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Shade affects fine-root morphology in range-encroaching eastern redcedars (*Juniperus virginiana*) more than competition, soil fertility and pH

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ABSTRACT

Fine-root morphological variables are often directly correlated with soil nutrient availability and water absorption. Indirectly, these morphological variables could be related to nutrient depletion by other plants. The relationships of fine-root variables with shade, direct or indirect, are unknown. I tested the relationships between a number of soil quality variables (levels of nitrogen and lime), competition with a co-dominant tree, and shade for the range-encroaching eastern redcedar *Juniperus virginiana*. I measured seven fine-root parameters as indices of potential nutrient-uptake or competitive ability. I also assessed the relationships of these fine-root parameters with foraging return (i.e., tissue nitrogen, non-structural carbohydrates, relative growth rate and biomass). There were no significant (main) effects of soil amendments (added nitrogen and lime) or tree competition on any of the seven root parameters. There was a significant (indirect) effect of shade (affecting five out of seven fine-root morphological variables), and a significant interaction effect between fertilizer and lime amendments, although the last-mentioned interaction affected one fine-root variable only. There were two additional pieces of evidence that support the effect of shade: a significant difference between shaded and unshaded plants in the slope of the negative relationship between root diameter and specific root length, and a significant overall difference among all fine-root morphological variables in a principal components analysis between shaded and unshaded plants. I found that fractal dimension was the only variable for which there were positive correlations with foraging return, probably because a higher fractal dimension leads to more effective use of nutrients. I expected a negative correlation between foraging returns and fine-root characteristics that incurred substantial costs. I only found a significant cost for specific root length, which may be mediated by the unmeasured parameter of root lifespan.

1. Introduction

The strategies employed to acquire nutrients and water are known to be associated with the population growth and productivity of woody plants (Caplan et al., 2017; Chen et al., 2018). There is considerable variability in the morphology of fine roots, both within and among species (e.g., Comas and Eissenstat, 2009; Tobner et al., 2013; Valverde-Barrantes et al., 2013). Some of this phenotypic plasticity may be ascribed to phylogenetic differences (Kembel and Cahill, 2005; Ma et al., 2018; Valverde-Barrantes et al., 2015). However, root phenotypic plasticity is most commonly ascribed to variability in growth patterns that maximize uptake of nutrients and water (e.g., Coleman, 2007; Razaq et al., 2017; Wang and Cheng, 2004). As a consequence of this morphological plasticity in fine roots, some species may have a competitive advantage over other plant species (Wang and Cheng, 2004). While there have been many studies that have revealed strong associations between the direct effects of soil nutrients on fine-root morphology (e.g., Gordon and Jackson, 2000; Hendricks et al., 1993; Razaq et al., 2017), there have been few that have examined indirect effects, such as interspecific competition (Hodge, 2004; Tomlinson et al., 2012; Wigley et al., 2019). Despite the fact that shading has been frequently examined in aboveground studies (e.g., Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013; Vadigi and Ward, 2013) it has seldom been considered in belowground studies (Freschet et al., 2015; Wahl et al., 2001; Wigley et al., 2019; Xue and Li, 2017). Shading may have an indirect effect on fine-root morphology because there may be a reduced rate of evapotranspiration and consequently reduced rates of water loss, as well as alterations in the uptake of certain nutrients (Bilbrough and Caldwell, 1995; Jackson and Caldwell, 1992; Marshall, 1986). In forests, plants growing in shade may experience very different climatic conditions from those growing in open habitats (Xue and Li, 2017; Zadworny et al., 2017, 2018; Ward, 2020). Furthermore, restricted light availability may cause there to be a reduction in the amount of carbon acquired by the plant that results in reduced

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allocation of carbon to the roots. This might have consequences for the uptake of nutrients and water. Consequently, the degree of plasticity of fine-root morphology may differ considerably between shaded and unshaded habitats.

Eastern redcedars *Juniperus virginianus* are the most widely distributed conifers in the eastern United States (Lawson, 1990; Nackley et al., 2017). They are rapidly expanding their range across the Great Plains, as far as the 100th meridian (Briggs et al., 2002; Nackley et al., 2017; Tomiolo and Ward, 2018). They are also altering their niche to include old fields and other disturbed habitats. They are a primary successional forest species (Lawson, 1990; Meneguzzo and Liknes, 2015), and are considered shade intolerant (Ormsbee et al., 1976). Eastern redcedars respond positively to nitrogen fertilization (Henry et al., 1992). They are frequently dominant on limestone habitats (Hoff et al., 2018; Pierce and Reich, 2010). Edmeades et al. (1981) and Kennedy et al. (2004) found that plants grew better on limestone, perhaps because of increased nitrogen mineralization on lime. These limestone habitats are often termed “cedar barrens” because of their dominance by eastern redcedars and the relative absence of other tree species (Baskin and Baskin, 2004; Mills, 2008). However, it is possible that it is not a preference for high pH substrates by eastern redcedars on these “cedar barrens”, but absence of competition with other plants, especially trees (Ward, 2020). In natural succession, eastern redcedars are often replaced by oaks and hickories (Hoff et al., 2018).

