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Contrasting Root System Structure and Belowground Interactions between Black Spruce (*Picea mariana* (Mill.) B.S.P) and Trembling Aspen (*Populus tremuloides* Michx) in Boreal Mixedwoods of Eastern Canada

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Abstract: This study explored the underground interactions between black spruce and trembling aspen in pure and mixed stands to understand how their soil resource use help these species coexist in the boreal mixedwoods of Western Quebec. We analyzed species-specific fine root foraging strategies (root biomass and root tissue density) along three soil layers (organic, top 0–15 cm, and bottom 15–30 cm mineral soil), using 180 soil cores. We collected cores in three sites, each containing three 20×50 m^2 plots of pure spruce, pure aspen, and mixed spruce and aspen stands. Spruce had a shallow rooting, whereas aspen had a deep rooting in both types of stands. Compared to pure spruce stands, spruce had a lower fine root biomass (FRB) and a higher root tissue density (RTD) in the organic layer of mixed stands. Both patterns were indicative of spruce's more intensive resource use strategy and competitive advantage over aspen in that layer. Aspen FRB in the organic soil did not differ significantly between pure and mixed stands, but increased in the mineral soil of mixed stands. Since we did not observe a significant difference in the nutrient content of the mineral soil layer between pure aspen and mixed stands, we concluded that aspen may experience competitive exclusion in the organic layer by spruce. Aspen exhibited an extensive nutrient uptake strategy in the organic layer of mixed stands: higher FRB and lower RTD than spruce. In mixed stands, the differences in aspen rooting patterns between the organic and mineral layers suggested the use of contrasting nutrient uptake strategies along the soil profile. We speculate that the stronger spatial separation of the roots of spruce and aspen in mixed stands likely contribute to a higher partitioning of their nutrient uptake along the soil profile. These results indicate the competitive exclusion of aspen by spruce in boreal mixedwoods, which likely occurs in the soil organic layer.

Keywords: diversity-productivity relationships; nutrient uptake strategy; fine root biomass; root tissue density; stand compositional diversity

1. Introduction

Understanding the relationship between diversity and productivity in forest ecosystems is critical to quantifying ecosystem responses to past and future environmental variability. A consistent and positive relationship between these two variables has been demonstrated on the global scale [1–3] in temperate and tropical forests [4–7]. Forest stands with high structural and species richness support a greater diversity of species (understory vascular plants, birds, insects, and mammals) and functions [8,9], and have greater productivity [10–13]. More diverse stands have been shown to exhibit a higher resilience to environmental variability [14–16]. On the other hand, a few studies have highlighted an insignificant or even negative effect of species diversity on forest productivity [17,18] and tree growth [14]. Earlier studies of biodiversity–productivity relationships (BPR) in boreal mixedwoods focused primarily on the effects of variability in climate [14,19] and light conditions [17]. Evaluating variability in tree diameter and volume growth, these studies indicated the potential role of competition, facilitation, and niche complementarity in shaping tree productivity [12,20,21]. Apart from theoretical interest, a mechanistic model explaining BPR in the boreal forest could be of critical importance in developing silvicultural practices in boreal mixedwoods.

Black spruce and trembling aspen are two of the most dominant and economically-valuable species in the boreal forests of eastern Canada [22,23]. These species have broadly different habitat requirements and successional trajectories, and are hypothesized to temporally coexist in the stands in the absence of fire [24] because of the competitive exclusion of aspen by spruce over time. These ecological considerations, the absence of a general trend concerning BPR [20,25], and the concern of operationalizing tree harvest in mixed stands of the two species have led forest managers to opt for monosilvicultural practices (clearcutting on ~85% of the total harvested area in Canada [26]). The growing evidence of a positive species mixing effect on stand productivity [12] and resilience to major disturbances, such as fire [27] and insect outbreaks [28,29], including the projected northward shift in the range of distribution of aspen with future climate conditions [30,31], calls for the consideration of silvicultural practices focused on mixed stands.

Aspen (TA) and spruce (BS) can modify their environment through the impact of their litter and root system on soil chemistry [10]. As compared to pure spruce stands, mixed stands of spruce and aspen have higher soil fertility, due to increases in soil temperature, humidity, nutrient content, and pH. In turn, this leads to a positive relationship between aspen abundance in mixed stands and the total stand productivity [25], despite a negative effect of spruce on aspen growth [20]. The accumulation of spruce litter and reduced rates of its decomposition in mixed stands of spruce and aspen compared to pure aspen stands [10] could be among the factors behind such a negative effect on aspen growth. The use of soil nutrients appears to be the central element in species interactions affecting growth rates, species geographic distribution, and nutrient cycling within forest stands. However, the contribution of underground interactions has seldom been quantified, and their effects on productivity remain to be demonstrated.

Underground interactions between tree species occur through the effect on the availability of resources, chemical signaling, and the secretion of allelochemical compounds [32]. These can either be positive, neutral, or negative [33]. Positive interactions exhibit themselves when one species has a positive effect on other species by increasing the availability of soil nutrients [34,35], whereas negative interactions occur through resource depletion, allelopathy, or interference competition [36–38]. These mechanisms can simultaneously occur in the same plant community [39]. Therefore, there is a need to consider resource competition and the physiological response of plants to competition in order to better understand the underground interactions between species in mixed forest stands.

Fine roots are of immediate importance for tree nutrient uptake [40], and their biomass, tissue density, nutrient content, and distribution along the soil profile have been viewed as proxies for nutrient uptake strategies [41–45]. The filling of soil volume by fine roots (hereafter referred to as *fine root biomass*) has been shown to reflect nutrient availability [44,46], and has an impact on tree aboveground biomass production [47], ultimately determining tree competitive ability for resources [48,49]. On

the other hand, the root tissue density (RTD, the amount of structural material invested by the unit volume of roots) is related to the availability of nutrients, and reflects the response of species to competition for resources [50]. Root biomass and tissue density are, therefore, essential metrics which define species' underground niche, in terms of its resource use [42,48].

