	0.44 (0.02)[a]	0.68 (0.13)[a]
Mid age	30-36	0.79 (0.22)[a]	0.27 (0.02)[b]	0.39 (0.10)[ab]
Old growth	65-76	0.73 (0.34)[a]	0.14 (0.01)[c]	0.28 (0.09)[b]

Within columns, means denoted with the same lower-case letter were not significantly different ($P < 0.1$).

Figures

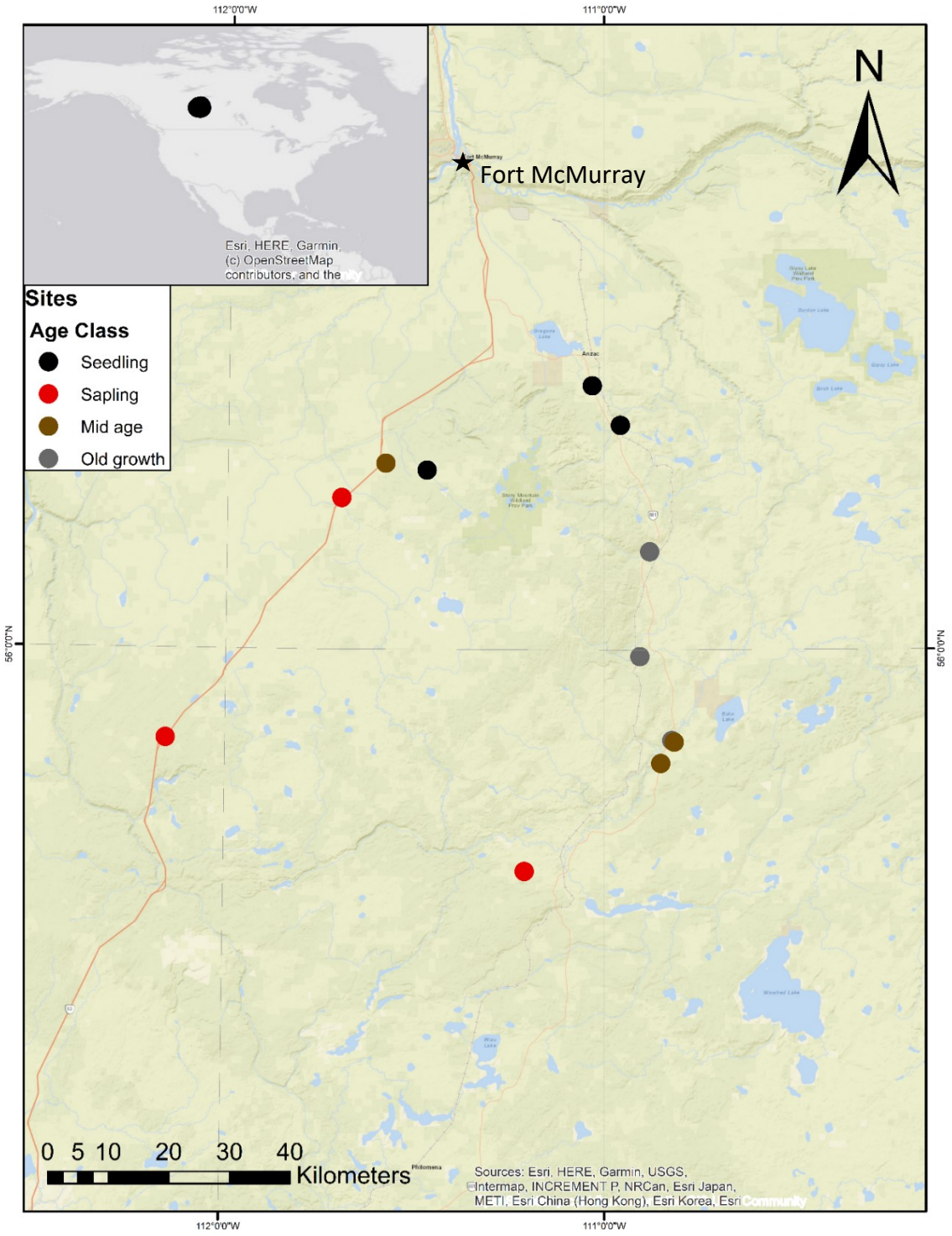

Figure 1. Map of plot locations situated in *Pinus banksiana* stands in northeastern, Alberta, Canada. (n = 12).

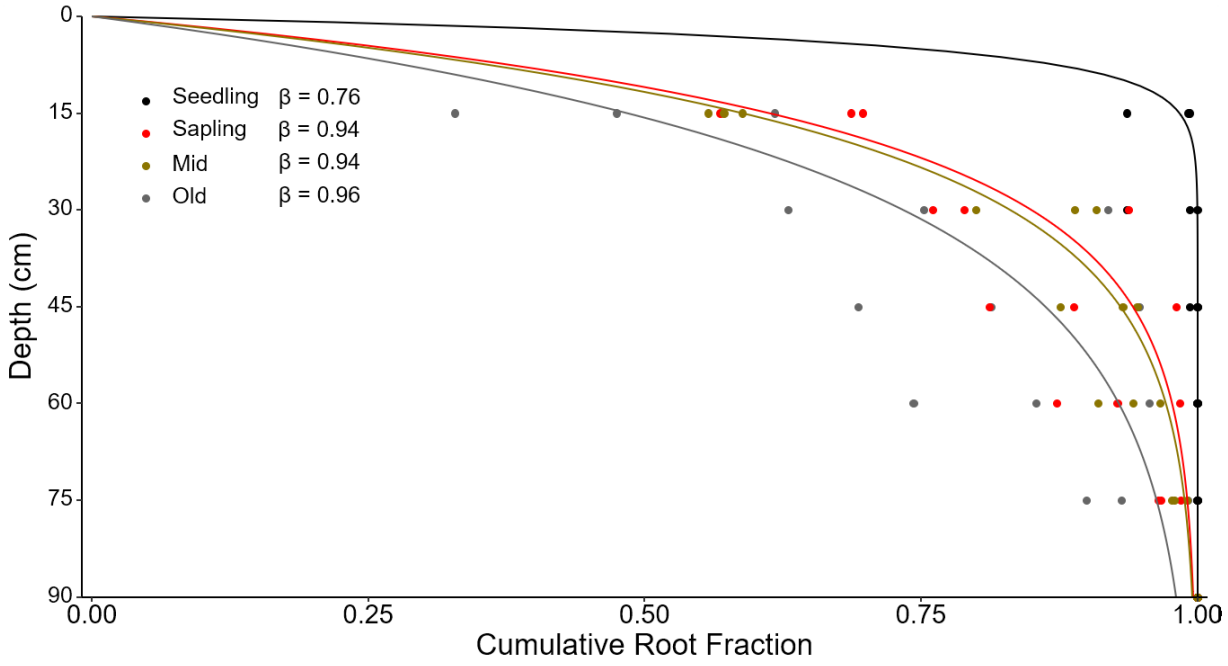

Figure 2. Cumulative root fraction at each 15 cm soil increment of *Pinus banksiana* stands in northeastern, Alberta, Canada, as a function of stand age class. Seedling: 2–5 years; Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. A nonlinear regression line is fit to data of each stand age class: $Y = 1 - \beta^D$, where Y is cumulative root fraction and D is soil depth increment.