Fine roots ≤2 mm in diameter are considered to be the most active parts of the root system, being involved in both nutrient uptake (Eissenstat, 1992; Freschet et al., 2017; Hendricks et al., 1993, 2006) and water absorption (Kazda and Schmid, 2009; Ryser, 2006). However, using a threshold of ≤2 mm diameter groups together both roots involved in transport and absorptive roots (i.e., roots that are responsible for resource acquisition) (Mucha et al., 2019; Pregitzer et al., 1993, 2002; Pregitzer, 2002; Zobel, 2003). However, Guo et al. (2008) have indicated that about 75 % of fine roots ≤2 mm diameter are involved in absorption, when averaged across 23 temperate woody species. A meta-analysis by Freschet and Roumet (2017) recognized that there are problems with defining an arbitrary cut-off for fine roots (such as ≤2 mm diameter), or by differentiating according to fine-root order (first through third order, beginning distally), or by function (absorptive vs. transport). However, Freschet and Roumet (2017) recognized that fine roots of woody species were useful to differentiate when ≤2 mm diameter (see also McCormack et al., 2015). An additional issue is that Kong et al. (2014) have demonstrated that fine-root branching traits represent an additional dimension of root trait variation, independent of the absorption-transport spectrum.

I designed an experiment to simultaneously test the effects on fine-root morphology of eastern redcedars of nutrient fertilization (specifically nitrogen), lime supplementation, competition with a common native tree, the post oak, and with shade. I hypothesized that:

1) direct effects, such as soil nutrients, should have stronger effects on fine-root morphological variables than indirect effects, such as interspecific competition and shade (Edwards et al., 2004; Ostonen et al., 2007).
2) There should be differences in the slopes of regressions between pairs of fine-root morphological variables for the various treatments (shade, fertilization, lime, competition) and the absence thereof.
3) the degree of phenotypic integration of complex traits within a single species (Niklas, 2004; Pigliucci, 2003) would result in strong positive correlations between root foraging strategies (specifically, fractal dimension (FD) (Eshel, 1998; Kong et al., 2014; Wang et al., 2009), specific root tip abundance (SRTA) (Hertel et al., 2003; Meinen et al., 2009), average link length (Dupuy et al., 2010; Kong et al., 2014; Pregitzer, 2002)) and foraging return as measured by root nitrogen, total nonstructural carbohydrates (TNC), relative growth rate (RGR), and total biomass (Ostonen et al., 2017; Prieto et al., 2015). This is because these parameters indicate that the root is focusing on creating branches in a specific area (large FD) with more root tips (high SRTA) and have longer link lengths to reach those high-resource sites (Borden et al., 2020; Caplan et al., 2017; Farley and Fitter, 1999; Johnson and Biondini, 2001). Contrastingly, I predicted that there would be trade-offs (negative correlations) between the cost of organ investment in specific root length (SRL) (Ostonen et al., 2007; Ryser, 1996), specific root surface area (SRA), root tissue density (RTD) (Eissenstat, 1992) and average diameter (Coleman, 2007; Comas et al., 2002; Valverde-Barrantes et al., 2013) and foraging return. Furthermore, for those treatments that demonstrated a significant relationship with fine-root morphological variables, there should be differentiation between the treatment and absence thereof as manifested in a multivariate analysis.

2. Methods

2.1. Experimental design and treatments

All eastern redcedar saplings were of similar size when purchased from Pineland’s Nursery in Columbus, New Jersey and were about 18 months old. Mean ± S.E. initial heights of eastern redcedars at the start of the experiment on 23 June 2016 were 138.4 ± 2.54 mm, and mean ± S.E. initial stem diameters were 2.6 ± 0.06 mm.

I ran an experiment investigating the controls on growth rates of eastern redcedars (see Ward, 2020) from May 2016 until August 2018. I manipulated the levels of shade, fertilizer, lime, and competition with the post oak *Quercus stellata* in a greenhouse (see Ward, 2020). I used 95 L containers (n = 120) (depth =70 cm; 55 cm diameter) so that the trees would not be constrained by soil availability, with a single eastern redcedar placed in each container. I used a split-plot experimental design, with shade (and control) the *whole* plot (replicated six times) and the *sub-plots* completely randomized and consisting of the remaining factors (nutrients, lime, and competition). The soil used was Scott’s® Hyponex Potting Soil (nitrogen–phosphorus–potassium ratio = 0.07−0.01-0.03 (N-P-K); initial pH range = 5.5–6.2; derived from peat, forest products, perlite and/or compost [exact contents not explicitly stated]).

