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*Research Paper***Temporal and spatial distribution of fine root length of *Quercus castaneifolia* and *Cupressus arizonica* seedlings**Hashem Habashi^{1*}, Yasamin Sharifpour²

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Abstract

Fine roots have the function of water and nutrient uptake specially a critical role on carbon delivery from plant to soil. Spatial and temporal variability of fine roots characteristic have affected by environmental and genetic factors. The aim was comparatively investigated of temporal and spatial distribution of fine root lengths of *Quercus castaneifolia* and *Cupressus arizonica* seedlings. Soil samplings have done in different distance and direction of 10 seedlings in 11 different interval periods with auger sampler in 0-20 cm soil depth. Fine roots separated by washing and root lengths determined by Newman method. Results showed two species seedlings were not same fine roots spatial distributions also significantly differences were observed in temporal variability. Total fine root length of cypress seedlings was more than oak seedlings. Finally we concluded environmental parameters have more affected on growing fine roots than genetic factors.

Key words: Fine root, Seedling, *Quercus castaneifolia*, *Cupressus arizonica*.

Introduction

Fine roots mediate C delivery from plants to soil microbes via exudation (Qualls *et al*, 1991; Grayston *et al*, 1997). Investigation of fine root dynamics is very important because fine roots may be short lived, but are replaced by the plant in an ongoing process of root 'turnover'. Fine roots may input more carbon to soil than from leaves (Stover *et al*, 2010; Chen *et al*, 2003, Jackson *et al*, 1997), have the function of water and nutrient uptake (Zobel, 2008). They are often heavily branched and support mycorrhizas, therefor temporal and spatial distribution of fine roots is of especial interest (Kochsiek *et al*, 2013).

Trenches, monoliths and cores that are used most commonly by many investigators (Jackson *et al*, 1996; Atkinson, 1985) in contrast, root tracing techniques which constrain root growth along a transparent viewing surface (Glinski *et al*, 1993) and rhizotron viewing panes (Atkinson, 1985; Taylor & Klepper, 1971; Taylor *et al*, 1971) are modern technique and may be the best choice for study of changes in root abundance over time because high spatial variability confounds the use of destructive sampling (Atkinson, 1985). Anyway because lack of technical facilities we chose to use cores to examine temporal and spatial changes in fine root length in *Quercus castaneifolia* and *Cupressus arizonica* seedlings.

Quercus castaneifolia is an endemic species of Hyrcanian forest. This species compose pure and mixed association with *Carpinus betulus*. Oak seedling has tap root and in adults has fibrous root system. This species is planted very wide in plantations (Sabeti, 2004).

Cupressus arizonica is exotic species that has significant resistance against drought, severe aridity, corn snow and air pollution because of vigorous and depth root system. This species in wet sites with heavy soils may create shallow root system (Zare, 2002). This species were also widely planted in north forestation of Iran. In destroyed forest land are well established because of resistant.

Millikin and Bledsoe (1999) investigated root systems of six blue oak trees using soil cores. Rooting depth for the main root system ranged from 0.5 to 1.5 m, with an average of 70% of excavated root biomass located above 0.5 m. Fine root biomass decreased with depth while at surface depths (0–20 cm), small-fine (< 0.5 mm diameter) roots accounted for 71%, large-fine (0.5–2.0 mm) for 25%, and coarse (> 2 mm) for 4% of total root biomass collected with cores.

John *et al*, (2001) studied vertical distribution and seasonal changes of fine root mass in pine forests. Their results confirmed that vertical distribution of fine roots are limited to top of 30cm of soil profile. Results also revealed that soil humidity has important role on fine root distribution. The most and least amount of fine root mass were in rainy season and autumn respectively.

These studies have mainly focused on differences in fine root length, fine root temporal and spatial distribution of roots between chestnut-leaved oak and Arizona cypress. The objective of the present study is to comparative investigation of the spatial and temporal variability in fine root length of oak and cypress.

Methods and materials

The selected area are located on flatted slopes (<5°), hill landform at elevations of between 200 and 250 m a.s.l in Naman village near to Gorgan city. The soil types are generally weak-drained Cambisols (WRB classification) and soil structure was granular and angular. The climate is moderate humid, and based on Domarton climatic methods is semi-humid (Shamooshak Forestry plan, 2004).

