



Nonstructural carbohydrates, carbon and nitrogen concentrations in fine roots of *Quercus variabilis* secondary forests after two different periods of regeneration

Chuang Ma¹, Yinghua Li¹, Haizhou You², Hong Long¹, Weiwei Yu¹, Yunchang Gao², Yuanhong Wang¹

¹College of Horticulture and Landscape, Tianjin Agricultural University, Jinjing Road 22, 300384 Tianjin, China; ²Hebei Academy of Forestry Science, Xuefu Road 75, Shijiazhuang, 050061 Hebei, China

Abstract

Aim of study: *Quercus variabilis* is a sclerophyllous oak with strong resprouting capabilities and whose regeneration is facilitated by the development of stump shoots following disturbance. During secondary forest regeneration, fine roots are important organs relative to changes in stand characteristics. Here, we aimed to provide novel insights into the chemical composition variations in roots with seasonality and root order hierarchy in a *Q. variabilis* forest at different periods of regeneration.

Area of study: The forest is located next to the Baxianshan National Reserve in the southern part of the Yanshan Mountains, Tianjin, China.

Material and methods: Six plots were established in stands with either eight or 40 years of regeneration for the repeated sampling of fine roots during the growing season of 2019. All roots were classified by branch order. The first three root orders were collected to analyse the concentrations of nonstructural carbohydrate, carbon, and nitrogen.

Main results: Short-term regeneration stands showed a reduction in soil moisture and an increase in soil temperature because of the lower canopy cover, compared to long-term stands. Soluble sugar and starch were lower in roots of short-term stands than in those of long-term stands, and the decreasing ratio of both parameters was observed in short-term stands. Less carbon and greater nitrogen concentrations of fine roots were found in short-term stands than in long-term stands, which resulted in weaker C/N ratio values. Nonstructural carbohydrate was stored more in higher order roots than terminal roots and presented greater sensitivity to forest regeneration. Redundancy discriminate analysis demonstrated that the nonstructural carbohydrate concentrations in roots were affected positively by canopy cover and negatively by soil temperature.

Research highlights: The seasonal dynamics and branch allocation of chemical reserves in fine roots varied in the different periods of forest regeneration because of the discrepancy between the canopy cover and soil traits. Less nonstructural carbohydrate and a lower C/N ratio at the onset of forest regeneration may elevate the risk of root death.

Keywords: soluble sugar; starch; forest regeneration; root order; C/N ratio; redundancy discriminate analysis.

Authors' contributions: Conceived and designed the experiments: CM and YHL. Performed the experiments: CM, WWY and YCG. Analysed the data: CM, HL and YCG. Contributed reagents/materials/analysis tools: CM, YHL and YHW. Wrote the paper: CM, YHL and HL.

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Correspondence should be addressed to Chuang Ma: machuang_tjau@126.com

Introduction

Fine roots are a key component of the root system in trees, as they play an important role in nutrient uptake and

carbon (C) cycling. Although root production rarely represents more than 5% of the total tree biomass, it contributes approximately 33–67% of total annual net primary production (Jackson *et al.*, 1997). As has been confirmed

in various forest stands, the growth of fine roots is affected by several factors, such as air temperature, precipitation, stand age, and elevation (Pregitzer *et al.*, 2000; Zeleznik *et al.*, 2016; Pei *et al.*, 2018). Recently, a considerable number of studies have suggested that forest regeneration often shifts the fine root biomass, which may reach a minimum after several years of thinning prior to recovery upon the initiation of aboveground organ regrowth (Lopez *et al.*, 2003; Yuan & Chen 2013; Mosca *et al.*, 2017). However, little information is available on the chemical composition of fine roots in response to regeneration after forest damage or harvest.

Carbohydrates are the most important compounds in trees and constitute more than 90% of the available C in plants (Boldingh *et al.*, 2000; Kosola *et al.*, 2002; Hoch & Körner, 2003). Soluble sugars, including glucose, sucrose, fructose, and maltose, offer C and energy directly for plant growth. Starch, on the other hand, is stored in the roots and is generally used to buffer reductions in free sugar when an imbalance of photosynthesis occurs (Noland *et al.*, 1997; Sayer & Haywood, 2006). In regenerating tree stands, the pre-damage carbohydrates in roots are the only source of C supporting aboveground tissue growth until they are replenished by assimilate production in the new leaves. Fang *et al.* (2007) reported that clipping accelerated the reduction of starch in the roots and increased sucrose flux. Luostarinen & Kauppi (2005) found that recent coppicing clearly reduced both sugar and starch concentrations in the roots. These results indicate that soluble sugar and starch in roots are closely related to aboveground growth. Therefore, the detection of root carbohydrate, especially the seasonal dynamics of nonstructural carbohydrate reserves in the roots, may provide further insights with which to understand forest regeneration.

Carbon concentration in fine roots is associated with growth costs; nitrogen (N) concentration, on the other hand, is more related to root metabolic and respiratory activities (Pregitzer *et al.*, 1997; Wang *et al.*, 2017). Numerous studies have shown that C and N concentrations are consistent with root morphology and lifespan (Pregitzer, 2002; McCormack *et al.*, 2012). With seasonal growth, C and N may be reallocated among the root tissues or other sink tissues, leading to changes in their concentrations (Cerasoli *et al.*, 2004; Chen *et al.*, 2017). In mature temperate deciduous trees, C reserves are formed during the growing season, reaching maximal concentrations at leaf senescence (Bazot *et al.*, 2013; Gilson *et al.*, 2014). C fluctuations have been found to occur more significantly in branches and belowground organs than in other compartments (Genet *et al.*, 2010), suggesting that roots play a more important role in C storage. The main nitrogen stored as proteins is synthesized between the end of summer and leaf senescence, and remobilized in the spring for the growth of the youngest parts of the trees, such as

medium-sized and fine roots, branches, and the youngest rings (Valenzuela Nunez *et al.*, 2011, Bazot *et al.*, 2013). The C and N stores are key to understanding tree survival; however, few studies have focused on the C and N chemical composition of fine roots responding to forest regeneration. Recently, Terzaghi *et al.* (2013) confirmed that a significant change in the C and N concentrations in fine roots occurred after regrowth of a coppice into a tall forest. However, no more specific records regarding the influence of environmental variables on these chemical reserves during this process were provided.

Chinese cork oak (*Quercus variabilis*) is an important woody species both for economic development and for ecosystems in China, Korea, and Japan (Xue *et al.*, 2014). An increasing number of trees are being cut because of the increasing market share for utilizable cork. Root systems in this species are maintained completely in the stumps and often support the growth of multiple sprouts through mineral absorption from the soil (Johnson *et al.*, 2002; Xue *et al.*, 2013). Previously, we reported on the effect of thinning on the biomass and morphological characteristics of fine roots in oak forests (Ma *et al.*, 2013; 2015). In the present study, we focused on the nutrient reserves in fine roots during the process of forest regeneration. In the experiment reported herein, nonstructural carbohydrates and C and N concentrations of the first three root orders were investigated in *Q. variabilis* forest stands after regeneration periods of eight and 40 years. Specifically, this study aimed to (1) test the hypothesis that the chemical composition of fine roots varies with the period of forest regeneration; (2) describe the seasonal dynamics of non-structural carbohydrates, C, and N across the first three fine root orders; and (3) detect the relationships between the chemical composition of fine roots and environmental factors.