I used Green-Tek® knitted 80 % shade cloth (BFG Supply, Burton, Ohio) and a control (unshaded). I checked the shading effect using an AccuPAR model LP-80 ceptometer (Decagon Devices, Inc., Pullman, Washington) in photosynthetically active radiation (PAR) in the 400–700 nanometer waveband during biweekly measurements over the study period (32 measurements). I confirmed that there was a highly significant reduction in the effect of sunlight caused by the shade cloth (78.7 % ± 0.53 reduction; minimum = 73 % reduction; maximum = 84 % reduction). I used Vigoro® fertilizer 16-4-8 N-P-K, containing 1.6 % ammoniacal nitrogen and 14.4 % urea nitrogen with 14.3 % slowly available nitrogen from coated urea. I fertilized half the containers with 30 g m^{-2} nitrogen twice per year (henceforth “full fertilization”), following Tilman (1987). The remainder of the containers were fertilized at 15 g m^{-2} twice per year (henceforth “half fertilization”). The lower level of fertilization was used to minimize potential volatilization and to ensure a constant supply of nutrients from the fertilizer (Pillay and Ward, 2020) because of the low levels of these nutrients from the potting soil and high compaction of the potting soil after repeated watering over two years. I also raised the level of alkalinity in the soil by adding lime to half the containers to simulate the commonly seen association of eastern redcedars with limestone habitats and a control. At the end of the experiment, there was a significant difference in soil pH of the lime treatment (mean pH ± S.E. = 5.7 ± 0.26) and controls (mean pH ± S.E. = 5.4 ± 0.35) (see Ward, 2020). I also introduced competition with a common tree, the post oak *Quercus stellata*, by growing a single oak tree in half the containers, with the remainder being controls. My rationale was that it may not be lime (i.e., alkalinity *per se*) that causes eastern redcedars to be so abundant on cedar barrens but rather because there are relatively few competitors, particularly trees (Baskin and

Baskin, 2004; Mills, 2008). Interspecific competition may have a negative indirect effect on the availability of nutrients to roots. I purchased post oaks from Mossy Oak Nativ Nursery in Westpoint, Mississippi. Mean ± S.E. initial heights of post oaks were 195.6 ± 7.75 mm, and mean ± S.E. initial stem diameters were 4.6 ± 0.11 mm. Water availability was not manipulated and was provided *ad libitum* by means of drip irrigation.

All fine roots were thoroughly cleaned prior to analysis. I measured key variables pertaining to fine root dimensions ($<$ 2 mm diameter), with one set of fine roots measured per plant. The entire root system was not analyzed. Each root sample was scanned using a high-resolution flatbed scanner (800 DPI resolution, 256-level gray-scale, TIFF format; Epson Scanner STD4800, USA) and WinRhizo software (2015 Pro version, Regent Instruments, Quebec, Canada). I analyzed seven different root traits (following Prieto et al., 2015; Ostonen et al., 2017; Valverde-Barrantes et al., 2013): FD, SRTA, average link length, SRL, specific root surface area (SRA), root tissue density (RTD) and average diameter. A high value of FD is closely related with the branching pattern of roots (Tatsumi et al., 1989; Walk et al., 2004), and is a measure of the density and of the complexity of a branching system (Eshel, 1998; Mandelbrot, 1982; Wang et al., 2009).

For assessments of storage, I measured total non-structural carbohydrates (TNC) in the roots using a standard protocol, separating TNC into soluble sugars and starch (Tomlinson et al., 2012; Ward, 2016). All TNC measurements were performed in a single laboratory (Landhäusser et al., 2018). I also recorded % nitrogen in the roots using a Rapid N Exceed® Elementar nitrogen analyzer. Additionally, I measured total (dry) biomass and relative growth rate (RGR) in height (relative to initial height) of the trees.

2.2. *Statistical analyses*

I performed a general linear model for repeated measures for a split-plot design. The design was unbalanced and, consequently, a Type III model was employed in SPSS (Ward, 2020). I used a MANOVA (including all interaction effects) to control for Type I statistical error because I measured multiple (seven) dependent variables. The independent variables were shade (whole plot), nutrients, lime, and competition (all sub-plots). All fine-root variables were log$_{10}$-transformed to fulfill the requirements of normality and homogeneity of variance, with the exception of fractal dimension which was normally distributed. For the significant variables in the MANOVA only, I ran univariate ANOVA to test for the significance of individual fine-root variables. I also ran pairwise Pearson product-moment correlations between the above-mentioned fine-root variables, nitrogen, TNC, soluble sugars, starch, relative growth rate (RGR) in height, and total biomass. To test for trade-offs, I ran regressions among significant variables, and tested the significance of the interaction effects to demonstrate differences in slopes (Zar, 1999).

I also ran a principal components analysis (PCA) of the fine-root variables to examine integration of these variables. I used a Euclidean distance metric to compare the first two PC axes in multi-dimensional space (Collins et al., 2000; Heikinheimo et al., 2012). I then tested the significance of Euclidean distances using a bootstrap resampling test for the main effects (shade, nutrients, lime, competition), with 2000 iterations each (Efron and Tibshirani, 1994; Manly, 1997; Anderson and Walsh, 2013). I used Real Statistics Resource Pack software version 7.2 for analysis of Euclidean distances (Zaiontz, 2020). All other analyses were done using SPSS v. 26.