We tested for differences in root foraging strategies between black spruce and trembling aspen, by analyzing their fine root biomass and tissue density along the soil profile in pure and mixed stands. We tested the general hypothesis that species coexistence in ecosystems is maintained through differences in resource use [51]. More specifically, we first hypothesized (H1) that the two species have different rooting depth (shallow for spruce and deep for aspen) in pure stands. Second, based on the concept of "functional equilibrium" [52], we hypothesized (H2) that the fine root biomass of spruce will decrease in mixed stands, and that (H3) the fine root biomass of aspen will increase in mixed stands. The functional equilibrium hypothesis assumes that in ecosystems subject to nutrient stress, plants will allocate more biomass towards the roots to enhance the uptake of resources when nutrients availability becomes limiting, and contrarily when soil fertility increases. Since the nutrient content increases mostly in the organic layer of mixed stands compared to pure spruce stands [10], we hypothesized that (H4) the spruce would exhibit lower FRB and higher root tissue density, and that the opposite pattern would be observed in aspen in the organic layer of mixed stands, compared to pure spruce and pure aspen stands. We expected, therefore, an intensive nutrient uptake strategy for spruce and extensive strategy for aspen, which are characterized by generally low and high carbon investments in root production, respectively [50,53–55]. Finally, we hypothesized that (H5) the increasing aspen FRB in mixed stands compared to pure aspen stands would lead to a stronger spatial separation of spruce and aspen roots (shallower for spruce and deeper for aspen), and consequently, contrasting nutrient uptake strategies in different soil horizons. Knowledge about such a pattern will help guide future forest management decisions in the boreal zone of Quebec.

2. Materials and Methods

2.1. Study Area

The study area was located in the black spruce-feathermoss bioclimatic domain in north-western Quebec (49°08′ N to 49°11′ N and 78°46′ W to 78°53′ W). The boreal forest in the area is dominated by black spruce, which grows on deep clay soils developed from proglacial deposits of Barlow and Ojibway lakes at the time of their maximum expansion during the Wisconsonian glaciation [56]. The mean annual temperature and total annual precipitation of the study area are 1.5 °C and 675.7 mm, respectively [57]. Fires and insect outbreaks are the primary factors of natural forest dynamics in the area. Selected stands were growing on moderately dry, clay-dominated soil [58], and originated from the same fire that occurred in the area in ca. 1916 [10].

The studied sites (three in total) were between 2 and 15 km away from each other (Figure 1). We established three 20 × 50 m² (0.1 ha) rectangular samples plots in each site, whereby each represented a stand which was uniform in terms of topography and vegetation. These comprised one pure (monodominant) stand of trembling aspen, one pure black spruce, and one mixed stand (Figure 1).
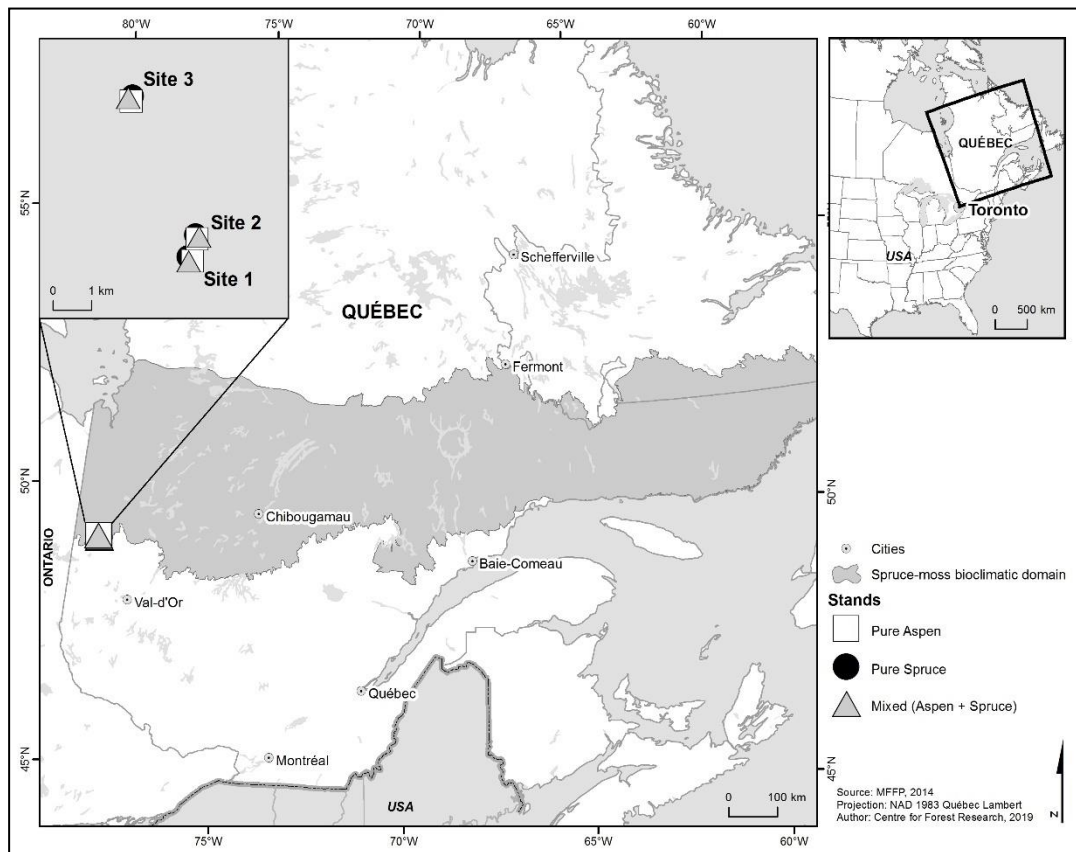
Figure 1. Location of the study sites in the spruce feathermoss bioclimatic domain of Quebec.