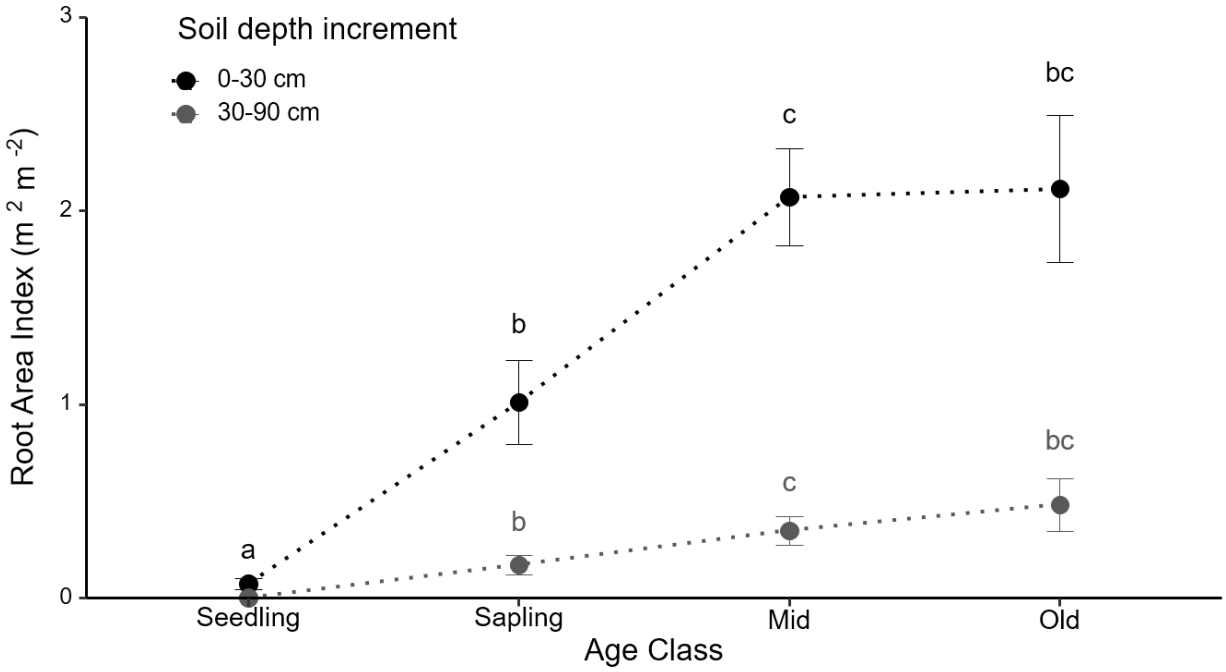

Figure 3. Mean (± SE; n = 3) fine root (<2 mm diameter) area index of *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of stand age class. Seedling: 2–5 years; Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. Means calculated with raw data. Within a given soil depth, means denoted with the same lower-case letter were not significantly different ($P > 0.1$).

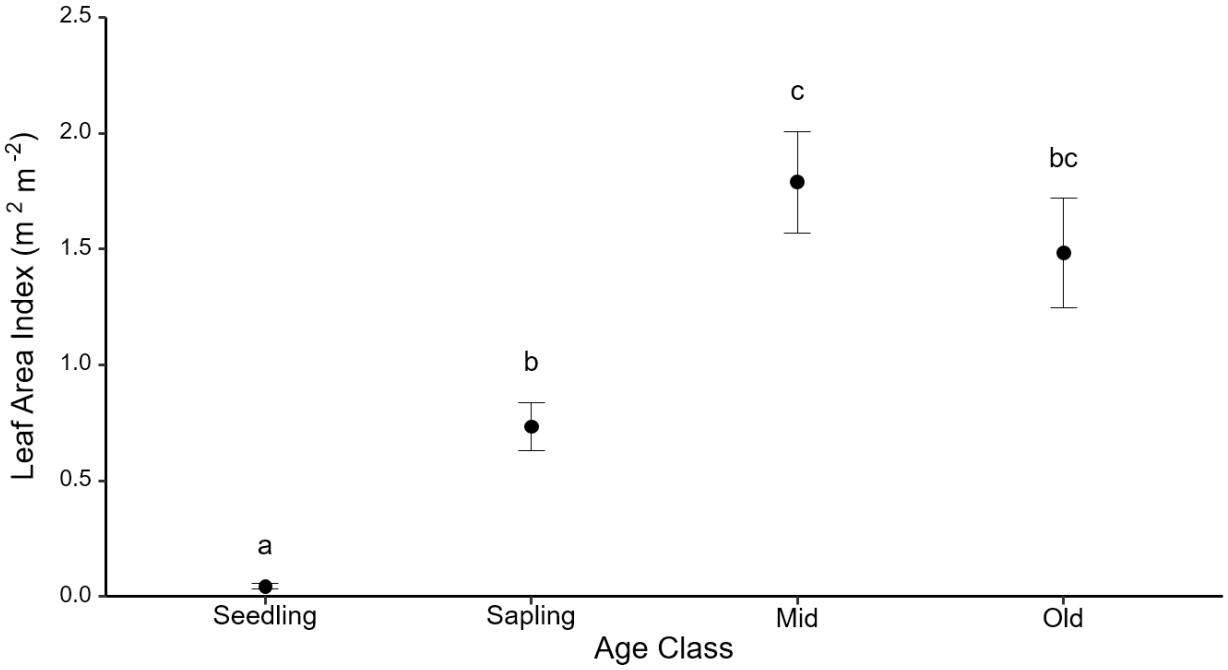

Figure 4. Mean leaf area index (± SE; n = 3) of *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of stand age class. Seedling: 2–5 years; Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. Means calculated with raw data. Means denoted with the same lower-case letter were not significantly different ($P > 0.1$).

Figure 5. Estimated number of Distance mycorrhizas in *Pinus banksiana* stands located in northeastern, Alberta, Canada, as a function of stand age class. Seedling: 2–5 years; Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. Raw averages (± SE) for the upper 0–30 cm soil increment (I), and lower 30–90 cm soil increment (II). Within soil depths, means denoted with the same lower-case letter were not significantly different ($P > 0.1$).