3. Results

I did not find that direct effects (e.g., nutrients) had a greater effect than indirect effects (e.g., shade). I found a significant overall main effect for shade (MANOVA: Wilks' $\lambda = 0.637$, $p < 0.001$), but no other significant main effect (Table 1). There was also a significant interaction effect between fertilizer and lime (Table 1). No other interaction effects showed significant differences ($p > 0.05$) (Table 1).

Table 1
MANOVA for shade, fertilizer addition, lime amendment, and competition, and interaction effects. The only significant variables were Shade and the Fertilizer X Lime interaction. There were 96 error d.f.

Independent Variable	Wilks' Lambda	Equivalent F	p
Shade	0.637	7.800	**<0.001****
Fertilizer	0.939	0.893	0.515
Lime	0.903	1.472	0.186
Competition	0.922	1.162	0.332
Shade * Fertilizer	0.898	1.563	0.156
Shade * Lime	0.920	1.185	0.319
Shade * Competition	0.943	0.833	0.563
Fertilizer * Lime	0.834	2.731	**0.013***
Fertilizer * Competition	0.921	1.182	0.320
Lime * Competition	0.900	1.524	0.168
Shade * Fertilizer * Lime	0.964	0.513	0.822
Shade * Fertilizer * Competition	0.964	0.507	0.827
Shade * Lime * Competition	0.957	0.609	0.747
Fertilizer * Lime * Competition	0.888	1.726	0.112
Shade * Fertilizer * Lime * Competition	0.942	0.851	0.548

There was a significant (univariate) ANOVA difference between shaded and unshaded redcedars in most comparisons (range in $F = 17.809–48.106$, all $p < 0.001$), with the exception of log$_{10}$ fine-root tissue density (RTD) ($F = 2.237$, $p = 0.138$) and log$_{10}$ Average Link Length ($F = 0.372$, $p = 0.544$). SRTA and SRL had smaller values for unshaded redcedars than shaded redcedars, while surface area, root diameter, and fractal dimension had larger values for unshaded redcedars than shaded redcedars (Table S1).

The single significant interaction effect (Fertilizer X Lime; Table 1) was only significant for one of the seven fine-root variables, viz. log$_{10}$ surface area ($F = 12.754$, $p = 0.001$) in a subsequent (univariate) ANOVA (Fig. 1). The log$_{10}$ surface area for full fertilization without lime was greater than for half fertilization (Fig. 1). When lime was added, there was a reduction in log$_{10}$ surface area in the full fertilization treatment in comparison to half fertilization (Fig. 1).

Regarding my second hypothesis, I found a significant difference in the slopes of the regressions for shaded and unshaded plants ($F = 4.581$, $p = 0.034$ – tested for significance of interaction effect; McDonald, 2014). There was a significant negative correlation between log$_{10}$ average diameter and log$_{10}$ SRL ($r = -0.886$, $p < 0.001$ – Table 2, Fig. 1). For the shaded trees, log$_{10}$ Average Root Diameter = -0.29 log$_{10}$ Specific Root Length + 0.39 ($r^2 = 0.68$, $p < 0.001$) and for unshaded trees log$_{10}$ Average Root Diameter = -0.37 log$_{10}$ Specific Root Length + 0.48 ($r^2 = 0.77$, $p < 0.001$). The shaded plants had significantly larger values for

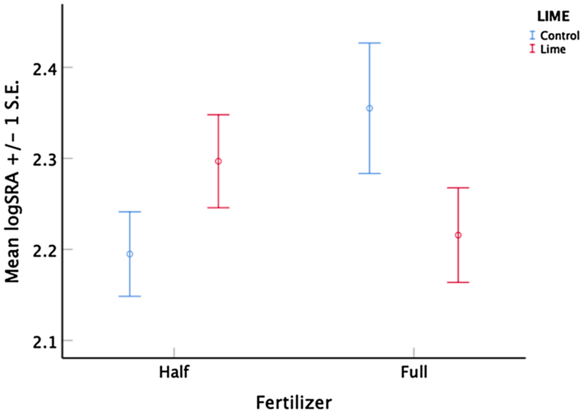

Fig. 1. The effects of fertilization and lime amendment on the mean log$_{10}$ Specific Root Area (SRA). Fertilization alone (full) resulted in an increase in SRA whereas lime amendment resulted in a decrease under full fertilization.

Table 2

Correlation matrix for size, growth rate (RGR), nitrogen, nonstructural carbohydrates (TNC), soluble sugars, starch and fine-root variables (Specific Root Tip Abundance (SRTA), Specific Root Length (SRL), Root Tissue Density (RTD), Root Surface Area, Root Diameter, Fractal Dimension, and Average Link Length).