Materials and methods

Study site and plant material

This study was carried out in a *Q. variabilis* forest in the southern part of the Yanshan Mountains (40°10'–40°14'N, 117°30'–117°36' E), Tianjin, China (Fig. 1). This region belongs to the warm temperate zone, with an annual mean temperature of 10.1 °C and an annual precipitation of 970 mm during the entire research period. More than 50% of the annual rainfall occurs in July, August, and September. The soils of the experimental plots are luvisols and cinnamon soils (FAO classification) with depths of 15 to 25 cm. The region belongs to the main distribution area of *Q. variabilis*. Numerous deciduous shrubs are distributed in the area, including *Vitex negundo*, *Grewia biloba*, and *Deutzia scabra*, as well as herbaceous sub-shrubs, including *Carex tristachya* and *Clematis heracleifolia*.

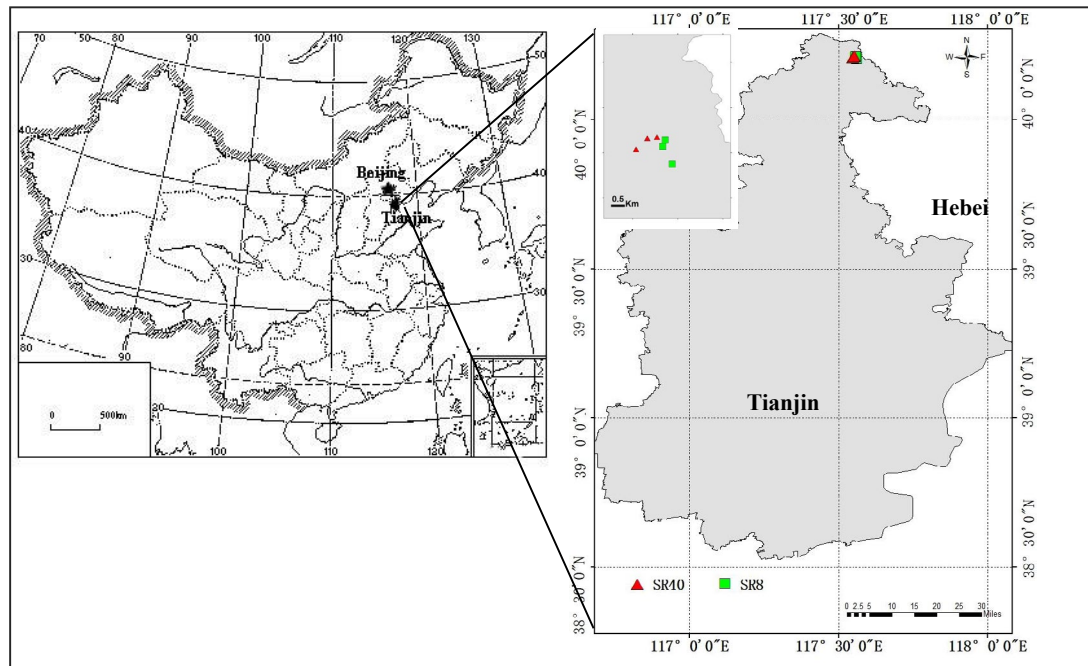


Figure 1. Location of the study sites in the Baxianshan National Reserve, Tianjin, China.

A 1.5 ha area of *Q. variabilis* forest was clear-cut 40 years ago. Since then, it has been allowed to re-grow from stumps without any further disturbance. This area served as the long-term regeneration stands (SR40). Other small areas of *Q. variabilis* forest were clear-cut in the spring of 2011 to meet the demand for cork and timber by national law and local regulations, and then allowed to regrow for eight years. These areas serve as the short-term regeneration stands (SR8). After clear-cutting, the stems were removed, while the stumps and roots were maintained on site for new shoot regeneration. Six 20 m × 20 m plots were established in both types of forest stand with different regeneration periods. The two types were adjacent to each other and no significant differences in soil chemistry were recorded.

Stand characteristics

The diameter at breast height (DBH) was recorded using a measuring tape at a height of 1.3 m from the ground in September 2019. Canopy cover was measured using the hemispherical photo method (Rich, 1990) and height was measured using the VertexIV instrument (Haglöf Inc. Sweden). All data were correlated to the stand area and corrected for the slope angle. The accumulation of the basal area and stem density were expressed per hectare. Ten points on each plot were selected randomly for soil sampling, and the soil moisture and temperature at 10 cm depth below the soil surface were recorded weekly with a Theta KIT TK3-BASIC meter (DELTA-T Inc., UK) and a HI8751 probe type thermometer (Hanna Inc.,

Italy), respectively, in the experimental months. A total of 120 values in each month were used to calculate the average soil temperature or moisture of each regeneration forest type.

Root sampling

Quercus variabilis is a deciduous tree and its growth season in this area lasts from the end of March (budburst) to the beginning of October (leafless). Root samples were collected at the three key phenological stages of *Q. variabilis* in this area in 2019, namely leaf expansion in spring (April 25th), leaf maturity in summer (June 30th), and leaf senescence in early autumn (September 22nd). Nine *Q. variabilis* individuals with the same stem basal diameter were chosen along the two diagonal paths within each plot. Blocks of soil (20 cm × 20 cm × 10 cm), gathered under the crown to avoid the roots of neighbor trees or shrubs, were sampled in four cross directions at distances of 0.2 m from each trunk or stump. Roots from *Q. variabilis* were easily distinguished by their dark red color, high elasticity, and the intensive cohesion of stele and cortex or periderm compared with the roots of shrubs. At each sampling time, 216 soil blocks (108 per regeneration stand), collected from three plots per stand, were separately placed in plastic bags and then transported to the laboratory in an ice box. The large intact branch roots were carefully retrieved from the soil with forceps under water at 4 °C. Live roots were identified by their bright color, elasticity, and the strong cohesion between stele and cortex or periderm (Vogt & Persson,

1991). Live roots were washed thoroughly with distilled water at 4 °C and then dissected into branch orders (Fitter, 1987; Pregitzer, 2002). The most distal root tips were labeled as the first order roots and the more proximal root tips were labeled as the second and third order roots (Pregitzer *et al.*, 2002; Wang *et al.*, 2006). The first three root orders were collected for analysis in this study (Guo *et al.*, 2004).