Root sampling and analyses

We randomly selected 5 healthy seedlings of each two species, allometric characteristic were measured. To record fine root length in the two species we did an inventory in each of the 8 radial direction and 3 intervals distance (20, 50, 100 cm) of collar seedlings. At 8 randomly selected sampling locations of around collar species, root samples were taken with a soil corer (5.5 cm in diameter) from the mineral soil down to 20 cm soil depth. In order to avoid clumping of the sampling locations, while at the same time minimizing soil heterogeneity effects, all subsequent samples were taken at a 15° compared to earlier sampling. After sampling, the trench was filled with soil. The soil samples were transported to the laboratory at the GAUSNR University, where the stored samples (4°C) were processed within 12 weeks. In the laboratory, the samples were soaked in water and cleaned of soil residues using a sieve with a mesh size of 0.25 mm (Bohm, 1979). Then they were kept in 0.9% NaCl solution until investigated using a dissecting microscope. Large root fractions (> 10 mm in length) were picked out by hand. Only fine roots (roots < 2 mm in diameter) were included in the analyses. In order to make an estimation of temporal changes in fine root length, fine root sampling with the sequential coring method (Persson, 1978; Vogt & Persson, 1991; Fahey & Hughes, 1994; Yang *et al.*, 2004) was carried from March 2009 until June 2009. Fine root length was calculated by Newman (1966) method. The intersections of roots with axes of vertical and horizontal graph paper were counted. The intersections according to dimensions of graph paper were multiplied by the specific factor (Bohm, 1979, Alizadeh, 2001; Rafiee, 2004; Taylor *et al.*, 2013).

$$L = 0.786 N \quad (1)$$

L= root length (cm)

N= number of intersections with axes of graph paper.

Differences between species were examined with independent sample T- Test, temporal and spatial variability of fine root length were analyzed by repeated measurements ANOVA test. Statistical analyses were conducted using SPSS Statistical Software (version 16.0). Normality of data distribution was analyzed with Kolmogorov- Smirnov test.

Results

Five healthy seedlings of each species were selected, cutting and allometric characteristics were measured (table. 1). Aboveground biomass of oak seedling was ranged 64.6-84.0 while cypress was ranged 70.3- 86.4 gr.m² that were split to stem and canopy biomass. Oak seedlings have more stem biomass than cypress, while cypress canopy biomass was more and differences were significant in 0.001 confident levels (table. 1). Seedling cypress height was significantly more than oak.

Table I. Mean allometric seedling characteristics (g.m², mean ± SD) in oak and cypress stands. Values followed by different letters on the same row indicate significant differences at $P < 0.001$.

Seedling allometric characteristic	Oak	Cypress	Oak	Cypress	Oak	Cypress
	Mean ± Std.Dev		Coef.Var.		Range	
Stem Biomass (gr/m²)	16.2 ± 3.8 ^a	9.6 ± 1.4 ^b	5.8	3.6	11.5-21.3	7.6-11.4
Canopy Biomass (gr/m²)	57.8 ± 3.5 ^b	68.1 ± 4.9 ^a	1.5	1.8	53.1-62.7	62.7-75.0
Root Biomass (gr/m²)	45.1 ± 4.9 ^a	42.6 ± 4.3 ^a	2.7	2.5	40.0-53.2	37.5-48.2
Aboveground Biomass (gr/m²)	74.0 ± 7.2 ^a	77.7 ± 6.2 ^a	2.4	2.0	64.6-84.0	70.3-86.4
Height (Cm)	34.5 ± 1.0 ^b	48.3 ± 4.3 ^a	0.7	2.3	33.0-35.5	42.5-52.5
Canopy Diameter (cm)	5.9 ± 0.7 ^a	6.7 ± 0.5 ^a	2.9	1.9	5.0-6.7	6.3-7.5

Spatial variability of fine root length

Horizontal spatial variability of fine root length density around collar seedlings was analyzed with one way ANOVA revealed significantly differences for cypress and not significantly differences for oak (20, 50, 100cm) in 95% confidence level (Figure1). This result revealed oak and cypress were not same horizontal fine root distribution around the seedlings collar while distribution in different geographic direction were same.

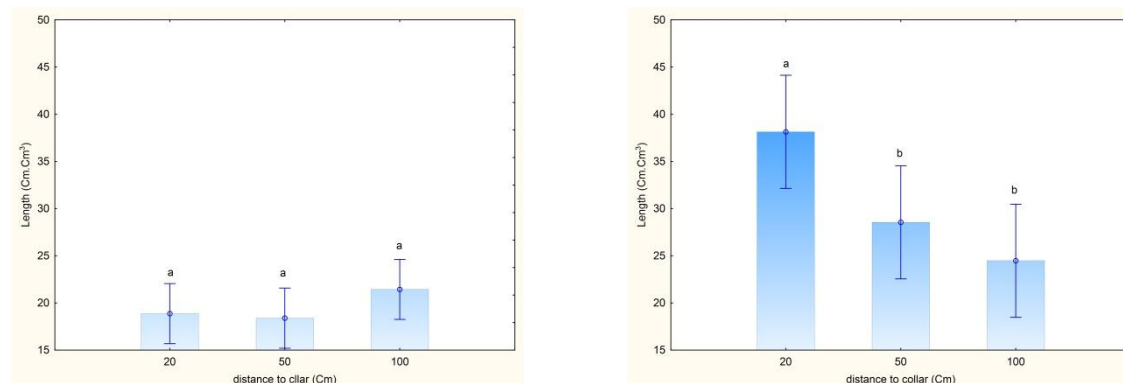


Fig I. The root length density around collar seedlings of oak (left) and Cypress (right). different letters on the histogram indicate significant differences at $P < 0.05$

Temporal variability of fine root length

We used repeated measures ANOVA to determine the effects of sampling directions and distance to collar over time, results showed in table 2.