Variables	$\log_{10}$ Total Biomass	Root Nitrogen	Root TNC	Root Soluble Sugars	Root Starch	$\log_{10}$ SRTA	$\log_{10}$ SRL	$\log_{10}$ RTD	$\log_{10}$ Surface Area	$\log_{10}$ Root Diameter	Fractal Dimension	$\log_{10}$ Average Link Length
RGR Height	0.848**	**−0.527** **	0.316**	0.612**	ns	**−0.399** **	**−0.428** **	ns	0.272**	0.335**	0.430**	ns
$\log_{10}$ Total Biomass		**−0.466** **	0.418**	0.640**	ns	**−0.420** **	**−0.454** **	0.217*	0.360**	0.362**	0.478**	ns
Root Nitrogen			ns	**−0.212** **	ns	**−0.297** **	**−0.356** **	**−0.263** **	ns	ns	ns	ns
Root TNC				0.790**	0.735**	**−0.257** **	**−.0210** **	ns	0.253**	0.247**	0.287**	ns
Root Soluble Sugars					ns	**−0.294** **	**−0.216** *	ns	0.253**	0.293**	0.326**	ns
Root Starch						ns	ns	ns	ns	ns	ns	ns
$\log_{10}$ SRTA							0.866**	**−0.553** **	ns	**−.0525** **	**−0.631** **	ns
$\log_{10}$ SRL								**−0.605** **	ns	**−0.886** **	**−0.818** **	ns
$\log_{10}$ RTD									ns	ns	0.197*	ns
$\log_{10}$ Surface Area										0.339**	0.516**	**−0.265** **
$\log_{10}$ Root Diameter											0.916**	0.245**
Fractal Dimension												ns

* $p < 0.05$.

** $p < 0.01$. Negative correlations appear in bold font.

$\log_{10}$ SRL than the unshaded plants ($F = 3.855$, $p < 0.001$), and the unshaded plants had significantly larger $\log_{10}$ average root diameters ($F = 24.603$, $p < 0.001$). Mean ± S.E. $\log_{10}$ average diameter for unshaded plants was 0.209 ± 0.011 mm vs. 0.119 ± 0.011 mm. Mean ± S.E. $\log_{10}$ SRL of shaded plants was 0.921 ± 0.029 m g^{-1} and for unshaded plants was 0.712 ± 0.031 m g^{-1}. I also tested the significance of the differences in the slopes of the FD vs SRL regression (which was also significant; Table 2) for shaded and unshaded plants. However, there was no significant difference in the slopes of shaded and unshaded plants for this relationship ($F = 2.530$, $p = 0.114$) (tested for significance of interaction effect; McDonald, 2014).

I tested the third hypothesis by running correlations among the fine-root variables and relative growth rate in height, total biomass, total nonstructural carbohydrates in the roots, and nitrogen of the roots (Table 2) and examined differences in the significant main effect of shade (Table S1).

There were 21 significant positive correlations among pairs of these variables and 16 significant trade-offs (negative correlations) (Table 1). Regarding significant correlations between root nitrogen and the seven fine-root variables, there were two positive correlations (SRTA and SRL) and one negative correlation (RTD). Of the significant correlations between root total nonstructural carbohydrates and the fine-root variables, there were three positive (Surface Area, Root Diameter, FD) and two negative correlations (SRTA, SRL). These significant correlations were the same as with root soluble sugars (Table 1). There were no significant correlations between root starches and any of the fine-root variables (Table 1). There were significant correlations between size of eastern redcedars (total biomass) and growth rate (RGR) of eastern redcedars and the seven fine-root variables: there were four positive (RTD, Surface Area, Root Diameter, FD) and two negative correlations (SRTA, SRL) between total biomass and the fine-root variables. I also found a negative correlation between $\log_{10}$ SRL and $\log_{10}$ belowground biomass ($r = 0.452$, $F = 29.757$, $p < 0.001$). The significant correlations between RGR_{height} and the fine-root variables were three positive correlations (Surface Area, Root Diameter, FD) and two negative correlations (SRTA, SRL). Thus, the only difference between the total biomass and RGR_{height} comparisons was an additional significant correlation for RTD and total biomass. Root nitrogen was negatively correlated with both total biomass ($r = -0.466$, $p < 0.001$) and RGR_{height} ($r = -0.527$, $p < 0.001$), but there was no significant correlation between root nitrogen and root TNC ($p > 0.05$). However, there was a significant trade-off of root nitrogen with root soluble sugars ($r = -0.212$, $p < 0.001$). Root nitrogen was positively correlated with SRTA and SRL, and negatively correlated with RTD. There were four fine-root variables that were not significantly correlated ($p > 0.05$) with root nitrogen (Surface Area, Root Diameter, FD, Average Link Length). There were significant positive correlations between root TNC (nonstructural carbohydrates) and both total biomass ($r = 0.418$, $p < 0.001$) and RGR_{height} ($r = 0.316$, $p < 0.001$). There were also 17 non-significant correlations, many of which were between $\log_{10}$ average link length and other fine-root variables, the exceptions being one significant negative correlation (Surface Area) and one significant positive correlation (Root Diameter) with $\log_{10}$ average link length.