Assay of soluble sugar and starch

Root samples from the same order in four soil blocks of each trunk or stump were pooled together and stored in a freezer at -80 °C. The root samples were freeze-dried for 72 h and then ground to a fine powder in liquid N₂ with a mortar and pestle. Fifty milligrams of the powdered root samples were homogenized in 80% ethanol, and then bathed at 80 °C for 1 h. After the homogenates were centrifuged at 3 000 rpm for 20 min, the supernatant was collected for soluble sugar analysis. Because most of the ethanol was removed by evaporation at 80 °C, the supernatant was brought to the same volume and to 25 ml with distilled water (Smith, 1969).

Starch was extracted from the residue by adding 2 ml of 52% perchloric acid and then shaking it for 15 min in an orbital shaker (Bowen & Pate, 1993). After the extract was centrifuged at 3 000 rpm for 20 min, the supernatant was moved into a 50 ml flask using a pipette. The residue was repeated three times to extract starch with perchloric acid. The supernatant from three extractions was pooled together into the same flask.

To quantify the soluble sugar and starch concentrations, a 0.5 ml extract in a glass tube was mixed with 1.5 ml of distilled water and 0.5 ml anthrone solution followed by the addition of 5 ml of concentrated H₂SO₄. After the solution was placed in a boiling water bath for 1 min and then cooled to room temperature (20–25 °C), the absorbance was measured, respectively, at 630 nm by an ultraviolet and visible spectrophotometer (UV1800, Shimadzu, Japan; Dubois *et al.*, 1956). The concentration of soluble sugar was determined by reference to the standard curve previously constructed for glucose, and the starch was quantified as glucose equivalents (Barbaroux & Breda, 2002). The soluble sugar divided by the starch concentration in the same roots was calculated as the soluble sugar/starch ratio (SS/ST).

Assay of root carbon and nitrogen

Root samples were classified according to root branch order and oven-dried at 120 °C for 2 h and then at 80 °C for 24 h to a constant mass. After being ground in liquid N₂ with a mortar and pestle, the total organic C and N in roots from the same branch order were determined using an elemental analyzer (VarioELIII, Elementar Inc. Germany) and the C/N ratio was then calculated by dividing total C by total N.

Statistical analysis

The Shapiro-Wilk test was used to test the probability of the data fitting the normal distribution before the chemical traits were subjected to a three-way analysis of variance (ANOVA) with regeneration periods, root order, and season (sample timing) as the main factors. For each chemical trait, a one-way ANOVA and the Duncan pairwise multiple comparison test were performed among seasons within each regeneration period. The independent samples t-test was performed for the pair-wise comparison between the two regeneration periods within each season. Differences at $P < 0.05$ were considered significant. The SPSS statistical package (PROC GLM Procedures, SPSS Inc. 2009) was used for all calculations and graphs were constructed using SigmaPlot 18.0.

Redundancy discriminate analysis (RDA) was conducted using CANOCO (Windows v5.5, Netherlands) and revealed that the variation in chemical reserves was decomposed into variation related to environmental variables. In the biplots diagram, each canonical axis is a linear combination of all explanatory variables (stand situations; Legendre & Gallagher 2001).

Results

Stand characteristics

In the short-term regeneration stands (SR8), there was more than one shoot per stool, causing high stem density. The average height in the short-term regeneration stands was 54.1% of that in the long-term regeneration stands (SR40), whereas the DBH in SR8 ranged from 4.1 cm to 7.2 cm, which was only 37.2% of SR40 (Table 1).

Table 1. Above-ground characteristic for the investigated stands. Values are the mean of three replicate plots (SE)

Stands	Canopy cover (%)	Density (ha ⁻¹)	DBH (cm)	Basal area (m ² ·ha ⁻¹)	Height(m)
SR40	92.4±0.8	978±60	15.3±1.3	18.7±2.8	13.7±2.1
SR8	66.1±2.4	1025±56	5.7±1.4	3.8±1.2	7.4±1.7

Consequently, canopy cover decreased by 28.5% in SR8 compared with S40. The low canopy cover in SR8 caused the seasonal variation of soil moisture and temperature to appear more drastic. Compared to SR40, average soil moisture in SR8 decreased by 21.8% in June, whereas the average soil temperature in June (19.3–27.5 °C daily) and September (16.7–23.9 °C daily) significantly increased by 16.7% and 20.7%, respectively (Table 2).

Soluble sugar and starch

The three-way ANOVA demonstrated the significant effect of the regeneration period and season on soluble sugar, starch, and the ratio of both (Table 3). The effect of root order was significant for soluble sugar and starch, but not significant for the ratio of both. The interaction among regeneration period, season, and root order was more significant for starch than soluble sugar.

A significantly lower soluble sugar concentration was observed in SR8 than in SR40, except for the first order roots in April (Fig. 2). In the first-order roots, the soluble sugar concentration decreased by 12.2%, 54.8%, and 38.6% in April, June, and September, respectively, com-

pared with SR40 (76.1 mg·g⁻¹, 64.3 mg·g⁻¹, and 77.9 mg·g⁻¹, respectively). The same parameter in the second-order roots of SR40 was observed as 26.4%, 42.1%, and 36.9% in April, June, and September, respectively (68.5–104.9 mg·g⁻¹), while those of the third-order roots were 43.3%, 26.5%, and 35.1%, respectively (73.3–158.1 mg·g⁻¹). The highest concentration of soluble sugar was found in April (76.1–158.1 mg·g⁻¹ for SR40 and 66.8–89.7 mg·g⁻¹ for SR8) and the lowest was found in June (64.2–73.3 mg·g⁻¹ for SR40 and 29.1–53.7 mg·g⁻¹ for SR8). In September, a slight increase in soluble sugar compared to April and June was observed in the first three orders of both regeneration forests.

For starch, the concentration in the roots increased with the branch order (4.7–17.2 mg·g⁻¹ for SR40 and 2.9–14.8 mg·g⁻¹ for SR8; Fig. 2). Starch concentration in the first-order roots was higher in SR40 than in SR8 without significance. However, in the second- and third-order roots, the starch concentration in SR8 in September decreased significantly by 26.8% and 23.7%, respectively, compared with SR40 (Fig. 2). The minimum starch concentration value was observed in June in both SR40 and SR8, and a significant difference between both regeneration forests was observed in the third-order roots.

