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

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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.

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.
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, 2010).
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 ℃ 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 ℃, 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 ℃), 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).

| Stands | Canopy cover (%) | Density (ha⁻¹) | DBH (cm) | Basal area (m²·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    |

Table 1. Above-ground characteristic for the investigated stands. Values are the mean of three replicate plots (SE)
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, compared 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. | 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. | 16.711*** | 7.377*** | 3.077** | 5.388*** |
| Period * root order * season | F | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
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.
Nonstructural carbohydrates, carbon and nitrogen concentrations in fine roots

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 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.](image)

![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.](image)
activities (Noland \textit{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 \textit{et al.} (2002) suggest that this resulted from the shortage of photosynthetic material 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 \textit{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 \textit{et al.}, 2007). Lacointe \textit{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 \textit{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 \textit{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 \textit{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 \textit{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 \textit{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 \textit{et al.}, 2013; Terzaghi \textit{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 \textit{et al.}, 2013). However, interpretation of the root lifespan is often complicated. Jones \textit{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 \textit{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 \textit{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.

Acknowledgments

The authors thank Bolin Wang, Chen Xu, Huiyun Guo, and Yiteng Zheng for assistance in the field and laboratory. We also thank Hongyan Cheng to determine C and N concentration with an elemental Analyzer. The authors gratefully acknowledge the two anonymous referees for their valuable comments.

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