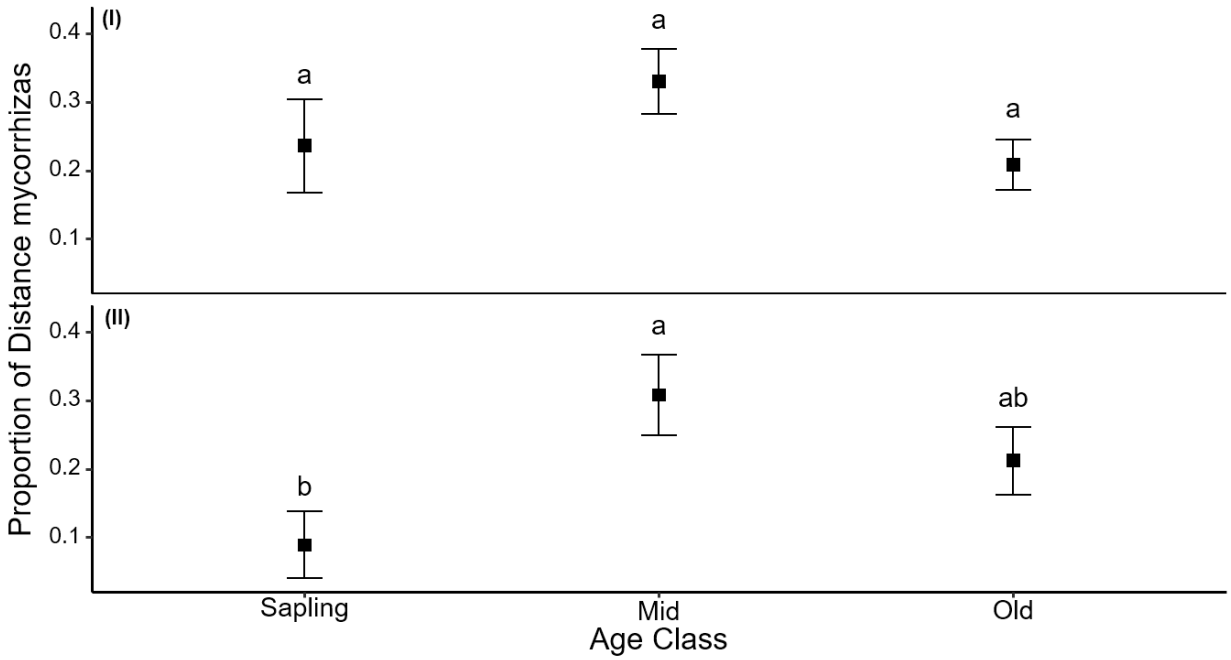

Figure 6. Proportion of colonized root tips categorized as Distance mycorrhizas in *Pinus banksiana* stands located in northeastern, Alberta, Canada, as a function of stand age class: Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. Raw averages (± SE) for the upper 0–30 cm soil increment (I), and lower 30–90 cm soil increment (II). Within soil depths, means denoted with the same lower-case letter were not significantly different ($P > 0.1$).

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Appendix

Appendix 1 – Supplemental Materials

S1. Stand age

To estimate the age of trees comprising a plot, I took two cores per tree near the base of the stem for 15 trees. These cores were scanned on a flatbed scanner at 1200 dpi (Epson Perfection V600 Photo), and I used CooRecorder software (Cybis Dendrochronology, Sweden) to count the tree rings on each increment core. The files produced from this process were used with CDendro software (Cybis Dendrochronology, Sweden) to build a tree-ring chronology for the local area, which also served to verify ring counts on questionable cores and to identify missing and false rings. The age of each plot was established using the dplR package in R statistical software (Bunn et al., 2018; R Core Team, 2019). When trees were too small for increment boring, we took cross sections at the base of 15 trees, counted the rings and used average ring count as an estimate of stand age. Finally, for Seedling plots, age was approximated from fire history.

S2. Leaf mass and area

I used two approaches for estimating leaf area and compared the results with those obtained through destructive harvesting. The first method estimated leaf mass from DBH through an equation published by Hegyi (1972). The second method estimated leaf area as a function of sapwood area (Deblonde, Penner, & Royer, 1994).

To measure sapwood area, two increment cores per tree (or cookies) were taken at 1.37 m (diameter at breast height; DBH), for 15 trees per plot. I immediately measured sapwood width in the field based on water content and translucent appearance. Taking measurements in the field allowed us to more easily identify the sapwood versus heartwood and minimized the effect of core shrinkage or breakage. Once measured, the cores were placed in paper straws and frozen. Sapwood area was calculated from the average of the two cores taken at DBH. I then used an equation developed by Deblonde, Penner, & Royer (1994) for *Pinus banksiana*, to estimate the leaf area index from sapwood area.

To estimate leaf area directly, I felled two trees per plot (3 age categories × 3 replicates × 2 trees = 18 trees total), and measured diameter and length of each branch. One branch per 0.5 m or 1 m section was collected and oven-dried at 65 °C for 96 hours. Needles were removed from each branch and weighed. Regression equations were developed for each stand age category to predict dry needle weight of each branch from basal diameter, length and crown section (see Table S1). Full tree dry needle weight was calculated as the sum of branch dry needle weight. The total leaf area per tree was estimated using the specific leaf area and the results were compared with those from published leaf area equations (Deblonde et al., 1994; Hegyi, 1972).

Estimates of leaf area using the Deblonde et al. (1994) and Hegyi (1972) equations were on average 3.8 m^2 (± 1.2 SE) and 1.2 m^2 (± 0.5 SE) per tree smaller than direct estimates, respectively. I decided to use the Hegyi (1972) equation for plot level measurements since it produced better results than the sapwood equation (Deblonde et al., 1994), and there was a strong relationship between direct estimates and the estimates produced via this equation ($R^2 = 0.98$) (Fig. S1).

S3. Classification of ectomycorrhizal root tips

I classified colonized root tips based on the presence of emanating fungal tissue (Fig. S2). Root tips were classified as 'Distance' type if (a) rhizomorphs originated from the same root cluster or (b) they possessed abundant emanating hyphae. Roots were classified as 'Contact' type if they possessed no or very few emanating hyphae and no rhizomorphs.