Table 2. Monthly soil moisture and temperature in the 10 cm depth below the surface for the investigated stands. The data of each stage is denoted as mean ± standard error. Different letters (a, b, c) indicate significant differences (*p* < 0.05) across the seasons. * means significant differences (*p* < 0.05) between SR40 and SR8 in the same time (n=120)

Stands	Soil moisture (%)		Soil temperature (°C)	
	SR40	SR8	SR40	SR8
April	8.7 ± 1.6 b	7.6±1.5 b	8.3 ± 1.9 c	11.7±1.7 c
June	17.3 ± 1.5 a	13.6±1.3 a*	20.9 ± 2.5 a	24.4±2.1 a*
September	18.5 ± 1.3 a	16.1±1.4 a	15.9 ± 2.3 b	19.2±2.4 b*

Table 3. *F* value of the effects of regeneration periods, root orders and sample times on the soluble sugar, starch, SS/ST ratio, C concentration, N concentration and C/N ratio through a mix-level (2×3×3) three-way ANOVA. Significance level: ***p*<0.01, ****p*<0.001. n.s. means that the effect was not significant and removed from the model

		Soluble sugar	Starch	SS/ST ratio	C	N	C/N ratio
Period	F	202.21***	53.236***	89.499***	652.448***	23.708***	116.784***
Root order	F	77.558***	107.658***	14.391***	64.049***	132.527***	151.273***
Season	F	63.463***	297.580***	187.862***	196.015***	n.s.	48.220***
Period * root order	F	n.s.	4.815**	n.s.	n.s.	n.s.	4.281**
Period * season	F	6.936***	23.732***	35.297***	13.185***	n.s.	4.182**
Root order* season	F	3.924**	n.s.	16.671***	7.377***	3.077**	5.388***
Period * root order * season	F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

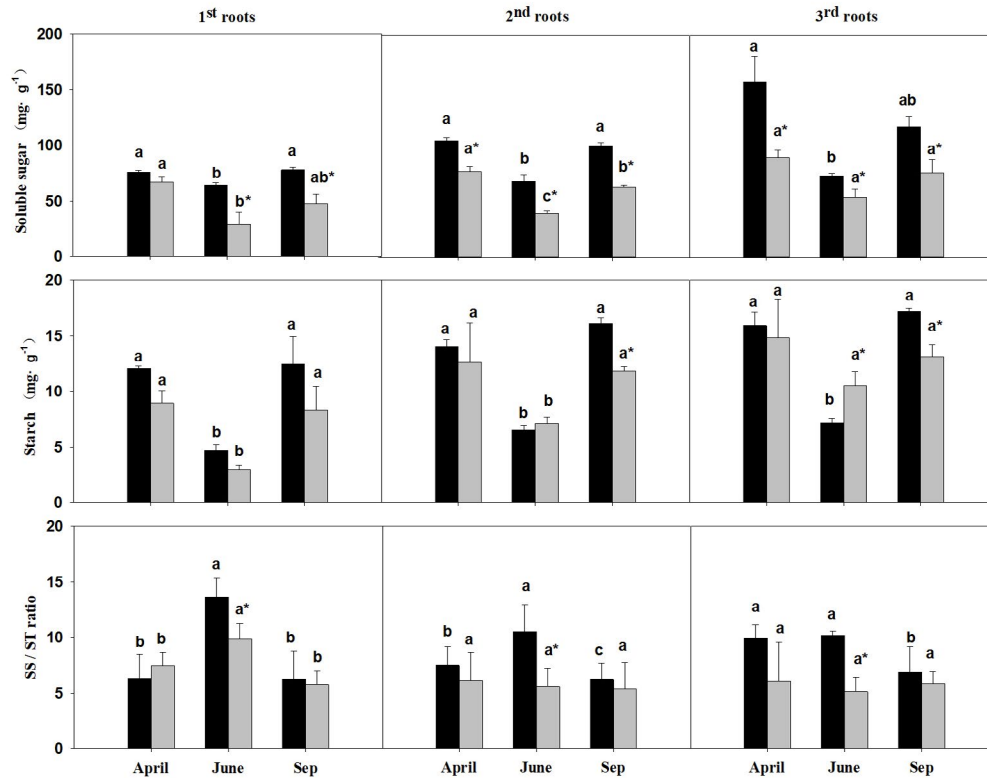


Figure 2. Soluble sugar, Starch concentrations and the ratio of both (SS/ST) along the experiment time (mean±SE). Different letters (a, b, c) indicate significant differences (*p* < 0.05) across the seasons in the same order. * means significant differences (*p* < 0.05) between SR40 (black bar) and SR8 (grey bar) in the same time.

SR40 exhibited a higher SS/ST ratio than SR8 in the roots, except for the first-order roots in April (Fig. 2). In June, the SS/ST ratio in SR8 fell significantly by 27.6% for the first-order roots, 46.8% for the second-order roots, and 49.7% for the third-order roots in contrast to SR40. No significant differences in the SS/ST ratio in SR8 were observed in April and September.

Carbon and nitrogen concentrations

The regeneration period and root orders significantly affected the roots' C concentration, N concentration, and the ratio of both, while seasonality showed a significant effect on the C concentration and C/N ratio (Table 3). No significance was observed regarding the interaction between the regeneration period and the season on the C concentration, N concentration, or the C/N ratio. The interaction between the regeneration period, root orders, and season took a slight impact on the N concentration and C/N ratio, but did not significantly impact the C concentration (Table 3).

C concentration increased with branch position from the first-order to the third-order roots (383.4–453.5 mg·g⁻¹ in SR40 and 352.3–437.4 mg·g⁻¹ in SR40; Fig. 3). Significant differences across seasons were found in both periods

of regeneration with regard to the C concentration, which increased significantly from April to June, and then decreased slightly in September (Fig. 3). For a given season, a lower C concentration was observed in the first three root orders in SR8 compared with SR40, particularly in the second- and third-order roots in June. Compared with the C concentration in SR40, the average corresponding value in SR8 decreased by 7.1% and 4.6% in the first- and third-order roots, respectively.

N concentration displayed an opposite pattern to that of C concentration. A significantly greater N concentration was found in SR8 (9.5–14.7 mg·g⁻¹) than in SR40 (8.6–12.9 mg·g⁻¹; Fig. 3). N concentration in SR8 increased by 16.9% in the first-order roots and 12.1% in the third-order roots in comparison with SR40. A lower N concentration was measured in June compared to that measured in the other months, although seasonal differences were not significant (Table 3).

The C/N ratio ranged approximately from 29 to 77 in SR40 and from 23 to 46 in SR8 (Fig. 3). However, the average values of the C/N ratio declined by as much as 20.8% in the first-order roots and 26.1% in the third-order roots of SR8 compared with SR40. The maximum values for the C/N ratio for the stands in both periods of regeneration were observed in June with significant differences.