I ran a principal components analysis (PCA) of the fine-root variables to assess overall similarity (Table 3). The variance explained by PC1 was 53.8 %, with a further 22.9 % explained by PC2. The most important parameters affecting PC1 were $\log_{10}$ SRL (*-0.970*), $\log_{10}$ SRTA (*-0.926*), FD (*0.911*), and $\log_{10}$ diameter (*0.903*). Regarding PC2, the most important parameters were $\log_{10}$ surface area (*0.814*) and $\log_{10}$ average link length (*-0.800*).

The only significant difference across the first two axes was in the

Table 3

Weightings of the variables measured for the first two principal components of the PCA on fine root parameters. PC1 explained 53.8 % of the variance in the data, and PC2 explained a further 22.9 % of the variance. SRTA = specific root tip abundance.

Variable	PC1	PC2
$\log_{10}$ Specific Root Length	−0.970	0.135
$\log_{10}$ Surface Area	0.240	0.814
$\log_{10}$ Root Tissue Density	0.511	−0.407
$\log_{10}$ Root Diameter	0.903	0.069
Fractal Dimension	0.911	0.303
$\log_{10}$ SRTA	−0.926	0.161
$\log_{10}$ Average Link Length	0.058	−0.800

effect of shade. I used a Euclidean distance metric to compare these first two axes. There was a significant difference due to shade (bootstrap resampling test: $p < 0.001$) (Fig. 3). There were no significant effects of fertilization ($p = 0.874$), lime ($p = 0.616$), or competition ($p = 0.896$) on Euclidean distances.

4. Discussion

I did not find that direct effects, such as soil nutrients, were stronger than indirect effects. There was a significant interaction between soil nutrients and lime for only one of the seven fine-root variables I measured, Specific Root Area (SRA) (Table 1, Fig. 2). I found that plants receiving full fertilizer had larger SRA than those receiving half fertilizer. Contrastingly, plants receiving lime had smaller SRA values for the full fertilizer than for half fertilizer, presumably because of the negative effect of increased alkalinity (raising pH) on nitrogen application (Ward et al., 2017a, b). However, there was no significant effect for any other fine-root variables for the fertilizer X lime interaction. Moreover, there was no significant main effect for either fertilizer or lime. The most important treatment effect was the indirect effect of shade (Table 1; see also Table S2), which affected five out of seven fine-root variables (i.e., all but root tissue density (RTD) and average link length).

In this experiment, I found that there were no significant direct (main) effects (nutrients and lime). Possible reasons for the absence of an effect of nitrogen fertilization is that either there was sufficient nitrogen supplied by the potting soil, and/or that additional nitrogen would not further benefit growth, i.e., according to Liebig's law of the minimum, there was another nutrient that was limiting (Tilman, 1987). However, phosphorus and potassium were also manipulated here. Furthermore, the level of nutrients in the potting soil were extremely low (see *Methods*). It would appear that there is only support for Liebig's law of the minimum from agricultural examples (e.g., Paris, 1992; Van der Ploeg et al., 1999; Niles et al., 2015). In ecological studies, there is little evidence that only a single nutrient is limiting. Several studies indicate that there is co-limitation of nutrients and not just nitrogen (e.g., Danger et al., 2008; Craine and Jackson, 2009; Harpole et al., 2011). Furthermore, increased levels of nitrogen have caused an increase in growth, including in eastern redcedars (Henry et al., 1992; Skrabka et al., 1979; Smart et al., 2013; Vinton and Goergen, 2006; Yolcu and Serin, 2009). However, they did not in this study (Table S2).

In a review of the responses of fine roots to direct and indirect effects of changes in nutrient availability, Hodge (2004) found that exploitation of phosphate was dependent on photosynthetic carbon supply (Cui and Caldwell, 1997; Jackson and Caldwell, 1992), indicating that shaded environments were depriving plants of the ability to take up phosphate. Hodge (2004) also found that shading in some studies (but not all) reduced the uptake of N (Bilbrough and Caldwell, 1995; Cui and Caldwell, 1997; Jackson and Caldwell, 1992), which may be related to N demand by the plant (Hodge, 2004). Hodge (2004) also noted that RGR of roots and investments in nonstructural carbohydrates could also be affected by shading (Bilbrough and Caldwell, 1995), as I also found (Table S2: Ward, 2020).