The selection criteria for stand types were similar to those of the previous studies investigating tree species' mixing effects in natural forest stands, i.e., pure stands had more than 80% of the stand basal area made up by a single species, whereas in mixed stands, none of the species had a basal area beyond 80% of the total stand basal area [47,59]. Our selection criteria also followed the definition for pure stands in the Quebec forest resource inventory. There, a single conifer species should contribute to more than 75% of stand basal area for the stand to be classified as *pure*, while in mixed stands, 25% to 50% stand basal area should be composed of coniferous trees [60]. To validate the attribution of stand type, we recorded diameter at breast height (DBH), i.e., at 1.3 m, for all trees above 10 cm, and calculated the basal area (BA) and density of each species (Table 1).

Selected stands had an overstory dominated by healthy aspen or/and spruce, the same age of the dominant canopy cohorts (100 years; [10]), low proportions of jack pine (*Pinus banksiana* Lamb.) in the canopy (<15%) and balsam fir (*Abies balsamea* (L.) Mill.) in the understory (field observation), and low abundance of *Sphagna* on the forest floor. These stand properties have been reported to influence the biomass of fine roots [59,61] and nutrient partitioning [62] in boreal forests. The selected plots were at least 150 m away from any other sampled point in a given site (Figure 1). We selected mixed stands within a transition zone of two pure stands of BS and TA. The 150-m distance between the two stands was designed to avoid border effects. To this end, we kept in mind the results of an earlier study, where the effect of aspen on the soil's physical and chemical properties was reported to persist within a distance of 7 m from a trembling aspen-dominated stand [10].

Table 1. Characteristics of sampled stands. BS, TA, and M refer to pure spruce, pure aspen, and mixed stands, respectively.

| | | Site 1 | | | Site2 | | | Site 3 | | |
		BS	TA	M	BS	TA	M	BS	TA	M
Species proportion (% number of stems)	*Populus tremuloides* Michx.	2	60	34	2	70	38	2	74	43
	Picea mariana Mill.	83	33	50	89	22	51	97	20	54
	Abies balsamea (L.) Mill.		6	3		2			6	1
	Pinus banksiana Lamb.	15	1	10	9	5	11	1		1
	Betula papyrifera Marshall			1						1
	Populus balsamifera L.			2		1				
Density (stems ha^{-1})		3100	1410	1160	3000	1220	1610	2382	900	1680
Organic soil layer properties	pH	3.8	4.0	4.9	4.2	5.0	4.7	3.6	5.01	4.4
	Total C (g kg^{-1})	311	194.2	301.2	337.7	321.5	321.2	376.2	287.2	348.7
	Total N (g kg^{-1})	7.8	10.5	13.7	10.67	11.5	11.8	7.7	17.3	11
	Mineral N (mg kg^{-1})	29.2	31.7	27.5	34.2	43.5	42.0	22.5	72.0	37.2
	C:N ratio	39.9	18.3	21.8	31.6	26.6	27.1	48.8	16.6	31.7
	P (mg kg^{-1})	81.5	36.2	59.7	69.5	79.0	61.5	129.7	90.5	161.2
	K (mg kg^{-1})	407	331	444.7	552.2	585.7	480.7	460.7	524.2	635.7
Basal area (BA, m^2 ha^{-1})		61.42	113.46	61.63	66.6	91.6	78.55	65.48	78.07	74.53
Basal area percentage per species (%)	*Populus tremuloides*	3.32	81.93	58.82	5.50	87.05	63.52	2.86	90.47	68.93
	Picea mariana	72.9	16.11	26.83	80.00	7.50	24.26	96.02	8.47	29.14
	Abies balsamea		0.94	0.78		0.27			1.06	0.20
	Pinus banksiana	23.78	1.02	12.21	14.50	4.35	12.22	1.12		0.78
	Betula papyrifera			0.46						0.25
	Populus balsamifera			0.90		0.83				0.70
Organic layer depth (cm)		17.3	6.2	10.16	20.0	7.5	11.5	14.2	9.76	13

C, N, P, and K in soil properties denote carbon, nitrogen, phosphorus, and potassium, respectively.

2.2. Sample Collection and Preparation

At each plot, 20 soil cores were extracted using a 7-cm diameter manual auger every 10 m along four 50 m long transects, spaced 4 m apart from each other. The sampling took place between July 15 and August 20, 2016. The chosen timing broadly corresponded to the period when fine roots are at their maximum length in this part of the boreal forest [47]. We collected samples from the organic horizon

(F and H layer combined) and from the top 0–15 cm (later referred to as *Min1*) and bottom 15–30 cm (*Min2*) of the mineral soil. The samples were placed in plastic bags, transported to the laboratory in an ice-filled cooler, and stored at a temperature of −20 °C prior to analyses.

The 180 soil cores were pooled per transect and for each soil horizon to form a set of 12 composite samples for each plot. The composite samples were allowed to thaw over six hours and then soaked in water for at least one hour. The mixture was then stirred carefully to separate roots from the soil particles and poured through a 750 μm mesh round sieve. The sieve was suspended under a distilled water bath and shaken continuously, until the roots were free from any soil particle. The roots were collected and live and dead roots were separated, based on visual appearance and a manual extension test [47]. Live roots were light in color and resistant to breakage, whereas dead roots were a grey to dark color and were easily fragmented. We discarded dead roots from further analyses. Live fine roots were sorted by species, using a combination of morphological characteristics established from prior root reference samples collected on known younger and older trees of each studied species in the study stands. The morphological criteria used were color, size, ramification, and presence/absence of root hairs [47]. Aspen roots were less ramified, with colors ranging between white and pale, whereas the roots of coniferous trees were ramified, finer in structure, and of a color ranging between red and brown. Compared to other coniferous trees, spruce roots were more reddish in color with a black bark. Balsam fir (*Abies balsamea* (L.) Mill.) had roots with bark color ranging from yellowish to orange, whereas jack pine had roots with a texture similar to spruce but with a lighter, brown bark color. The presence of root hairs was mostly used to separate nontree roots (commonly with root hairs) from tree roots. We extracted only the roots of spruce and aspen based on these criteria. We discarded roots larger than 2 mm in diameter, measuring them with a caliper. The separated fine roots were gently dried between two filter papers at room temperature to remove water, and were immediately weighed to obtain the fine roots' fresh mass. A representative fresh root subsample per soil layer was subsequently taken per transect in each stand, in such a way that their weight would be comparable among transects [63]. Following this protocol, we took two grams of fresh live roots in the FF and in the top *Min1* soil section, whereas only 0.5 g was taken in the lower *Min2*, where fine root weights in the samples were the lowest. The composite samples were oven-dried at 60 °C for 48 h and reweighed to obtain the root dry mass.