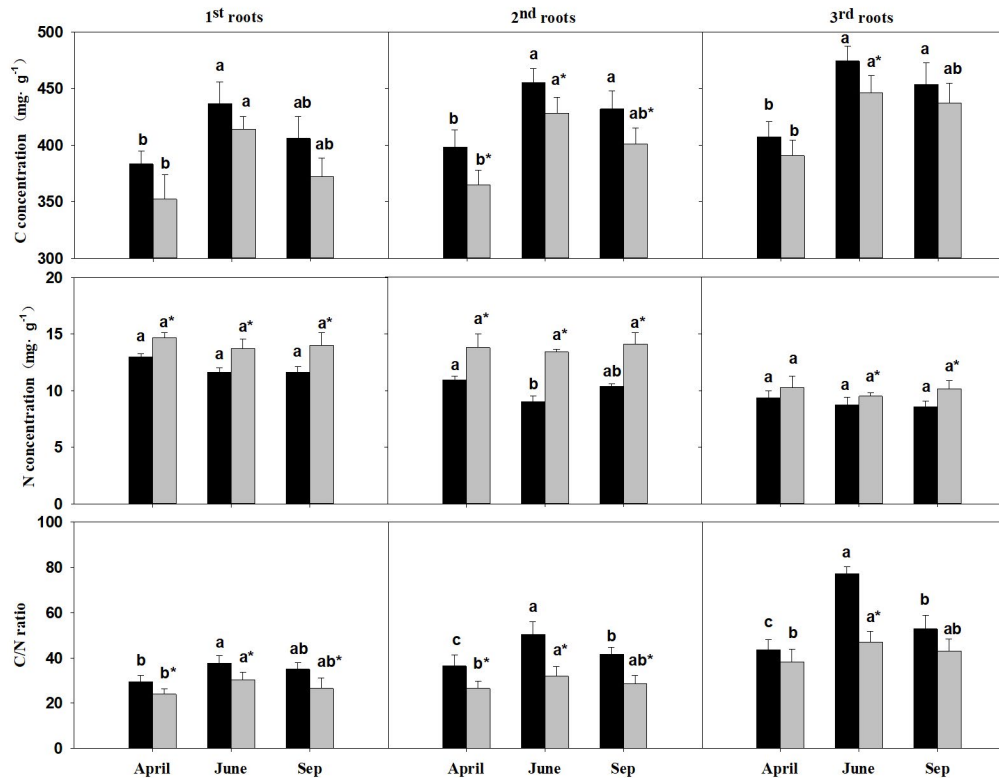


Figure 3. Total C, N concentrations and C/N ratio along the experiment time (mean±SE). Different letters (a, b, c) indicate significant differences ($p < 0.05$) across the season in the same order. * means significant differences ($p < 0.05$) between SR40 (black bar) and SR8 (grey bar) in the same time.

Redundancy discriminate analysis

The result of the RDA was a significant relationship between the chemical composition of the fine roots and the stand situations. The first two axes accounted for 51.1% and 13.5% of the variation of the chemical reserves (Fig. 4). The soluble sugar concentration was found to be primarily influenced by canopy cover ($p < 0.01$, $r = 0.546$), while the correlation between starch and canopy coverage was poor ($p > 0.05$, $r = -0.135$). The C concentration in the roots was related to canopy cover ($p < 0.05$, $r = 0.353$). Additionally, soil moisture and temperature were observed to affect carbon positively ($p < 0.001$, $r = 0.583$; $p < 0.001$, $r = 0.480$) and soluble sugar negatively ($p < 0.05$, $r = -0.353$; $p < 0.01$, $r = -0.461$; Fig. 4).

Discussion

Effect of regeneration periods on chemical reserves in roots

Nonstructural carbohydrate reserves (starch and soluble sugars) are critical for the survival of trees during forest regeneration (Kaelke & Dawson, 2005); further, they determine root respiration, absorption, and transport

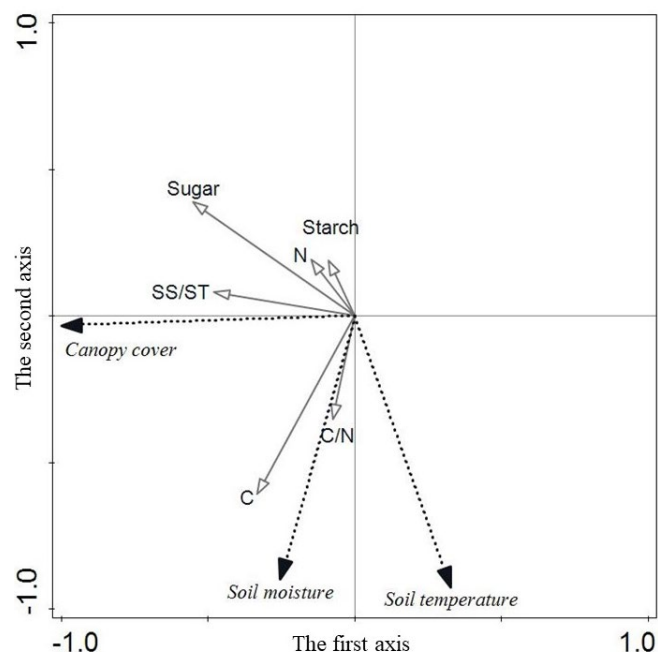


Figure 4. Biplots of the redundancy analysis (RDA) of chemical composition in roots, including soluble sugar, starch, SS/ST ratio, C concentration, N concentration and C/N ratio, and stand situations, including soil temperature, soil moisture, canopy cover. The vector length indicates the strength of the influence, pointing in the same direction as root reserves arrows means positive, opposite direction negative correlation.

activities (Noland *et al.*, 1997; Stobrawa & Lorenc-Plucinska, 2007). In this study, a clear decline in nonstructural carbohydrates occurred in short-term regeneration stands (Fig. 2), which is in agreement with the results of a previous study that showed a striking decrease in the sugar and starch concentrations in roots after the removal of whole birch stems (Luostarinen & Kauppi, 2005). Kosola *et al.* (2002) suggest that this resulted from the shortage of photosynthate available for roots. However, McCormack & Guo (2014) reported that fine roots are greatly influenced by temperature and water availability. Recent literature also emphasized that the root growth rate is high under a moderate soil temperature (19–21 °C; Di Iorio *et al.* 2016).

It is surmised, therefore, that the increasing soil temperature (within a certain range) characterized in the short-term regeneration stands of this study promotes the activities of enzymes related to nonstructural carbohydrates utilized in roots. In addition, a lower ratio of soluble sugars to starch was found in these stands (Fig. 2), indicating that the conversion of soluble sugars into starch occurred during the preliminary stage of forest regeneration. In many resprouting plants, carbohydrates are stored as starch in roots and will be mobilized to support aboveground tissue regrowth after shoot damage (Fang *et al.*, 2007). Lacoite *et al.* (2016) suggest that starch could be of importance to plants in the case of total defoliation. Di Iorio (2016) has reported that starch accumulation was induced by thermal acclimation in fine roots that originate from exposure to high soil temperature. In our case, the declining ratio of soluble sugars to starch may be an ecological strategy which gradually provides materials and energy for roots in the case of adverse environmental conditions during forest regeneration.

An average value of approximately 50% for C concentration has been widely used (Makineci *et al.*, 2015), whereas a low C concentration was observed in short-term regeneration stands in this study (Fig. 3). This finding could be related to the reduction of nonstructural carbohydrate in order to support newly developing tissues. Terzaghi *et al.* (2013) has confirmed that starch concentration changes explained 38% of the C concentration variation. In other studies, secondary metabolites such as lignin have been confirmed to take a greater contribution of the C content in tissue than cellulose or other sugars (Chua & Wayman, 1979; Krässig, 1993). Terzaghi *et al.* (2016) observed that the C concentration of roots decreased in five years after felling as a consequence of a higher cellulose to lignin concentration ratio. Therefore, we speculate that the low C concentration in fine roots of short-term regeneration stands could be due to less lignin, which merits further investigation.