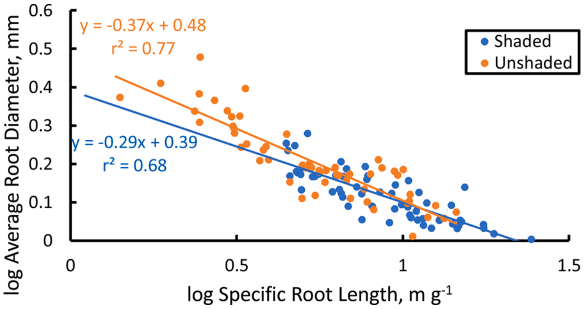

Fig. 2. Negative correlation between log$_{10}$ average root diameter (mm) and log$_{10}$ specific root length (SRL) (m g^{-1}) ($r = -0.89$, $p < 0.001$). There was a significant difference in the slopes of the regression relationships for shaded and unshaded plants (see *Results*).

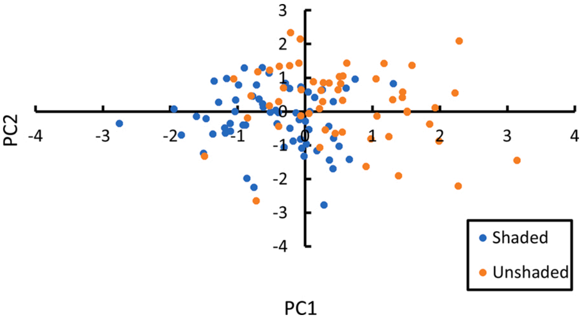

Fig. 3. There was a significant difference in Euclidean distance between shaded (blue) and unshaded (orange) plants for the first two components of the PCA. Weightings are indicated in Table 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

I predicted that there would be differences in the slopes of regressions between pairs of fine-root morphological variables. I found this pattern for the relationship between average root diameter and SRL only for the comparison of shaded and unshaded plants (Fig. 1) (see also Valverte-Barrantes et al., 2013). This may not reflect a trade-off *per se*, but rather a necessary negative correlation (i.e., a geometric constraint) between root diameter and SRL when fine-root density remains constant, as it did in this study. This may be more complex than it first appears. For example, Wahl et al. (2001) found that there was no significant effect of nutrient fertilization but that shade caused modifications of the water-conducting system and produced tissue with lower construction costs per volume in three *Bromus* and five *Poa* grass species. The decrease in tissue mass density in their study was especially pronounced when sunlight became growth-limiting. Wahl et al. (2001) concluded that such a response may have allowed the plants to maintain root length and nutrient-acquisition capacity, albeit at the expense of tissue density. A similar conclusion was reached for two *Dactylis* species which were able to maintain their total root length when shaded (30 % daylight) although the relative biomass allocation to roots was strongly reduced (Ryser and Eek, 2000), as I found was the case with the eastern redcedars I studied (Table S1: Ward, 2020). However, there was no significant difference in root tissue density in my study, but there was a negative correlation between SRL and root diameter, with shaded plants having higher SRL and lower root diameters than unshaded plants (Fig. 2). I note that Giertych et al. (2015) were unable to detect a trade-off in shade-intolerant plants such as the species I studied, with the exception of a reduction in belowground biomass. Similarly, Freschet et al. (2015) found no significant difference between high- and low-light treatments in SRL or root mass fraction in 12 herbaceous species. However, these authors did find significant differences for the two levels of nutrients they manipulated. Hecht et al. (2016) found that sowing density had an effect on SRL, which occurred because of reduced light availability at high sowing densities. Bearing in mind that the consequence of being in the shade was reduced total and belowground biomass for eastern redcedars in my study (Table 2: Ward, 2020), I speculate that shade plants sacrifice root diameter to maximize SRL because of the shortage of carbon. Such a strategy is consistent with the observations of Eissenstat (1992), Hodge (2004) and Luquet et al. (2005), and the results obtained by Kong et al. (2014) for 96 subtropical forest species.

I predicted that fine roots of eastern redcedars associated with root foraging strategies would result in more efficient uptake of nutrients and, consequently, with increased size and growth rates. The fine-root variables associated with foraging strategies are fractal dimension (FD) (Eshel, 1998; Kong et al., 2014; Wang et al., 2009), specific root tip abundance (SRTA) (Hertel et al., 2003; Meinen et al., 2009) and average link length. Only FD was significant in the expected direction (i.e., greater FD in unshaded conditions) (Table S1). I also found that fractal dimension (FD) was positively correlated with RGR_{height}, total biomass and TNC (although there was no significant correlation with root N – Table 2). These results are consistent with the conclusions of Dannowski and Block (2005) and Kong et al. (2014) who showed that root branching can be enhanced to rapidly exploit resource-rich patches. Additionally, I found that fine-root surface area and root diameter were also larger with more sunlight (Table S1). However, SRTA was smaller (Table S1), indicating that there were fewer root tips rather than more. SRTA was negatively correlated with RGR_{height}, total biomass, and TNC, indicating that there was an organ cost to the construction of more root tips (Valverde-Barrantes et al., 2013), but there was a significant positive correlation with root N (Table 1). There was no significant relationship between foraging returns and average link length, which is inconsistent with the findings of Ostonen et al. (2017); Prieto et al. (2015), and Valverde-Barrantes et al. (2013).