Table 2 Repeated measures ANOVA for Oak and Cypress. Multivariate Tests of Significance, Sigma-restricted parameterization. ** indicate significant difference at $p < 0.001$

Effect	Value		F		df	p	
	Oak	Cypress	Oak	Cypress		Oak	Cypress
Intercept	0.33	0.38	294.37	232.05	2	0.00	0.00
Time	0.70	0.61	11.10**	15.98**	10	0.00	0.00
Distance	0.99	0.95	0.66	4.08**	2	0.62	0.00
Direction	0.98	0.98	0.81	0.97	7	0.56	0.44
Time*Distance	0.96	0.90	0.63	1.62**	10	0.89	0.04
Time*Direction	0.89	0.88	1.14	1.31	35	0.28	0.13
Distance*Direction	0.96	0.96	0.88	1.03	6	0.57	0.42
Time*Distance*Direction	0.85	0.81	0.84	1.05	49	0.80	0.39

According to results of table 2, the effects of time (temporal variability) on fine root length density for both species (*Quercus castaneifolia* and *Cupressus arizonica*) were significant. Figure 2 revealed significantly differences of fine root length between growing and dormant season in two species.

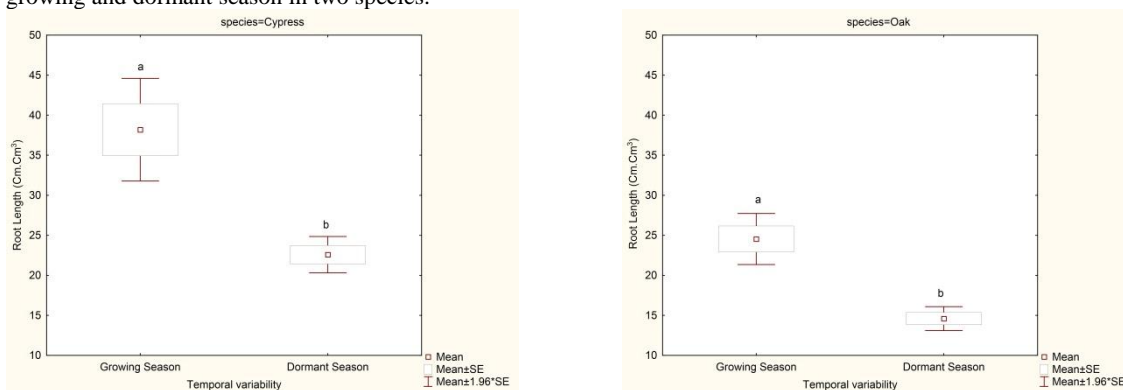


Fig 2. Temporal variability of fine root length in cypress and oak species. Different letters on the histogram indicate significant differences at $P < 0.05$

The time interval and distance to collar seedling (temporal & spatial variability) were significantly affected on fine root length of Cypress in 95% confidence level whereas in the oak species only temporal variability was true. In the 0–20 cm soil layers there were consistent differences between oak and cypress fine root length. Thus, cypress had a more shallow fine roots length than cypress (figure 3).

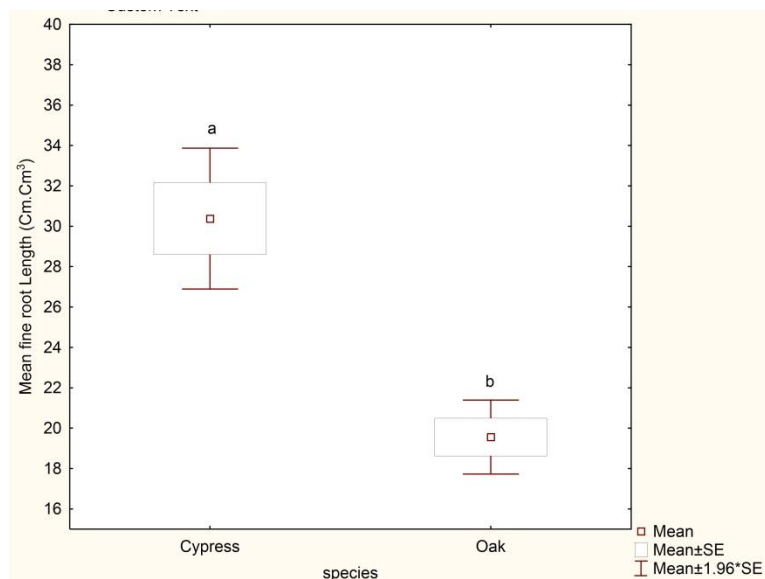


Figure 3. Mean fine root length of *Quercus castaneifolia* and *Cupressus arizonica*. Different letters on the histogram indicate significant differences at $P < 0.05$