2.3. Chemical Analysis

An aliquot (50 g) was taken from each soil sample taken per transect and air-dried to a constant weight for a week [64], ground, and sieved (<2 mm). For each soil horizon layer, soils were extracted with 2M KCl (10:1 solution soil ratio, 30 min). Nitrate (NO_3) and ammonium (NH_4) concentrations were measured, respectively, by ion chromatography (Dionex2120i, Thermo Fisher Scientific Inc., Sunnyvale, CA, USA) and colorimetrically with a Technicon Autoanalyzer (Technicon AA2, Seal Analytical Inc., Mequon, WI, USA). Since the observed nitrate concentrations were close to the detection limit (limit of <1 mg kg^{-1} for extractable NO_3), we only report NH_4 results. Total C and N concentrations were measured with an elemental analyzer (Carlo Erba, Milan, Italy). The bulk soil pH in water was analyzed following the method described by Carter and Gregorich [64] using a Thermo Scientific Orion 2 pH meter (Thermo Fisher Scientific, Waltham, MA, USA). Exchangeable cations and phosphorus (P) were measured by extraction in Mehlich-3 solution [65], and were further summed to estimate the effective cation exchange capacity (CEC). A summary of these soil chemical properties is provided in the Supplementary Materials Table S1. Chemical analyses were conducted at the laboratory of organic and inorganic chemistry of the Ministry of Forests, Wildlife and Parks of Quebec.

2.4. Data Analysis

2.4.1. Metrics Calculation

Four metrics were used to test the hypotheses. The fine root biomass (FRB) was calculated as the dry mass of living fine roots per volume of soil (kg·m^{-3}) in each sample. The vertical heterogeneity index (VHI) was assessed as the standard deviation of fine roots biomass percentages in each of the three soil horizon layers over transect. VHI indicates how fully and evenly fine roots occupy the belowground space [66]. The mixture effect ratio (Ro) was calculated as the ratio between the observed biomass (B$_o$) to the expected biomass (B$_{exp}$) of fine roots in mixtures. B$_{exp}$ was calculated by multiplying the fine root biomass of each species in pure stands by their respective basal area proportion in mixed stands [67]. Finally, the root dry matter content (RDMC) was derived as the ratio between root dry mass and root fresh mass, in g·g^{-1}. This metric is used as a proxy of root tissue density (RTD, [63]), and represents the amount of structural material invested by unit volume of roots. RDMC is an easily measurable and less susceptible to errors metric, as compared to metrics based on image analysis [63]. A potential increase in the volume of data for roots may advance our understanding of underground interactions between plant species [68,69].

2.4.2. Statistical Analysis

We used several approaches to test our hypotheses. First, we used (1) a repeated measures analysis of variance (repeated measures ANOVA) to analyze differences in species-specific FRB, and a Kruskal-Wallis rank test to analyze differences in species-specific RDMC. We ran layer-wise comparisons between pure and mixed stands, and comparisons of soil layers within mixed stands. We regarded data collected on the transects as "multiples samples" (repeated measures). The ANOVA consisted of a mixed model with FRB as the response variable. Stand types and transects were fixed-effect factors, and the site identity was the random factor. The normality and the homoscedasticity assumptions were met for FRB data. In contrast, for the RDMC, we performed the Kruskal-Wallis and Dunn tests using R package *dunn.test*, since the normality and the homoscedasticity was not met, even after log10 transformation. Second, we assessed the spatial heterogeneity of fine root distribution by comparing species-specific roots VHI between pure and mixed stands (H1). Lower VHI values are indicative of a more even distribution of fine roots with soil depth [47]. Third, we tested for the effect of species mixing on the total fine root biomass within each soil horizon layer by comparing computed Ro values with the threshold value 1 (H2 and H3). Values of Ro greater than one indicate a positive effect of mixture on fine root biomass, while values below one indicate a negative effect [67]. The Ro value helped, therefore, to assess the effect of mixing upon tree rooting patterns. Fourth, to test hypotheses H4 and H5, we performed a principal component analysis (PCA) on the species rooting attributes (FRB and RDMC) and soil chemical properties of the three soil layers over transect to characterize species-specific rooting strategies in pure and mixed stands [70]. The analyzed soil chemistry included C:N ratio, pH, carbon (C), total nitrogen (N$_{tot}$), ammonium (N$_m$), phosphorus (P), potassium (K), and exchangeable cation (CEC) content. Finally, we performed an analysis of covariance (ANCOVA) to compare the explanatory power of each nutrient on the variation of FRB and RDMC between stand types. The ANCOVA assessed the effect of stand types (pure vs. mixed) on variation of species FRB and RDMC while considering the variability of nutrient content from one stand type to another. The analysis was performed independently for each nutrient and soil layer, since the method considers only linear association between one response variable and one explanatory variable [71]. As a regression model, the ANCOVA compared the slopes (i.e., the mixed effect) and the intercept of the regression lines (i.e., the effect of stand type) between pure and mixed stands. The model allowed us to determine whether differences in species rooting between pure and mixed stands resulted from the species mixture effect on nutrient availability. Ultimately, the analysis allowed us to test whether the two species in mixed stands retain the same rooting and nutrient uptake strategy as in their respective pure stands.