Why shading had the opposite effect on SRTA (i.e., bigger under shade) or no significant effects (average link length) is not known. A possible reason for SRTA being bigger under shade may have to do with the positive correlation between SRTA and SRL (Table 2). In other words, if there are correlations with other fine-root variables, selection may favor one of these variables (presumably for reasons not tested here), or several variables in tandem, resulting in the absence of differences (e.g., average link length) or even the reversal of associations (e.g., SRTA). Hecht et al. (2016) have shown that sowing density may also have significant impacts on SRL, which in turn may affect SRTA. Furthermore, Weemstra et al. (2020) found that SRL was negatively correlated with root lifespan and fine-root biomass, leading to an optimal (non-linear) interaction, further complicating an already complex picture. The PCA of the first two principal components (Fig. 3) also verified the differentiation of shaded and unshaded environments, indicating support for the notion of phenotypic integration of multiple fine-root traits.

I also hypothesized that the degree of phenotypic integration of complex traits within a single species would result in trade-offs (negative correlations) between the cost of organ investment in specific root length (SRL), specific root surface area (SRA), root tissue density (RTD) and average diameter and foraging return, indicating that there were costs involved in the production of these fine-root variables. I found this pattern of negative correlations for SRL and foraging return only, but for fine-root surface area all such correlations were positive (except N, which was non-significant – Table 2). Root tissue density was significantly positive for biomass and TNC (including soluble sugars), and non-significant for N and RGR. Fine-root diameter was significantly positive for RGR, biomass, TNC (including soluble sugars) and non-significant for N. Thus, it was only SRL that showed the predicted pattern. In all, only FD and SRL showed the predicted correlations (positive and negative, respectively).

5. Conclusions

I found that the indirect effect of shade was more important than the direct effect of nutrients. Although I found that there was an effect of nutrients, it was as an interaction effect with lime only, and for one fine-root morphological variable only (SRA). I found three pieces of evidence supporting the importance of shade: Firstly, I found a significant main effect of shade in the MANOVA, which was manifested in five of the seven fine-root variables. Secondly, there was a significant negative correlation between root diameter and SRL, with a significant difference between the slopes of shaded and unshaded plants. Thirdly, in a multivariate analysis (PCA), I found that there was a significant difference in Euclidean distances between shaded and unshaded plants. All three of these relationships indicate that shade should be included as a possible factor of importance to the morphology of fine roots, as also indicated by Ryser and Eek (2000) and Wahl et al. (2001).

I note that the negative correlation between root diameter and SRL appears to be consistent with the notion of a Root Economic Spectrum (RES) (Ostonen et al., 2017; Prieto et al., 2015; Valverde-Barrantes et al., 2015; Weemstra et al., 2016). A recent meta-analysis of the Fine-Root Ecology Database (FRED; https://roots.ornl.gov/ - Iversen et al., 2017) by McCormack and Iversen (2019) also found a trade-off (negative correlation) between root diameter and SRL. McCormack and Iversen (2019) also found a significant relationship between root tissue density and root diameter (which I did not find in eastern redcedars). These authors suggested that this relationship between tissue density and diameter might constitute a second axis of the RES, along with the first axis of the trade-off between root diameter and SRL.

Regarding phenotypic integration of fine-root morphological variables and foraging returns, I found that several root foraging strategies (Table 1) were positively correlated with root N (SRL, SRTA), TNC (Surface Area, Diameter, Fractal Dimension), RGR (Surface Area, Diameter, Fractal Dimension) and overall plant size (Tissue Density, Surface Area, Diameter, Fractal Dimension), indicating that there were positive effects of fine-root foraging on important parameters of plant size and nutrient composition. These results have been found previously (Ostonen et al., 2017; Prieto et al., 2015), suggesting their general importance for root foraging strategies. However, it was fractal dimension only that was positively correlated with all foraging return variables (except N). Fractal dimension is likely an important fine-root characteristic because it demonstrates that the plant is investing heavily in a particular area of the rhizospace (Valverde-Barrantes et al., 2013). Contrastingly, it was only specific root length that was found to be costly to the plant. In this case, there may be correlations with other variables such as root lifespan (unmeasured here) and fine-root mass that ultimately cause the trade-off I found (Weemstra et al., 2020). Further studies incorporating root lifespan should be included to better represent the trade-offs between SRL, fine-root mass and root lifespan.

McCormack and Iversen (2019) included mycorrhizal colonization rate with the SRL:diameter trade-off or negative correlation. I did not record mycorrhizal colonization in this study. For an AMF species such as the eastern redcedar (Liang et al., 2017), high-precision nutrient foraging may be achieved by a thin root cortex, thin root diameter, low root tissue density, medium to high root branching, high root concentration, fast root turnover and low mycorrhizal concentration (Chen et al., 2018). Future studies should incorporate the interactions of fine roots with mycorrhizae in eastern redcedars.

Declaration of Competing Interest

I declare that I have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.pedobi.2021.150708.

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