Conclusion

The distribution of fine roots within soil depends on plant form, the spatial and temporal availability of water and nutrients, and the physical properties of the soil (Millikin & Bledsoe, 1999; John *et al.*, 2001). The deepest roots are generally found in temperate broadleaves forests; the shallowest in boreal forest (Stone, 1975). Some roots can grow as deep as the tree is high. The majority of roots on most plants are however found relatively close to the surface where nutrient availability and aeration are more favorable for growth (Tamm, 1991; Persson & Wiren, 1995; Finer *et al.*, 2011).

Many studies reported deeply vertical fine root distribution in oak (Stuart, 1979; Millikin & Bledsoe, 1999). The other hypothesis, the fine root distribution of hardwoods has deeper than softwoods. This study indicates that cypress in young pure stands has more fine root lengths concentrated to the forest floor (humus layer, 0–10 cm) and upper mineral soil horizons (10–20 cm). Thus, our results as well as support the hypothesis that broad-leaved tree species generally have deeper fine root distribution than conifers.

Siren (1955), who investigated the distribution of birch and spruce total fine root length in the soil profile in older mixed stands (>70 years), found a tendency for birch to have proportionally more fine roots in the mineral soil than Norway spruce. In contrast, Brandtberg *et al.*, (2000) who investigated the distribution of birch and spruce fine root mass in young (35–40 years) mixed stands and in pure spruce stands, found that the fine roots of both birch and spruce were concentrated to the humus layer and upper mineral soil and that there were no significant differences between birch and spruce or stand types in the vertical distribution of fine roots.

Results showed fine root lengths were same extent in different geographical directions so the both species have same fine root radial distribution around the collar seedlings in this age. We conclude gentle slope in the study area (< 8%) caused same radial rooting distribution in both species. Ji *et al.*, (2012) assigned the effects of spatial variation of tree root characteristics on slope stability. They explained root density, root length, root architecture, root tension are very important parameter that should be assayed for improvement slope stability by plants. These parameters have dependence to the ecological, physiological and genetic characteristics of plants that should be assayed accompanied by site characteristics such as soil type, soil depth, soil hydrological situations, soil nutrient, water and air for get to purpose of control and low depth massed movement during in a long period.

Although genetic structure effects on fine root distribution and root system architecture in initial ages of seedling but environmental factors could modify effect of genetic structure that is related to the circumstances so, it is possible different species had similar root distribution. John *et al.*, (2001) expressed decreasing fine roots in increasing age of forest stands that could be showed genetic factors were dominated on environmental parameters in higher ages of forest stands.

Quan *et al.*, (2010) showed that fine root depth, production and biomass had important differences among 5 understudied moderate forest types even if climate and age were similar. This showed that genetic factors in high ages of forest trees. Cheng *et al.*, (2005) proved and expressed that fine root production is variable and dependent to environmental circumstances.

Zobel (2008) 's studies on root system distribution of forest species showed that more than 95% of total root length had diameter less than 0.1 mm, fine root distribution of hardwoods and softwoods species are intensively sensitive to environmental factors somehow fine root diameter were showing their environmental circumstances. He concluded that environmental factors are very more important than genetic factors or root growth of species that can be expressed evenly root distribution around the collar seedlings of two species more clearly in this study. Wang xiao *et al.*, (2008) assayed that some environmental factors such as rains and temperature are very important on fine root production. Ganzhuo *et al.*, (2010) emphasized on root length density and water soil content. Baker (1999) issued on potential of the root production as indicator of forest ecosystem sustainability. Kramer *et al.*, (1996) expressed height as effective factor on some parameter such as biomass, length, penetration of tip roots and lateral roots. John *et al.*, (2001) know depth and extend are related to environment and genetic. They expressed that environmental factors usually effect on root growth are texture, structure, aeration, humidity and temperature soil as well as competition with other plants.

Van Hees & Clerckx (2000) studied root to shoot ratio and shade in three sapling of silver birch, pendunculate Oak and beech, they concluded that shading would cause decreasing root to shoot ratio and fine root biomass. So, this research studied another role of environmental factors on root growth and emphasized that environmental factors have large proportion on inter-specific root growth differences and it's comparison with other species.

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