3. Results

3.1. Fine Root Biomass

Spruce and aspen FRB showed a decreasing gradient with increasing soil depth (Figure 2a). Spruce FRB differed significantly between the organic and the top mineral layers in both pure spruce ($F = 34.91$, $p < 0.001$) and mixed ($F = 62.72$, $p < 0.001$) stands. As for spruce, aspen FRB was significantly different among the three soil layers in pure aspen ($F = 51.51$, $p < 0.001$) and mixed ($F = 36.9$, $p < 0.001$) stands.

The FRB of spruce decreased, while aspen FRB increased in mixed stands, compared to respective pure stands (Figure 2). The decreased spruce FRB and increased aspen FRB in mixed stands varied along soil profile (Figure 2b), and resulted from the species mixture effect (Figure 2d,e). Spruce FRB in the organic layer and in the top mineral layer was higher in pure spruce stands, and differed significantly from that of their corresponding soil layers in mixed stands (Figure 2a,c). Unlike spruce, aspen FRB in the organic and the bottom mineral layers did not differ between pure aspen and mixed stands, except in the top mineral layer (Figure 2c).

Figure 2. Differences in soil volume filling by fine roots of black spruce and trembling aspen in the three sampled soil horizon layers (a,c) between pure and mixed stands and (b) within mixed stands. Species mixture effect on fine roots biomass of (d) black spruce and (e) trembling aspen in different soil horizon layers. In (a–c), the box represents 50% of the data set, distributed between the 2nd and 3rd quartiles. The median divides the box into the interquartile range. The lower and upper whiskers represent the minimum and maximum quartiles. For each soil layer, the number of samples $n = 12$ (i.e., 3 sites × 4 transects). Null values indicate that no root biomass was recorded in the soil layer on the transect. In (b), points represent average Ro values ($n = 3$), bars the 95% confidence interval, and the red dotted line the threshold under or over which the effect is considered as negative or positive. Statistical differences among pairwise comparison at $p = 0.05$ within each soil layer are denoted by letters.

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Aspen roots were more evenly distributed along soil profile than spruce roots (Figure 3). Spruce root VHI did not differ significantly between pure and mixed stands ($t = 0.95$, $p > 0.05$). However, the VHI values of spruce roots exhibited lower variability in mixed than in pure stands. Like spruce, aspen root VHI did not show significant difference between pure aspen and mixed stands ($t = 0.17$, $p > 0.05$). The VHI values of aspen roots showed lower variability in pure stands, compared to mixed stands (Figure 3).
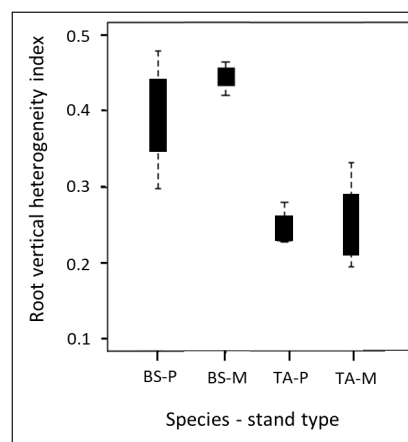

Figure 3. Fine roots vertical heterogeneity index describing how fully and evenly fine roots of black spruce (BS) and trembling aspen (TA) are distributed with soil depth in pure (P) and mixed (M) stands.

Overall, the PCA showed a shift in rooting patterns of spruce in mixed stands, compared to pure spruce stands, whereas aspen rooting did not differ between the two stand types (Figure 4).
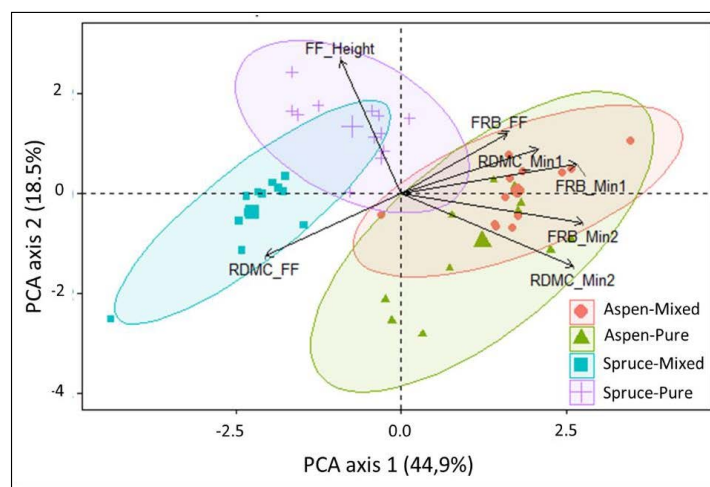

Figure 4. Principal component analysis of fine root biomass (FRB) and root dry matter content (RDMC) characterizing the rooting system of black spruce and trembling aspen in pure and mixed stands. The depth of organic horizon (FF_Height) was included in the analysis as a supplementary variable. FF, Min1, and Min2 refer to organic, mineral soil at 0–15 cm, and mineral soil at 15–30 cm, respectively.