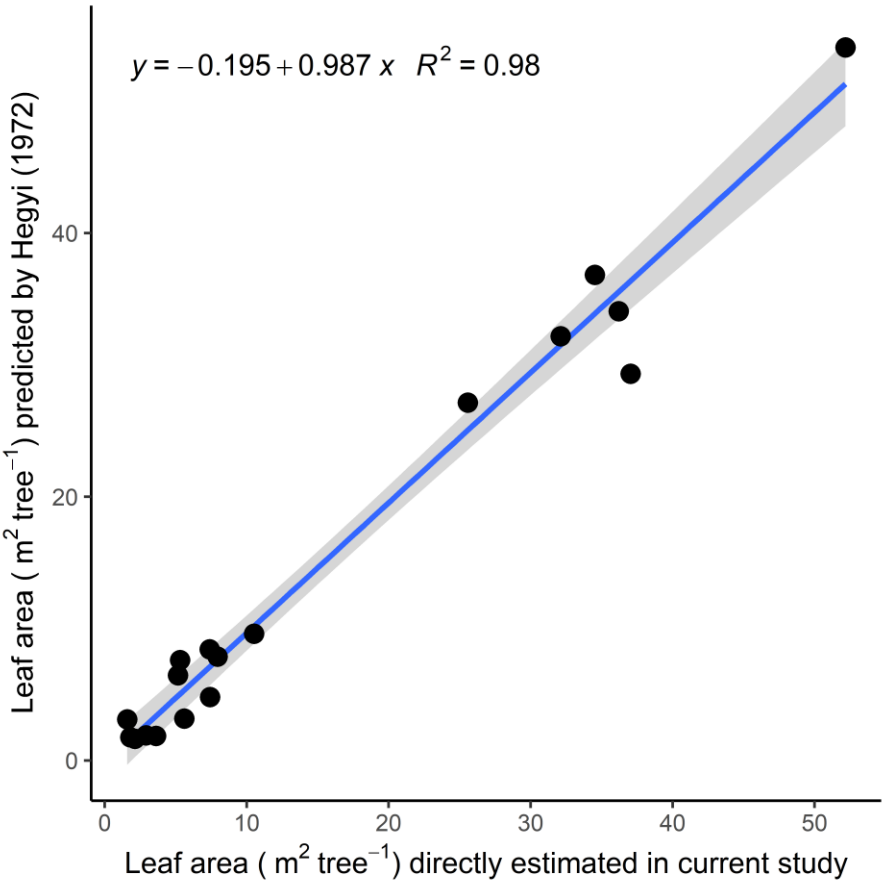

Figure S1. Comparison of leaf area estimates from Hegyi (1972) and destructive harvesting of 18 *Pinus banksiana* trees across three stand age categories: Sapling (12—16 years; color), Mid age (30—36 years; color), and Old growth (65—76 years; color). Stands are in north-eastern Alberta, Canada.

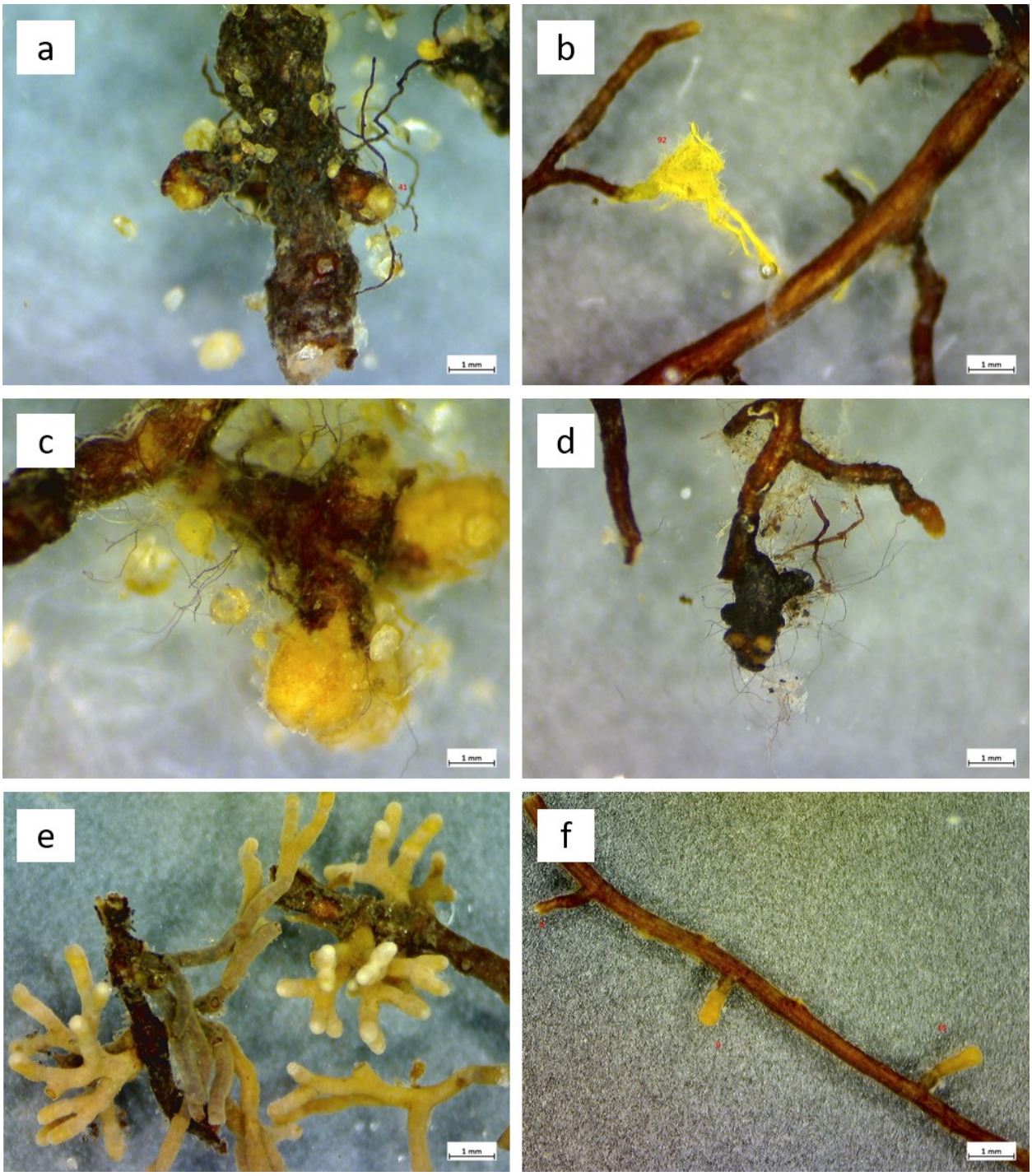

Figure S2. Photos of ectomycorrhizal root tips scored in this study. Photos (a-c) show mycorrhizal root tips with rhizomorphs, (c,d) show root tips with emanating hyphae and (e,f) show ectomycorrhizal roots without emanating tissues.