In this study, a slight increase in N concentration was observed in short-term stands (Fig. 3). This finding is consistent with the results of Terzaghi *et al.* (2013) who

found that N concentration was significantly higher in a coppice stand thinned five years previous to sampling compared to mature stands. The relatively high N concentration in roots is conducive to improving absorption for the renewed aboveground part, but accelerates root death (King *et al.*, 2005). Moreover, a decrease in the fine root C/N ratio in the short-term regeneration stands of this study also highlighted that the lower C investment in the construction of fine roots in these stands was balanced by their shorter lifespan. As reported previously, the longer-term removal of aboveground parts, along with limited C storage, clearly leads to increased root mortality (Eissenstat & Duncan, 1992; Hartmann *et al.*, 2013; Terzaghi *et al.*, 2016). This may be a reason why, in our previous work, fine root production decreased in stands clear cut for three years (Ma *et al.*, 2013). However, interpretation of the root lifespan is often complicated. Jones *et al.* (2003) created artificial forest gaps which increased local temperature variability along the gap edges and led to reduced root lifespan. Indeed, variation in soil traits has been observed in our previous and current work, which is an undeniable factor affecting root biomass and chemical reserves (Wang *et al.*, 2019).

Seasonal dynamics of chemical reserves in roots

During the experiment reported herein, soluble sugar and starch profiles after different periods of forest regeneration were similar across seasons, with minimum values found in June (Fig. 2). A decrease in the nonstructural carbohydrate reserves as a consequence of bud break in spring and active tree growth in early summer has also been observed in the roots of birch (Abod & Webster, 1991). Contrary to the seasonal dynamics of nonstructural carbohydrates, we confirmed that the C concentration in fine roots for both regeneration periods under study was higher in summer than in spring or autumn (Fig. 3). This finding is in agreement with the literature that observed a peak in C concentration in summer while nonstructural carbohydrate levels were lowest (Cerasoli *et al.*, 2004). This could be explained by the maximum vegetative activity which requires a reduction of nonstructural carbohydrates investment. Hishi & Takeda (2005) suggest that the seasonal fluctuations in root C concentrations reflect substantial growth of the fine roots. Furthermore, a slight decrease in root nitrogen content was observed in summer, strengthening the conclusion that the utilization of root N reserves supports newly developing tissues. However, there was no significant difference in N concentration over seasons, which may be explained by N retranslocation from stem to roots compensating for the loss (Eason & Newman, 1990). The fact has been confirmed that seasonal changes in fine root N concentration may be due to internal nutrient cycling (Gordon & Jackson, 2000).

Chemical reserves across root branching hierarchy

The position of an individual root in the branching root system appears to be important for understanding the function of fine roots (Pregitzer *et al.*, 2002; Hishi, 2007). In the present study, an increase in soluble sugar and starch concentrations was observed with increasing root order (Fig. 2). The short distance between the advanced root orders and buds indicates that soluble sugar in these roots can be transferred rapidly for aboveground regeneration (Zadworny *et al.*, 2015). This may explain why, in the experiment reported herein, the allocation of nonstructural carbohydrate in higher order roots was seemingly more sensitive to the process of forest regeneration compared to the primary roots. First-order roots are usually newly emerged and serve as actively physiological parts of the root hierarchy. Guo *et al.* (2004) showed that nonstructural carbohydrate was preferably maintained in lower-root orders for the uptake of water and minerals when longleaf pine was scorched. Furthermore, in this study, significant increases in N concentration were noticed in the first-order roots, indicating that more N concentration in the lower order roots was supplied for water and nutrient uptake in the short-term regeneration stands (Wang *et al.*, 2017). The results support our hypothesis that the chemistry reserves of fine roots vary across root orders with forest regeneration. Other aspects, such as the relocation of chemistry reserves in the roots with different positions, remain unclear and could be important topics for future research.

Conclusions

Our study showed that the chemical composition of fine roots in regeneration stands varied with the extent of the regeneration period. Compared to long-term stands, less canopy cover in short-term stands resulted in a drier and warmer soil surface. Short-term stands showed lower nonstructural carbohydrate in the first three root orders than the long-term stands, and greater sensitivity to forest regeneration was observed in the high order roots rather than the terminal roots. The ratio between soluble sugar and starch decreased in the fine roots of the short-term stands. Lower C concentration and higher N concentration were observed in the short-term regeneration stands. Consequently, a decline in the C/N ratio was found in the roots of the short-term stands. The results of the RDA showed that canopy cover with forest regeneration created a positive effect on soluble sugar and C concentration in roots, while soil moisture and temperature were negatively correlated with the soluble sugar in roots. These results suggest that the changes in the chemical storage profiles of fine roots are induced by the variance in the

stand canopy cover and soil microenvironment at different regeneration stages. Fine roots in short-term forest regeneration stands are extreme risk from lower nonstructural carbohydrate and C/N ratio. Future studies should be conducted on the dynamics of roots with forest regeneration in other woody species, especially on the secondary metabolites and tissue anatomy of fine roots in detail.

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References

- Abod SA, Webster AD, 1991. Carbohydrates and their effects on growth and establishment of *Tilia* and *Betula*: I. Seasonal changes in soluble and insoluble carbohydrates. *J Pomol Horticult Sci* 66(2): 235-246. <https://doi.org/10.1080/00221589.1991.11516150>
- Barbaroux C, Breda N, 2002. Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. *Tree Physiol* 22(17):1201-1210. <https://doi.org/10.1093/treephys/22.17.1201>
- Bazot S, Barthes L, Blanot D, Fresneau C, 2013. Distribution of non-structural nitrogen and carbohydrate compounds in mature oak trees in a temperate forest at four key phenological stages. *Trees Struct Funct* 27: 1023-1034. <https://doi.org/10.1007/s00468-013-0853-5>
- Boldingh H, Smith GS, Klages K, 2000. Seasonal concentrations of non-structural carbohydrates of five *Actinidia* species in fruit, leaf and fine root tissue. *Ann Bot* 85(4): 469-476. <https://doi.org/10.1006/anbo.1999.1094>
- Bowen BJ, Pate JS, 1993. The significance of root starch in post-fire shoot recovery of the resprouter *Stirlingia latifolia* R. Br. (Proteaceae). *Ann J Bot* 72(1): 7-16. <https://doi.org/10.1006/anbo.1993.1075>
- Cerasoli S, Maillard P, Scartazza A, Brugnoli E, Chaves MM, Pereira JS, 2004. Carbon and nitrogen winter storage and remobilisation during seasonal flush growth in two-year-old cork oak (*Quercus suber* L.) saplings. *Ann For Sci* 61(7): 721-729. <https://doi.org/10.1051/forest:2004058>
- Chen H, Dong Y, Xu T, Wang Y, Wang H, Duan B, 2017. Root order-dependent seasonal dynamics in the carbon and nitrogen chemistry of poplar fine roots.