At the species-specific level, the FRB of the two species was strongly related to the variation in soil chemical properties (Figure 5, Figure S1). In the organic and top mineral layers, spruce FRB correlation with pH, total N, C:N ratio, NH_4, and P was stronger in pure than in mixed stands (Figure 5a). These correlations were negative for pH, total N, NH_4, K, and CEC, and positive for P. Spruce FRB in mixed stands was mostly influenced by the nutrient content of the organic layer, whereas in
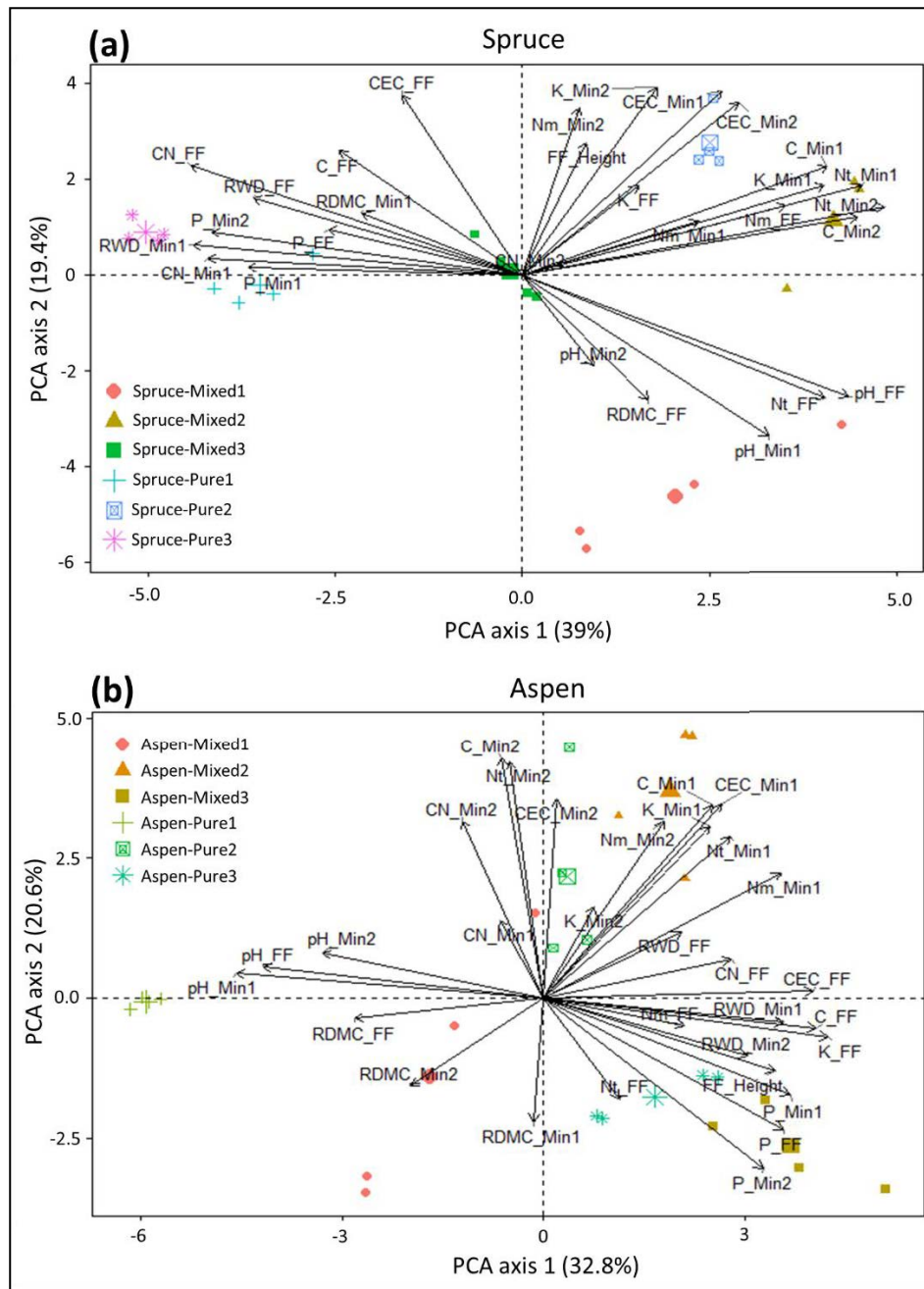
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Figure 5. Principal component analysis comparing rooting patterns of black spruce (**a**) and trembling aspen (**b**) between pure and mixed stands. Variables included in the analyses are C:N ratio, pH, total carbon (C), total nitrogen (Nt), Ammonium (Nm), phosphorus (P), potassium (K), exchangeable cations (CEC) and depth of the organic layer (FF height), fine root biomass (FRB), and root dry matter content (RDMC) of the two species in respective pure and mixed stands affected by site (1–3). FF, Min1, and Min2 refer to organic, mineral soil at 0–15 cm, and mineral soil at 15–30 cm, respectively.

Spruce FRB showed a stronger correlation with C, P, NH_4, K, and CEC in the pure than in the mixed stand (*p* for the effect of stand types <0.05; Table S2 and Figure S1). A similar pattern was observed for the effect of the depth of organic layer on spruce FRB between the two types of stands. In contrast, the pH and C:N ratio showed a stronger correlation with spruce FRB in mixed than in pure stands. The observed differences were, however, not the result of species mixture effect on the

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Aspen FRB was independent of nutrient content of the organic layer in pure and mixed stands, as suggested by PCA and ANCOVA (Figures 5b and Figure S1). However, aspen FRB in the top and bottom mineral layers was more strongly correlated to total N, NH$_4$, P, pH, K, and CEC in mixed than in pure stands (*p* for mixed effect <0.05; Table S3; Figure S1).