Table S1. Regression equations for estimating leaf mass of trees within *Pinus banksiana* stands in northeastern, Alberta, Canada by stand age category. Seedling: 2—5 years; Sapling: 12—16 years; Mid age: 30—36 years; Old growth: 65—76 years. Estimates of total needle mass from the Seedling regression equation were used for all subsequent analyses. Needle biomass estimates from the Sapling, Mid age and Old age equations were summed per tree and used to confirm reliability of Hegyi (1972) equation.

Age Class	Equation	ΔAIC	AIC weight	df	Pseudo R^2 (marginal)	Pseudo R^2 (conditional)
Seedling	$\ln(W) = (B_0 + a_i) + B_1 * \ln(H)$	24	1.00	5	0.83	0.92
Sapling	$\ln(W) = (B_0 + a_i) + B_1 * \ln(D) + B_2 * \ln(L) + B_3 * Section$	40	0.78	9	0.83	0.88
Mid	$\ln(W) = (B_0 + a_i) + B_1 * \ln(D)$	7	0.81	5	0.84	0.90
Old	$\ln(W) = (B_0 + a_i) + B_1 * \ln(D) + B_2 * \ln(L)$	11	0.69	6	0.84	0.84

Symbol

W	Dry needle mass
D	Branch base diameter
L	Branch length
Section	Crown section (bottom, mid or top)
H	Tree height
a_i	Random intercept specific to tree or site

Mixed models were used for each age class. Pseudo R^2 (Nagelkerke) statistics were calculated with r.squaredGLMM function in MuMIn package in R (Bartoń, 2019).

Table S2. Literature review of studies reporting LAI on *Pinus banksiana.* Table details the age of stands measured in each study and the estimate of leaf area index.

Study	**Site Age (years)**	**LAI**
Zha et. al (2013)	7	1.3
	11	0.7
	16	3
	28	2.8
	30	3.1
	90	2
Noormets et al. (2008)	8	0.52
	13-14	0.93
Deblonde et al. (1994)	30	1.6
	30	2.2
	30	1.7
	30	1.5
	60	2
Gower et al. (1997)	25	1.8
	65	2.2

Table S3. Regression equations for estimating 2012 DBH of trees within *Pinus banksiana* stands in northeastern, Alberta, Canada of three stand age classes. Sapling: 12—16 years; Mid age: 30—36 years; Old growth: 65—76 years. The 2012 diameter estimates were used in biomass equations, to estimate 5-year tree growth increment.

Age Class	Equation	ΔAIC	AIC weight	df	Pseudo R^2 (Nagelkerke)
Sapling	$Y = B_0 + B_1*D2017$	6.3	0.71	4	0.86
Mid	$Y = (B_0 + a_i) + B_1*D2017$	4.9	0.65	5	0.97
Old	$Y = B_0 + B_1*D2017$	0.9	0.62	4	0.99

Symbol	
Y	Tree diameter at 1.37 m in 2012
D2017	Tree diameter at 1.37 m in 2017/2018
a_i	random intercept specific to site (i)

Models were fit using nlme() package in R (Pinheiro et al., 2019), with power adjustments to reduce heteroscedasticity

Pseudo R^2 calculated with piecewiseSEM package in R statistical software (Lefcheck, Byrnes, & Grace, 2018)

Table S4. Summary of DNA results of EcM root tips from *Pinus banksiana* stands in Alberta, Canada of three age classes: Seedling: 2—5 years, Sapling: 12—16 years; Mid age: 30—36 years; Old growth: 65—76 years. Comparison of observed presence of emanating elements to reports from the literature. Rows represent EcM root tips identified to taxa.

NCBI Accession number	Unite Accession Number	Age	Organism	Emanating elements (observed)	Emanating elements (Literature)*	Source:
FJ554067	FJ554067	Old	*Amphinema* (U)	No	SD[3]	Agerer, 2006
KF617754	KF617754	Sap	Archaeorhizomyces (U)	No		
EU292346	EU292346	Mid	*Cadophora finlandica* (U)	Yes	EcM morphology unclear/undefined[1]	Vrålstad et al., 2002; Wang & Wilcox, 1985, 1987
KF428416	FJ553656	Sap	*Cadophora finlandica* (U)	No	EcM morphology unclear/undefined[1]	Vrålstad et al., 2002; Wang & Wilcox, 1985, 1987
FJ553656	FJ553656	Sap	*Cadophora finlandica* (U)	No	EcM morphology unclear/undefined[1]	Vrålstad et al., 2002; Wang & Wilcox, 1985, 1987
HM164565	HM164568	Mid	*Cadophora finlandica* (U)	No	EcM morphology unclear/undefined[1]	Vrålstad et al., 2002; Wang & Wilcox, 1985, 1987
JQ711896	KJ938039	Mid	*Cenococcum geophilum* (N, U)	Yes	SD	LoBuglio, 1999
KJ938039	KJ938039	Mid	*Cenococcum geophilum* (U)	Yes	SD	LoBuglio, 1999
MG136821	KY659394	Old	*Cortinarius psammocola* (N); *Cortinarius erythrinus* (U)	Yes	MD fringe[2]	Agerer, 2006
MG136821	KY659394	Old	*Cortinarius psammocola* (N); *Cortinarius erythrinus* (U)	Yes	MD fringe[2]	Agerer, 2006
JQ711911	UDB002214	Mid	*Cortinarius lux-nymphae* (U)	No	MD fringe[2]	Agerer, 2006
MH809978	KX516394	Mid	*Naganishia globosa* (N)	Yes	NON ECM	
KF617446	KF617446	Old	Helotiales (U)	No		
KF617927	KF617927	Old	Helotiales (U)	Yes		
KF617927	KF617927	Old	Helotiales (U)	Yes		
AB669499	AB669499	Old	*Humaria* (U)	No	SD, MD smooth[3]	Agerer, 2006
AM181405	KT692924	Old	*Humaria hemisphaerica* (U)	Yes	SD, MD smooth	Erős-Honti et al., 2008
KJ195392	KJ195392	Seed	*Hyaloscypha finlandica* (U)	No	EcM morphology unclear/undefined[1]	Vrålstad et al., 2002; Wang & Wilcox, 1985, 1987
FJ440910	FJ440910	Old	Hyaloscyphaceae (U)	Yes		
MK351729	UDB037066	Mid	*Hygrocybe conica* (U)	Yes	C, SD[2]	Agerer, 2006
MG882099	MG882099	Mid	*Hygrophorus siccipes* (N)	Yes	C, SD[2]	Agerer, 2006
MG882099	MG882099	Mid	*Hygrophorus siccipes* (N)	No	C, SD[2]	Agerer, 2006
MG882099	MG882099	Sap	*Hygrophorus siccipes* (N)	No	C, SD[2]	Agerer, 2006
MK307842	FJ845409	Old	*Hygrophorus hypothejus* (U)	No	C, SD[2]	Agerer, 2006
MG882099	MH087010	Sap	*Hygrophorus siccipes* (N); *Hygrophorus hypothejus* (U)	Yes	C, SD[2]	Agerer, 2006
MK307842	FJ845409	Sap	*Hygrophorus hypothejus* (U)	No	C, SD[2]	Agerer, 2006
MK307842	FJ845409	Sap	*Hygrophorus hypothejus* (U)	No	C, SD[2]	Agerer, 2006