- New Forest 48(5): 587-607. <https://doi.org/10.1007/s11056-017-9587-3>
- Chua MGS, Wayman M, 1979. Characterization of autohydrolysis aspen (*P. tremuloides*) lignins. Part 1. Composition and molecular weight distribution of extracted autohydrolysis lignin. *Can J Chem* 57: 1141-1149. <https://doi.org/10.1139/v79-187>
- Di Iorio A, Giacomuzzi V, Chiatante D, 2016. Acclimation of fine root respiration to soil warming involves starch deposition in very fine and fine roots: a case study in *Fagus sylvatica* saplings. *Physiol Plant* 156(3): 294-310. <https://doi.org/10.1111/ppl.12363>
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F, 1956. Colorimetric method for the determination of sugars and related substances. *Anal Chem* 28: 350-356. <https://doi.org/10.1021/ac60111a017>
- Eason WR, Newman EI, 1990. Rapid cycling of nitrogen and phosphorus from dying roots of *Lolium perenne*. *Oecologia* 82(4): 432-436. <https://doi.org/10.1007/BF00319782>
- Eissenstat DM, Duncan LW, 1992. Root growth and carbohydrate responses in bearing citrus trees following partial canopy removal. *Tree Physiol* 10(3): 245-257. <https://doi.org/10.1093/treephys/10.3.245>
- Fang X, Li Y, Xu D, Yang X, Gang W, 2007. Activities of starch hydrolytic enzymes and starch mobilization in roots of *Caragana korshinskii* following above-ground partial shoot removal. *Trees* 21(1): 93-100. <https://doi.org/10.1007/s00468-006-0100-4>
- Fitter A, 1987. An architectural approach to the comparative ecology of plant root systems. *New Phytol* 106 (s): 61-77. <https://doi.org/10.1111/j.1469-8137.1987.tb04683.x>
- Genet H, Breda N, Dufrene E, 2010. Age-related variation in carbon allocation at tree and stand scales in beech (*Fagus sylvatica* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.) using a chronosequence approach. *Tree Physiol* 30: 177-192. <https://doi.org/10.1093/treephys/tpp105>
- Gilson A, Barthes L, Delpierre N, Dufrene É, Fresneau C, Bazot S, 2014. Seasonal changes in carbon and nitrogen compound concentrations in a *Quercus petraea* chronosequence. *Tree Physiol* 34(7): 716-729. <https://doi.org/10.1093/treephys/tpu060>
- Gordon WS, Jackson RB, 2000. Nutrient concentrations in fine Roots. *Ecology* 81: 275-280. [https://doi.org/10.1890/0012-9658\(2000\)081\[0275:NCIFR\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[0275:NCIFR]2.0.CO;2)
- Guo DL, Mitchell RJ, Hendricks JJ, 2004. Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. *Oecologia* 140(3): 450-457. <https://doi.org/10.1007/s00442-004-1596-1>
- Hartmann H, Ziegler W, Trumbore S, 2013. Lethal drought leads to reduction in nonstructural carbohydrates in Norway spruce tree roots but not in the canopy. *Funct Ecol* 27: 413-427. <https://doi.org/10.1111/1365-2435.12046>
- Hishi T, 2007. Heterogeneity of individual roots within the fine root architecture: causal links between physiological and ecosystem functions. *J For Res* 12(2): 126-133. <https://doi.org/10.1007/s10310-006-0260-5>
- Hishi T, Takeda H, 2005. Dynamics of heterorhizic root systems: Protoxylem groups within the fine-root system of *Chamaecyparis obtusa*. *New Phytol* 167(2): 509-521. <https://doi.org/10.1111/j.1469-8137.2005.01418.x>
- Hoch G, Körner C, 2003. The carbon charging of pines at the climatic treeline: a global comparison. *Oecologia* 135(1): 10-21. <https://doi.org/10.1007/s00442-002-1154-7>
- Jackson RB, Mooney HA, Schulze ED, 1997. A global budget for fine root biomass, surface area, and nutrient contents. *Proc Natl Acad Sci USA* 94(14): 7362-7366. <https://doi.org/10.1073/pnas.94.14.7362>
- Johnson PS, Shifley SR, Rogers R, 2002. The Ecology and Silviculture of Oaks. CABI Publishing, Cambridge, UK. <https://doi.org/10.1079/9780851995700.0000>
- Jones RH, Mitchell RJ, Stevens GN, Pecot SD, 2003. Controls of fine root dynamics across a gradient of gap sizes in a pine woodland. *Oecologia* 134(1): 132-143. <https://doi.org/10.1007/s00442-002-1098-y>
- Kaelke C, Dawson J, 2005. The accretion of nonstructural carbohydrates changes seasonally in *Alnus incana* ssp *rugosa* in accord with tissue type, growth, N allocation, and root hypoxia. *Symbiosis* 39(2): 61-66.
- King JS, Pregitzer KS, Zak DR, Holmes WE, Schmidt K, 2005. Fine root chemistry and decomposition in model communities of north-temperate tree species show little response to elevated atmospheric CO₂ and varying soil resource availability. *Oecologia* 146(2): 318-328. <https://doi.org/10.1007/s00442-005-0191-4>
- Kosola KR, Dickmann DI, Parry D, 2002. Carbohydrates in individual poplar fine roots: effects of root age and defoliation. *Tree Physiol* 22(10): 741-746. <https://doi.org/10.1093/treephys/22.10.741>
- Krässig HA, 1993. Cellulose: structure, accessibility and reactivity. Philadelphia, PA, Gordon and Breach Science Publishers.
- Lacointe A, Kajji A, Daudet F, Archer P, Frossard JS, Saintjoanis B, Vandame M, 2016. Mobilization of carbon reserves in young walnut trees. *Bull Soc Bot Fr* 140(4): 435-441. <https://doi.org/10.1080/12538078.1993.10515618>
- Legendre P, Gallagher ED, 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia* 129: 271-280. <https://doi.org/10.1007/s004420100716>
- Lopez BC, Sabate S, Gracia CA, 2003. Thinning effects on carbon allocation to fine roots in a *Quercus ilex*