3.2. Root Dry Matter Content

The RDMC of spruce in mixed stands was significantly higher in the organic layer than in the top mineral (Figure 6a). However, in pure stands, spruce RDMC did not show a significant difference between the organic and the top mineral layers, despite the increasing trend with soil depth (Figure 6a). The RDMC of spruce in the organic layer was higher, with a greater variability in mixed than in pure stands (Figure 6a). In contrast, in the top mineral layer spruce RDMC was smaller in mixed than in pure stands (Figure 6a). No RDMC data was recorded for spruce roots in the 15–30 cm mineral layer of the soil, since spruce roots were limited in the organic and top 0–15 cm mineral layers.
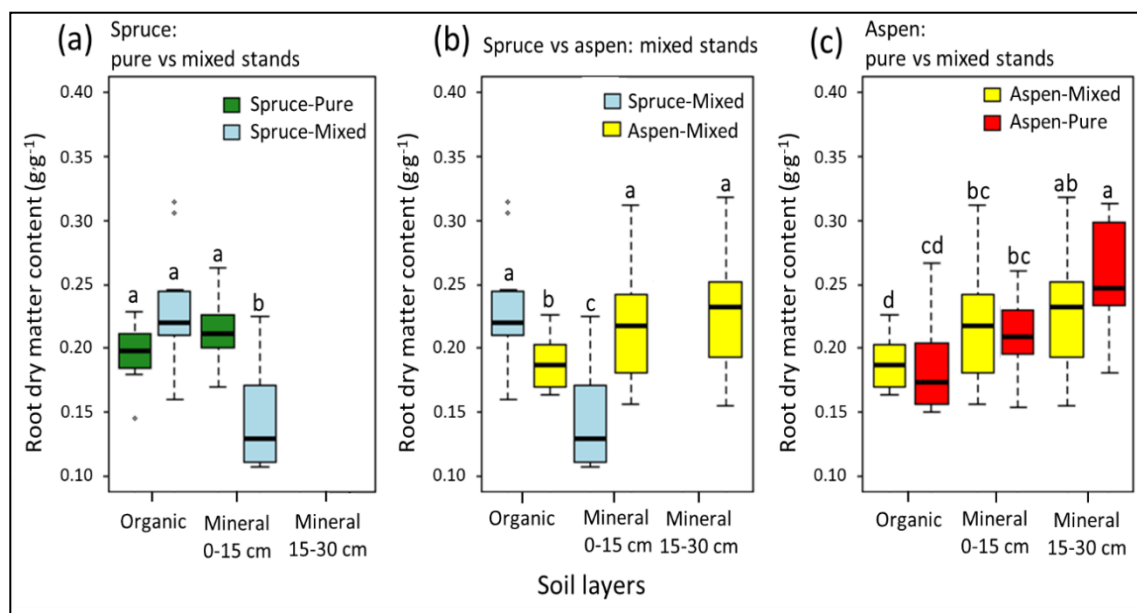

Figure 6. Differences in the root dry matter content (RDMC) of fine roots of black spruce and trembling aspen in the three sampled soil horizon layers (**a**,**c**) between pure and mixed stands, and (**b**) within trembling aspen in the three sampled soil horizon layers (a,c) between pure and mixed stands, and (b) within mixed stands. The box represents 50% of the data set, distributed between the 2nd and 3rd quartiles. The median divides the box into the interquartile range. The lower and upper whiskers represent the minimum and maximum quartiles. Statistical differences among pairwise comparison at *p* = 0.05 within each soil layer are denoted by letters.

In aspen, the RDMC increased with increasing soil depth (Figure 6c). Aspen RDMC was smaller in the organic layer than in the top mineral and bottom mineral layers in both pure and mixed stands (Figure 6c). There were, however, no significant differences in soil layer-specific RDMC between pure aspen and mixed stands (Figure 6c). Overall, spruce had a higher RDMC than aspen in the organic layer. By contrast, in the top mineral layer in mixed stands, spruce RDMC was lower than aspen RDMC (Figure 6b). Aspen RDMC was the highest in the bottom mineral layer (Figure 6b,c) and did not differ between pure and mixed stands (Figure 6c).

Spruce RDMC was correlated with the C:N ratio, total N, NH4, K, and pH. The effects of those nutrients on spruce RDMC varied with stand type and soil layer (Figure 5a; Table S2). The correlation between spruce RDMC was stronger for C, C:N ratio, and NH4 content in the organic layer in mixed

RDMC (Figure 6b). Aspen RDMC was the highest in the bottom mineral layer (Figure 6b,c) and did not differ between pure and mixed stands (Figure 6c).

Spruce RDMC was correlated with the C:N ratio, total N, NH4, K, and pH. The effects of those nutrients on spruce RDMC varied with stand type and soil layer (Figure 5a; Table S2). The correlation between spruce RDMC was stronger for C, C:N ratio, and NH4 content in the organic layer in mixed than in pure spruce stands. In contrast, in pure stands, those nutrients were strongly correlated with spruce RDMC in the top mineral horizon, as compared to mixed stands. Most of the nutrient concentrations did not differ significantly between pure aspen and mixed stands, which argued against the difference in the availability of soil nutrients as a driver of differences in aspen RDMC between the two types of stands (Table S3).

4. Discussion

Our results showed a shallow rooting for spruce and a deep rooting for aspen in pure stands (Figures 2 and 3), supporting hypothesis H1, i.e., that the two species have a contrasted rooting depth in their respective pure stands. We found a lower spruce fine root biomass in both the organic (23% decrease) and mineral layers (45% decrease) in mixed stands, compared to pure stands. The result supported the hypothesis that mixing decreases the spruce root biomass (H2). Although aspen root biomass in the organic and the bottom mineral layers did not differ significantly between pure and mixed stands, the 25% increase in FRB recorded in the top mineral layer of mixed stands indicated a positive effect of mixing on aspen root biomass, supporting hypothesis H3. For spruce, lower root biomass and higher RDMC were associated with shallower rooting in mixed than in pure stands. We speculate that the pattern was a response of spruce to increased nutrient availability in mixed, compared to pure, spruce stands, and was indicative of an intensive resource acquisition strategy, with a more efficient use of soil resources. The result, therefore, supported H4. By contrast, aspen had deeper rooting and exhibited a contrasting nutrient uptake strategy in the organic layer in both mixed and pure aspen stands, as compared to spruce. The similarity between the rooting pattern (fine root biomass and RDMC) of aspen in the top mineral soil with that of spruce in the organic layer pointed to the use of an intensive resource acquisition strategy by aspen in the mineral layer. This observation supported the hypothesis suggesting more contrasting rooting patterns along the soil profile with mixing of species (H5).