NCBI Accession number	Unite Accession Number	Age	Organism	Emanating elements (observed)	Emanating elements (Literature)*	Source:
KF617586	KF359590	Sap	*Infundichalara microchona* (U)	No	NON ECM	
HQ604601	HQ604601	Sap	*Inocybe lacera* (N, U)	No	SD	Agerer, 2006
HQ604492	HQ604492	Old	*Inocybe praetervisa* (N, U)	Yes	SD[2]	Agerer, 2006
AY750156	AY750156	Seed	*Laccaria proxima* (N, U)	No	MD smooth	Agerer, 2006
AY969885	MF755271	Mid	*Lactarius chelidonium* (U)	No	C, SD,MD[2]	Agerer, 2006
JF899563	JF899563	Old	*Lactarius resimus* (N, U)	No	C, SD,MD[2]	Agerer, 2006
FR682163	FR682163	Sap	*Malassezia* (U)	No	NON ECM	
CP033152	EU400587	Old	*Malassezia restricta* (N, U)	Yes	NON ECM	
MG597405	KC571767	Mid	*Phellodon tomentosus* (N, U)	No	MD mat[2]	Agerer, 2006
AY394921	AY394921	Mid	*Phialocephala fortinii (N, U)*	Yes	EcM morphology unclear/undefined[1]	Currah et al., 2008; Grünig et al., 2008
HQ021752	HQ022032	Mid	*Piloderma* (U)	Yes	SD, MD mat[3]	Agerer, 2006
AY884240	AY884240	Mid	*Piloderma* (U)	Yes	SD, MD mat[3]	Agerer, 2006
AY884240	AY884240	Mid	*Piloderma* (U)	Yes	SD, MD mat[3]	Agerer, 2006
AY884240	AY884240	Mid	*Piloderma* (U)	No	SD, MD mat[3]	Agerer, 2006
AY884240	AY884240	Mid	*Piloderma* (U)	No	SD, MD mat[3]	Agerer, 2006
AY884240	AY884240	Mid	*Piloderma* (U)	No	SD, MD mat[3]	Agerer, 2006
AY884240	AY884240	Mid	*Piloderma* (U)	No	SD, MD mat[3]	Agerer, 2006
AY884240	AY884240	Mid	*Piloderma* (U)	No	SD, MD mat[3]	Agerer, 2006
MH809947	KP814514	Sap	*Piloderma bicolor* (N, U)	Yes	SD, MD mat[3]	Agerer, 2006
HM488589	KP814518	Mid	*Piloderma olivaceum* (U)	Yes	SD, MD mat[3]	Agerer, 2006
KP814428	KP814428	Seed	*Piloderma olivaceum* (N, U)	Yes	SD, MD mat[3]	Agerer, 2006
JQ711944	JQ711944	Mid	*Piloderma olivaceum* (N, U)	Yes	SD, MD mat[3]	Agerer, 2006
KP814428	KP814428	Seed	*Piloderma olivaceum* (N, U)	Yes	SD, MD mat[3]	Agerer, 2006
KP814428	KP814518	Mid	*Piloderma olivaceum* (N, U)	Yes	SD, MD mat[3]	Agerer, 2006
JQ711930	JQ711930	Sap	*Piloderma sphaerosporum* (N, U)	No	SD, MD mat[3]	Agerer, 2006
JQ711930	JQ711930	Sap	*Piloderma sphaerosporum* (N, U)	No	SD, MD mat[3]	Agerer, 2006
JQ711930	JQ711930	Sap	*Piloderma sphaerosporum* (N, U)	No	SD, MD mat[3]	Agerer, 2006
JQ711930	JQ711930	Seed	*Piloderma sphaerosporum* (N, U)	No	SD, MD mat[3]	Agerer, 2006
EU557327	GQ267480	Mid	*Pseudotomentella rhizopunctata* (U)	Yes	D	Martini & Hentic, 2003
EU837230	KT968587	Mid	*Rhizopogon evadens* (N, U)	Yes	LD[2]	Agerer, 2006
FJ845432	FJ845432	Old	*Russula decolorans* (N, U)	No	C	Fransson, 2004
JQ711959	FJ845432	Old	*Russula decolorans* (N, U)	Yes	C	Fransson, 2004
FJ845432	FJ845432	Mid	*Russula decolorans* (N, U)	No	C	Fransson, 2004
FJ845432	FJ845432	Mid	*Russula decolorans* (N, U)	No	C	Fransson, 2004
FJ845432	FJ845432	Mid	*Russula decolorans* (N, U)	No	C	Fransson, 2004
FJ845432	FJ845432	Old	*Russula decolorans* (N, U)	No	C	Fransson, 2004
FJ845432	FJ845432	Old	*Russula decolorans* (N, U)	No	C	Fransson, 2004
FJ845432	FJ845432	Old	*Russula decolorans* (N, U)	No	C	Fransson, 2004
FJ845432	FJ845432	Old	*Russula decolorans* (N, U)	No	C	Fransson, 2004
FJ845432	FJ845432	Old	*Russula decolorans* (N, U)	No	C	Fransson, 2004
FJ845432	FJ845432	Old	*Russula decolorans* (N, U)	No	C	Fransson, 2004
KX812842	UDB022486	Old	*Russula delica* (N, U)	No	MD smooth	Beenken, 2001
KX812842	UDB022486	Old	*Russula delica* (N, U)	Yes	MD smooth	Beenken, 2001