- forest. *Tree Physiol* 23(17): 1217-1224. <https://doi.org/10.1093/treephys/23.17.1217>
- Luostarinen K, Kauppi A, 2005. Effects of coppicing on the root and stump carbohydrate dynamics in birches. *New Forest* 29(3): 289-303. <https://doi.org/10.1007/s11056-005-5653-3>
- Ma C, Zhang W, Wu M, Xue YQ, Ma LW, Zhou JY, 2013. Effect of above-ground intervention on fine root mass, production, and turnover rate in a Chinese cork oak (*Quercus variabilis* Blume) forest. *Plant Soil* 368(1-2): 201-214. <https://doi.org/10.1007/s11104-012-1512-0>
- Ma C, Zhang W, Zhou JY, Wu M, Xue YQ, Ma LW, 2015. Fine root architecture, morphology, and biomass response to cutting in a Chinese cork oak (*Quercus variabilis* Blume) forest. *Turk J Agri For* 38: 668-675. <https://doi.org/10.3906/tar-1312-36>
- Makineci E, Ozdemir E, Caliskan S, Yilmaz E, Kumbasli M, Keten A, Beskardes V, Zengin H, Yilmaz H, 2015. Ecosystem carbon pools of coppice-originated oak forests at different development stages. *Eur J For Res* 134(2): 319-333. <https://doi.org/10.1007/s10342-014-0854-y>
- McCormack ML, Adams TS, Smithwick EAH, Eissensat DM, 2012. Predicting fine root life-span from plant functional traits in temperate trees. *New Phytol* 195 (4): 823-831. <https://doi.org/10.1111/j.1469-8137.2012.04198.x>
- McCormack ML, Guo DL, 2014. Impacts of environmental factors on fine root lifespan. *Frontiers in Plant Science* 5: 1-11. <https://doi.org/10.3389/fpls.2014.00205>
- Mosca E, Montecchio L, Barion G, Dal CC, Vamerali T, 2017. Combined effects of thinning and decline on fine root dynamics in a *Quercus robur* L. forest adjoining the Italian Pre-Alps. *Ann Bot* 119: 1235-1246. <https://doi.org/10.1093/aob/mcx007>
- Noland TL, Mohammed GH, Scott M, 1997. The dependence of root growth potential on light level, photosynthetic rate, and root starch content in jack pine seedlings. *New Forest* 13: 105-119. <https://doi.org/10.1023/A:1006517820981>
- Pei Y, Lei P, Xiang W, Ouyang S, Xu Y, 2018. Effect of stand age on fine root biomass, production and morphology in Chinese fir plantations in subtropical China. *Sustainability* 10(7): 2280-2294. <https://doi.org/10.3390/su10072280>
- Pregitzer KS, 2002. Fine roots of trees - a new perspective. *New Phytol* 154(2): 267-270. https://doi.org/10.1046/j.1469-8137.2002.00413_1.x
- Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL, 2002. Fine root architecture of nine North American trees. *Ecol Monogr* 72(2): 293-309. [https://doi.org/10.1890/0012-9615\(2002\)072\[0293:FRAONN\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2002)072[0293:FRAONN]2.0.CO;2)
- Pregitzer KS, King JA, Burton AJ, Brown SE, 2000. Responses of tree fine roots to temperature. *New Phytol* 147(1): 105-115. <https://doi.org/10.1046/j.1469-8137.2000.00689.x>
- Pregitzer KS, Kubiske ME, Yu CK, Hendrick RL, 1997. Relationships among root branch order, carbon, and nitrogen in four temperate species. *Oecologia* 111(3): 302-308. <https://doi.org/10.1007/s004420050239>
- Rich PM, 1990. Characterizing plant canopies with hemispherical photographs. *Remote Sens Rev* 5: 13-29. <https://doi.org/10.1080/02757259009532119>
- Sayer MAS, Haywood JD, 2006. Fine root production and carbohydrate concentrations of mature longleaf pine (*Pinus palustris* P. Mill.) as affected by season of prescribed fire and drought. *Trees* 20(2): 165-175. <https://doi.org/10.1007/s00468-005-0022-6>
- Smith D, 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. *Wisconsin Coll. Agric. and Life Sci. Res. Rpt.* 41pp.
- Stobrawa K, Lorenc-Plucinska G, 2007. Changes in carbohydrate metabolism in fine roots of the native European black poplar, *Populus nigra* L. in a heavy-metal-polluted environment. *Sci Total Environ* 373 (1): 157-165. <https://doi.org/10.1016/j.scitotenv.2006.11.019>
- Terzaghi M, Di Iorio A, Montagnoli A, Baesso B, Scippa GS, Chiatante D, 2016. Forest canopy reduction stimulates xylem production and lowers carbon concentration in fine roots of European beech. *For Ecol Manage* 379: 81-90. <https://doi.org/10.1016/j.foreco.2016.08.010>
- Terzaghi M, Montagnoli A, Iorio AD, Scippa GS, Chiatante D, 2013. Fine-root carbon and nitrogen concentration of European beech (*Fagus sylvatica* L.) in Italy Prealps: possible implications of coppice conversion to high forest. *Front Plant Sci* 4: 1-8. <https://doi.org/10.3389/fpls.2013.00192>
- Valenzuela Nunez LMV, Gerant D, Maillard P, Breda N, Cervantes GG, Cohen IS, 2011. Evidence for a 26 kDa vegetative storage protein in the stem sapwood of mature pedunculate oak. *Interciencia* 36: 142-147.
- Vogt KA, Persson H, 1991. Measuring growth and development of roots. Boca Raton, FL, CRC Press.
- Wang CG, Brunner I, Zong SW, Li MH, 2019. The dynamics of living and dead fine roots of forest biomes across the Northern Hemisphere. *Forests*, 10, 15. <https://doi.org/10.3390/f10110953>
- Wang G, Liu F, Xue S, 2017. Nitrogen addition enhanced water uptake by affecting fine root morphology and coarse root anatomy of Chinese pine seedlings. *Plant Soil* 418(1-2): 177-189. <https://doi.org/10.1007/s11104-017-3283-0>
- Wang ZQ, Guo DL, Wang XR, Gu JC, Mei L, 2006. Fine root architecture, morphology, and biomass of different branch orders of two Chinese temperate tree species. *Plant Soil* 288(1-2): 155-171. <https://doi.org/10.1007/s11104-006-9101-8>

- Xue YQ, Zhang WH, Ma C, Ma LW, Zhou JY, 2014. Relative importance of various regeneration recruits in different recovery stages of *Quercus variabilis* forest after selective logging. *For Syst* 23(2): 199-208. <https://doi.org/10.5424/fs/2014232-03263>
- Xue YQ, Zhang WH, Zhou JY, Ma C, Ma LW, 2013. Effects of stump diameter, stump height, and cutting season on *Quercus variabilis* stump sprouting. *Scand J For Res* 28(3): 223-231. <https://doi.org/10.1080/02827581.2012.723742>
- Yuan ZY, Chen HYH, 2013. Effects of disturbance on fine root dynamics in the boreal forests of northern Ontario, Canada. *Ecosystems* 16(3): 467-477. <https://doi.org/10.1007/s10021-012-9623-2>
- Zadworny M, McCormack ML, Rawlik K, Jagodzinski AM, 2015. Seasonal variation in chemistry, but not morphology, in roots of *Quercus robur* growing in different soil types. *Tree Physiol* 35(6): 644-652. <https://doi.org/10.1093/treephys/tpv018>
- Zelesnik P, Vilhar U, Starr M, de Groot M, Kraigher H, 2016. Fine root dynamics in Slovenian beech forests in relation to soil temperature and water availability. *Trees* 30(2): 375-384. <https://doi.org/10.1007/s00468-015-1218-z>