We found an increase of spruce FRB in pure stands in response to lower nutrient availability, and its decline in mixed stands as a result of increased nutrient availability compared to pure stands (Figure 5a). This indicated an anisotropic response of spruce root biomass production towards nutrient-rich patches of soil [41]. The higher spruce FRB in the organic and top mineral horizon in pure stands, compared to mixed stands (Figure 2a), suggested more stressful conditions for spruce in pure stands [73]. This situation may result from nitrogen limitation [74] and competition with mosses and ericaceous species [61,75]. Mosses and *Ericaceae* were more abundant in pure spruce stands, while they were only found in small patches in mixed stands (field observations). Both groups immobilize and cycle nutrients at the top of the organic layer, making them less accessible to trees [10,76]. In mixed stands, the higher root and litter turnover (short lifespan and high decomposition rate) of vascular plants [77] and aspen [47,73] maintain a higher fertility within the organic layer [10,78–81], thereby modulating interspecific competition among understory plants and trees. The higher turnover of aspen roots in the organic layer results mainly from their lower RDMC, common to deciduous species in temperate and boreal forests [80,82,83].

The shift in spruce rooting patterns between pure and mixed stands (Figures 4 and 5a) points to its plastic response to nutrient availability and competition (morphological plasticity, census Bradshaw [84]), which is indicative of the use of both intensive and extensive nutrient uptake strategies [53]. An intensive strategy is generally associated with low C cost for root production to the plant, and the reverse for the extensive strategy (higher C cost to the plant) [54,55]. The spruce intensive strategy prevails in mixed stands, and resulted in lower FRB (Figure 2a) and higher RDMC

(Figure 4) in the organic layer of mixed stands, as compared to pure stands. This indicates a reduced carbon allocation into root biomass [85,86]. In pure stands, the extensive strategy exhibited itself through higher spruce FRB, its strong and negative correlation with nutrient content (N, NH_4, K, CEC), and higher RDMC in both the organic and the top mineral layers (Figure 6) [42,48,49].

The decline in spruce root biomass in mixed stands might also indicate a higher competitive ability of aspen in these stands, as has been suggested for mixed Norway spruce (*Picea abies* (L.) H. Karst) and beech (*Fagus sylvatica* L.) stands in Europe [87]. The intersite variability observed in the rooting patterns of spruce in mixed stands (Figure 5a) might indicate that the interspecific competition may increase with a higher aspen basal area. However, the limited number of sites in our study did not allow us to test such a hypothesis. To do so, it would be necessary to increase the sampling size to ensure adequate coverage of a range of canopy compositions. We speculate that the negative impact of aspen on spruce nutrient uptake would be minimal, due to the facilitative effect of aspen on soil nutrient availability compared to multiple competitive mechanisms (intraspecific competition, interference with Ericaceae and nutrient immobilization by moss layer) which are at play in pure stands [88].

The lack of a strong correlation between aspen FRB, RDMC, and nutrients in the organic layer in both types of stands (Figure 5b and Figure S1) suggested a strong selective placement of aspen roots within soil patches, pointing to an extensive nutrient uptake strategy [89]. A similar lack of correlations has been reported for aspen in pure aspen and mixed jack pine-aspen stands [47], two deep-rooted boreal species.

Aspen responded to tree species mixture by a greater allocation of roots to the mineral horizon (Figure 2), apparently avoiding competition with spruce in the organic layer [90]. The increasing aspen FRB and its higher RDMC in the mineral horizon, as well as the similarity between these rooting patterns of spruce with those of spruce as observed in the organic layer of mixed stands, pointed to an intensive strategy in nutrient acquisition [53]. Under such a strategy, roots exhibit competition avoidance, which generally leads to the specialization of the part of the root system free from interspecific competition in the uptake of locally abundant resources [91–93]. Indeed, a higher nutrient content in the mineral soil of mixed stands (Table S1) and their correlation with aspen FRB indicated that aspen nutrient uptake mainly takes place in the mineral horizon in mixed stands. This corroborates the significant correlation observed between the natural isotopic abundance of N in aspen leaves and that of the top mineral soil in mixed stands and the lack of such correlation in pure aspen stands (Ghotsa et al. unpublished data). However, it should be noted that, overall, compared to total root biomass of aspen in pure stands, the increased root biomass of aspen in mixed stands might suggest that aspen would allocate more biomass to fine roots compared to aboveground biomass in the mixed stands. These results suggest that two species with different types of root foraging strategies and competitive ability are more likely to coexist in a heterogeneous environment [94].

Mycorrhizae also play an important role in the nutrition of trees species in boreal forests. Spruce and aspen are known as obligate mycorrhizal species, with spruce linking exclusively with ectomycorrhizae [95], and aspen with both ecto-and arbuscular mycorrhizae [96]. They extend the uptake zone of their host beyond their root-soil interface, and their diversity and uptake capacity varies with stand tree species composition [69,97]. Although they were not studied here, a good understanding of their composition and distribution along the soil profile might be critical for an appropriate characterization of belowground interactions between spruce and aspen in mixed stands.

5. Conclusions

Overall, the results suggest that spruce and aspen accentuate their differences in rooting depth when mixed together. The process is likely controlled by tree-soil feedbacks and the intensity of interspecific competition, as has been suggested [87,98–101]. We provide new insight into underground interactions between the two species in boreal mixedwoods in northwestern Quebec, supporting the hypothesis of a competitive exclusion of aspen by spruce, as suggested in earlier studies [24], while emphasizing that the hypothesis is valid only in the organic layer of the soil. The facilitative

effect of aspen on nutrient availability, however, might contribute to minimizing the impact of spruce competitive pressure on aspen. Aspen respond to mixing by increasing their root biomass in the mineral soil, and the process likely contributes to a partitioning of the use of soil resources by both species along the soil profile. These findings, therefore, suggest that spruce may benefit from the presence of aspen by increasing its nutrient uptake in the organic soil layer while not limiting aspen nutrients acquisition. We speculate, based on the concept of "*functional equilibrium*", that increased spruce nutrient uptake in mixed stands would lead to higher aboveground total stand biomass. The observation that mixed spruce–aspen stands have a higher volume of spruce merchantable biomass, compared to pure spruce stands [25], is in agreement with this hypothesis.

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