NCBI Accession number	Unite Accession Number	Age	Organism	Emanating elements (observed)	Emanating elements (Literature)*	Source:
KX812842	UDB022486	Old	*Russula delica* (N, U)	Yes	MD smooth	Beenken, 2001
KX812842	UDB022486	Old	*Russula delica* (N, U)	No	MD smooth	Beenken, 2001
KX812842	UDB022486	Mid	*Russula delica* (N, U)	No	MD smooth	Beenken, 2001
KX812842	UDB022486	Old	*Russula delica* (N, U)	No	MD smooth	Beenken, 2001
KX812842	UDB022486	Old	*Russula delica* (N, U)	Yes	MD smooth	Beenken, 2001
KX812842	UDB022486	Old	*Russula delica* (N, U)	Yes	MD smooth	Beenken, 2001
KX812842	UDB022486	Old	*Russula delica* (N, U)	Yes	MD smooth	Beenken, 2001
KX812842	UDB022486	Old	*Russula delica* (N, U)	Yes	MD smooth	Beenken, 2001
KX812842	UDB022486	Old	*Russula delica* (N, U)	No	MD smooth	Beenken, 2001
KX812842	UDB022486	Old	*Russula delica* (N, U)	No	MD smooth	Beenken, 2001
FJ803979	KP783457	Mid	*Russula nigricans* (U)	Yes	MD smooth	Mleczko, 2004
EU222981	UDB024867	Mid	*Sarcodon calvatus* (U)	No	MD mat[2]	Agerer, 2006
KF618041	KF618041	Mid	*Sistotrema* (U)	No	D[3]	Di Marino et al., 2008
FJ845440	FJ845440	Sap	*Suillus brevipes* (N, U)	Yes	LD[2]	Agerer, 2006
FJ845440	FJ845440	Sap	*Suillus brevipes* (N, U)	Yes	LD[2]	Agerer, 2006
FJ554247	FJ845440	Sap	*Suillus brevipes* (U)	Yes	LD[2]	Agerer, 2006
FJ845440	FJ845440	Sap	*Suillus brevipes* (N, U)	Yes	LD[2]	Agerer, 2006
FJ845440	FJ845440	Sap	*Suillus brevipes* (N, U)	Yes	LD[2]	Agerer, 2006
FJ845440	FJ845440	Sap	*Suillus brevipes* (N, U)	Yes	LD[2]	Agerer, 2006
FJ845440	FJ845440	Sap	*Suillus brevipes* (N, U)	Yes	LD[2]	Agerer, 2006
FJ845440	FJ845440	Sap	*Suillus brevipes* (N, U)	No	LD[2]	Agerer, 2006
FJ845440	FJ845440	Mid	*Suillus brevipes* (N, U)	Yes	LD[2]	Agerer, 2006
KU721226	KU721226	Mid	*Suillus glandulosipes* (N, U)	Yes	LD[2]	Agerer, 2006
HQ257500	JF899573	Mid	*Suillus granulatus* (U)	Yes	LD	Agerer, 2006
FJ554247	JQ711923	Sap	*Suillus luteus* (U)	Yes	LD	Agerer, 2006
JQ711923	JQ711923	Mid	*Suillus luteus* (N, U)	Yes	LD	Agerer, 2006
JQ711950	JQ711950	Mid	*Suillus* (N, U)	Yes	LD[3]	Agerer, 2006
JN021100	JN021100	Mid	*Suillus tomentosus* (N, U)	No	LD[2]	Agerer, 2006
JQ711926	JQ711926	Sap	*Suillus variegatus* (N, U)	Yes	LD	Agerer, 2006
JF304371	JF304371	Old	Thelephoraceae (U)	No		
JQ711813	JQ711813	Mid	*Tomentella* (N, U)	No	C, SD,MD[3]	Agerer, 2006
AF458449	AF458449	Mid	*Tricholoma flavovirens* (N); *Tricholoma equestre* (U)	Yes	MD fringe	Agerer, 2006
JF899574	JF899574	Mid	*Tricholoma flavovirens* (N); *Tricholoma equestre* (U)	Yes	MD fringe	Agerer, 2006
MK607499	UDB037433	Old	*Tricholoma portentosum* (N, U)	No	MD fringe[2]	Agerer, 2006
MK607499	UDB037433	Old	*Tricholoma portentosum* (N, U)	No	MD fringe[2]	Agerer, 2006
KF617336	KF617336	Sap	Trichophaea (U)	No		
KF617336	KF617336	Mid	Trichophaea (U)	No		
MK131601	KP814142	Old	*Tylospora* (N, U)	No	SD	Agerer, 2006
HQ285379	HQ285379	Mid	Uncultured *Cortinarius* (N, U)	Yes	MD fringe[3]	Agerer, 2006
KY430553	KY430553	Mid	Uncultured *Malassezia* (N, U)	No		
KU727188	KU727188	Old	Uncultured *Phialocephala* (N, U)	Yes	EcM morphology unclear/undefined[1]	Currah et al., 2008; Grünig et al., 2008
FJ554452	FJ554452	Mid	Uncultured *Russula* (N, U)	No	C, SD,MD[3]	Agerer, 2006
EU711731	KP781018	Mid	Uncultured *Russula* (N, U)	No	C, SD,MD[3]	Agerer, 2006
JX003292	KP781018	Mid	Uncultured *Russula* (U)	No	C, SD,MD[3]	Agerer, 2006
KP781018	KP781018	Mid	Uncultured *Russula* (N, U)	No	C, SD,MD[3]	Agerer, 2006

NCBI Accession number	Unite Accession Number	Age	Organism	Emanating elements (observed)	Emanating elements (Literature)*	Source:
DQ069001	JQ975970	Old	*Wilcoxina rehmii* (N, U)	No	C	Rudawska et al., 2011
JQ975970	JQ975970	Mid	*Wilcoxina rehmii* (U)	Yes	C	Rudawska et al., 2011
JQ975970	JQ975970	Old	*Wilcoxina rehmii* (U)	No	C	Rudawska et al., 2011
[1]Dark septate fungi, but can also form ectomycorrhizas. Ectomycorrhizal exploration type is not described.						
[2]No exploration type assignment at the species level. Generalization based on described species within the same genus. Therefore, actual exploration type could differ for this particular species.						
[3]Only identified to genus. Generalization based on described species within the same genus. Therefore, actual exploration type could differ for this particular species.						
* C - contact exploration type, SD - short-distance exploration type, MD - medium-distance exploration type (smooth, mat and fringe subtypes), LD - long-distance exploration						
(N) - indicates that an organism description was obtained from the NCBI database						
(U) - indicates that an organism description was obtained from the UNITE database						
Age classes: Seedling 2–5 years, Sapling: 12–16 years, Mid: 30–36 years, Old: 65–76 years						

Appendix 2 – Results of statistical analyses

Table S5. Analysis of variance (ANOVA) for β regression coefficients describing cumulative root fraction of *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of stand age class (n = 3). Seedling: 2–5 years; Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. Non-linear regression was used to estimate β values for each site, with the formula: $Y = 1 - \beta^D$, where Y is cumulative root fraction and D is soil depth increment.

	Sum of Squares	DF	F-value	*p*-value
Age Class	0.57	3	31	$9.9e^{-5}$

Table S6. Linear mixed effects model of root area index (RAI) with random effect of soil core nested within site for *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of stand age class (n = 3) and soil depth increment. Seedling: 2–5 years; Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. Roots were sampled in two depth increments, 0–30 cm and 30–90 cm. Values computed using Wald's Type III Chi-square (χ^2) tests.

	DF	χ^2	*p*-value
Age Class	3	186	$2.2e^{-16}$
Soil Depth Increment	1	13	$4.1e^{-4}$
Age Class × Depth Increment	3	18	$5.4e^{-4}$

Table S7. Generalized least squares linear model of leaf area index (LAI) of *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of stand age class (n = 3). Seedling: 2–5 years; Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. The statistical model included a term for within-group heteroscedasticity structure.

	DF (numerator)	DF (denominator)	F-value	*p*-value
Age Class	3	8	48	$2.2e^{-16}$

Table S8. Generalized least squares linear model to compare ratio of LAI:RAI of *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of stand age class (n = 3). Seedling: 2–5 years; Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. The statistical model included a term for within-group heteroscedasticity structure.

	DF (numerator)	DF (denominator)	F-value	*p*-value
Age Class	3	8	0.05	>0.9

Table S9. Analysis of variance (ANOVA) to compare aboveground biomass increment (kg ha^{-1} $year^{-1}$) of *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of stand age class (n = 3). Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years.

	Sum of Squares	DF	F-value	*p*-value
Age Class	$2.4e^{6}$	2	3.5	0.1

Table S10. Generalized least squares linear model to compare biomass growth efficiency (kg y^{-1} m^{-2} leaf area) of *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of stand age class (n = 3). Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. The statistical model included a term for within-group heteroscedasticity structure.

	DF (numerator)	DF (denominator)	F-value	*p*-value
Age Class	2	6	137	$2.2e^{-16}$

Table S11. Analysis of variance (ANOVA) to compare leaf area to biomass (m^2 kg^{-1}) ratio of *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of stand age class (n = 3). Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years.

	Sum of Squares	DF	F-value	*p*-value
Age Class	0.14	2	129	$1.2e^{-5}$

Table S12. Analysis of variance (ANOVA) to compare fine root area to biomass (m^2 kg^{-1}) ratio of *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of stand age class (n = 3). Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years.

	Sum of Squares	DF	F-value	*p*-value
Age Class	0.26	2	3.7	0.09

Table S13. Negative binomial regression of number of Distance mycorrhizas in *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of stand age class (n = 3) and soil depth. Seedling: 2–5 years; Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. Roots were sampled in two depth increments, 0–30 cm and 30–90 cm. Model included a random effects term for soil core nested within site. Values in ANOVA table computed using Wald's Type III Chi-square (χ^2) tests. Pairwise comparisons showing results of Tukey HSD test between age classes at each soil depth.

ANOVA table

	DF	χ^2	*p*-value
Age Class	3	27	$5.2e^{-6}$
Soil Depth Increment	1	11	$1.1e^{-3}$
Age Class × Depth Increment	3	11	$1.1e^{-2}$

Pairwise Comparisons

Upper Soil	Rate Ratio (SE)	DF	t-ratio	*p*-value
Seedling – Sapling	0.146 (0.1)	108	2.7	0.04
Seedling – Mid Age	0.074 (0.05)	108	3.8	0.002
Seedling – Old growth	0.111 (0.08)	108	3.2	0.01
Sapling – Mid Age	0.50 (0.3)	108	1.4	0.5
Sapling – Old growth	0.76 (0.4)	108	0.5	>0.9
Mid age – Old growth	1.50 (0.7)	108	0.8	0.8

Lower Soil	Rate Ratio (SE)	DF	t-ratio	*p*-value
Seedling – Sapling	0.079 (0.09)	108	2.1	0.2
Seedling – Mid Age	0.010 (0.01)	108	4.0	$8.0e^{-4}$
Seedling – Old growth	0.012 (0.01)	108	3.8	0.001
Sapling – Mid Age	0.130 (0.08)	108	3.3	0.008
Sapling – Old growth	0.157 (0.1)	108	2.9	0.02
Mid age – Old growth	1.21 (0.6)	108	0.4	>0.9

Table S14. Negative binomial regression of number of Distance mycorrhizas in *Pinus banksiana* stands in northeastern, Alberta, Canada as a function of LAI. Model included a random effects term for soil core nested within site. Values computed using Wald's Type III Chi-square (χ^2) tests.

	DF	χ^2	*p*-value
Age Class	1	2.0	0.2

Table S15. Beta-binomial logistic regression for the proportion of Distance relative to Contact mycorrhizas colonizing *Pinus banksiana* roots from stands in northeastern, Alberta, Canada as a function of stand age class (n = 3) and soil depth. Sapling: 12–16 years; Mid age: 30–36 years; Old growth: 65–76 years. Roots were sampled in two depth increments, 0–30 cm and 30–90 cm. Model included a random effects term for soil core nested within site. Values in ANOVA table computed using Wald's Type III Chi-square (χ^2) tests. Pairwise comparisons showing results of Tukey HSD test between age classes at each soil depth.

ANOVA table

	DF	χ^2	*p*-value
Age Class	2	4.3	0.1
Soil Depth Increment	1	5.6	0.01
Age Class × Depth Increment	2	4.0	0.1

Pairwise Comparisons

Upper Soil	Odds Ratio (SE)	DF	t-ratio	*p*-value
Sapling – Mid Age	0.50 (0.3)	75	1.1	0.5
Sapling – Old growth	0.90 (0.5)	75	0.2	>0.9
Mid age – Old growth	1.80 (1.0)	75	1.0	0.6

Lower Soil	Odds Ratio (SE)	DF	t-ratio	*p*-value
Sapling – Mid Age	0.19 (0.1)	75	2.4	0.04
Sapling – Old growth	0.29 (0.2)	75	1.8	0.2
Mid age – Old growth	1.5 (0.9)	75	0.7	0.8

Table S16. Beta-binomial logistic regression of the proportion of Distance relative to Contact mycorrhizas colonizing *Pinus banksiana* roots as a function of root density (cm^2 cm^{-3} soil). Model included a random effects term for soil core nested within site. Values in ANOVA table computed using Wald's Type III Chi-square (χ^2) tests.

	DF	χ^2	*p*-value
Age Class	1	1.1	0.3

Table S17. Beta-binomial logistic regression of the proportion of Distance relative to Contact mycorrhizas colonizing *Pinus banksiana* roots as a function of leaf area index (m^2 m^{-2}). Model included a random effects term for soil core nested within site. Values in ANOVA table computed using Wald's Type III Chi-square (χ^2) tests.

	DF	χ^2	*p*-value
Age Class	1	1.2	0.3

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