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ARTICLE

Impacts of Defoliation on Morphological Characteristics and Non-Structural Carbohydrates of *Populus talassica* × *Populus euphratica* Seedlings

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ABSTRACT

Leaves are important 'source' organs that synthesize organic matter, providing carbon sources for plant growth. Here, we used *Populus talassica* × *Populus euphratica*, the dominant species in ecological and timber forests, to simulate carbon limitation through artificial 25%, 50%, and 75% defoliation treatments and explore the effects on root, stem, and leaf morphology, biomass accumulation, and carbon allocation strategies. At the 60th d after treatment, under 25% defoliation treatment, the plant height, specific leaf weight, root surface area and volume, and concentrations of non-structural carbohydrates in stem and root were significantly increased by 9.13%, 20.00%, 16.60%, 31.95%, 5.12%, and 9.34%, respectively, relative to the control. There was no significant change in the growth indicators under 50% defoliation treatment, but the concentrations of non-structural carbohydrates in the leaf and stem significantly decreased, showing mostly a negative correlation between them. The opposite was observed in the root. Under 75% defoliation treatment, the plant height, ground diameter, leaf number, single leaf area, root, stem, and total biomass were significantly reduced by 14.15%, 10.24%, 14.86%, 11.31%, 11.56%, 21.87%, and 16.82%, respectively, relative to the control. The concentrations of non-structural carbohydrates in various organs were significantly reduced, particularly in the consumption of the starch concentrations in the stem and root. These results indicated that carbon allocation strategies can be adjusted to increase the concentration of non-structural carbohydrates in root and meet plant growth needs under 25% and 50% defoliation. However, 75% defoliation significantly limited the distribution of non-structural carbohydrates to roots and stems, reduced carbon storage, and thus inhibited plant growth. Defoliation-induced carbon limitation altered the carbon allocation pattern of *P. talassica* × *P. euphratica*, and the relationship between carbon reserves in roots and tree growth recovery after defoliation was greater. This study provides a theoretical basis for the comprehensive management of *P. talassica* × *P. euphratica* plantations, as well as a reference for the study of plantation carbon allocation strategies in the desert and semi-desert regions of Xinjiang under carbon-limitation conditions.

KEYWORDS

Populus talassica × *Populus euphratica*; defoliation; carbon limitation; carbon allocation; non-structural carbohydrates

1 Introduction

Since the 1950s, large-scale artificial afforestation increasingly occurred globally [1]. According to The State of the World's Forests 2022 [2], 7% of the global forest area consisted of plantations in 2020, with an area of approximately 294 million hectares, and the plantation area's growth rate has shown a decreasing trend. Globally, approximately 36% of plantation area comes from the East Asia region [3], with China's plantation area currently being the largest in the world [4]. The construction of plantations aids in the recovery of ruined forests and land degradation, as well as in improving biodiversity. Additionally, plantations provide effective supplements to the timber supply, thereby relieving the pressure of natural forest harvesting. The management of afforestation species is very important for improving forest growth and development, and consolidating afforestation achievements. As an important 'source' organ for synthesizing organics, leaves provide carbon sources for the growth and development of trees. Defoliation or leaf damage is a common way in which environmental stresses or anthropogenic disturbances affect the growth of tree seedlings [5], causing changes in the supply of organic, thereby affecting or threatening the normal growth and physiological metabolism of trees.

Carbon limitation can be divided into two types. One is the limitation of photosynthesis, which leads to insufficient carbon sources (carbon supply) in the tree, triggering a carbon source limitation that affects tree growth; the other is that stress (including defoliation caused by drought, pest outbreaks, and animal feeding) can lead to a reduction in the ability to utilize carbon in the tree, triggering a carbon sink (carbon utilization) limitation that affects tree growth [6,7]. Non-structural carbohydrates (NSCs), which are important components of plant carbon storage, include starch and soluble sugars [8], and the two can be reversibly transformed during carbon storage and carbon utilization under certain conditions [9]. NSCs play roles in the growth, metabolism, and defense systems of trees, as well as in the recovery from external disturbances (grazing, drought, and fire) [10–12]. Thus, when carbon synthesis is limited, plants consume stored NSCs to maintain activities, such as growth and development, metabolism, and physiological defense. The reduction of NSCs to the minimum threshold required to maintain growth and development inhibits plant growth and may lead to the threat of carbon starvation and death [13]. Defoliation may lead to both carbon supply limitation and carbon utilization limitation in tree growth [14], and artificial defoliation treatments are often used in experiments to simulate carbon limitation to examine how carbon allocation in plants reacts to defoliation.

Defoliation directly leads to a decrease in the total number and area of tree leaves, resulting in limited carbon synthesis and insufficient available carbon supply, which triggers a carbon source limitation. For example, for three consecutive years, 100% artificial defoliation was performed on *Quercus velutina*, and it was found that the ground diameter, total biomass, and NSC concentration of *Q. velutina* were consistently lower than the control [5]. Piper et al. [15] argued that the NSC content in the *Nothofagus pumilio* stem significantly decrease under 50% defoliation treatment, with changes in starch content playing a dominant role. In addition, under defoliation, trees can improve long-term viability by adjusting carbon allocation pattern of various organs, leading to slower growth and increased carbon storage. Puri et al. [16] found that *Pinus pinaster* branch and stem growth significantly decreases after defoliation treatments. Additionally, the NSC concentration is lower during the initial growth stage, and then, it increases and may even exceed that of the control group. However, defoliation significantly affects tree growth but not necessarily the NSC content; consequently, it has been hypothesized that this growth inhibition is not due to carbon limitation [17]. Variations in these research findings could stem from the degree of carbon limitation in plants and their response strategies. Therefore, studying the carbon allocation pattern and dynamic response after defoliation is of great theoretical significance for understanding tree adaptation strategies and regulatory mechanisms. In addition, defoliation may also lead to changes in NSCs, which can affect the hydraulic characteristics of the trees [18]. The main manifestation is that defoliation reduces the total leaf area of the crown, reduces transpiration, affects the water diversion ability of the tree, reduces water transport efficiency [19,20], and causes carbon utilization limitations [14]. NSCs can also participate in hydraulic regulation, such as osmotic regulation, embolization repair, and other processes [13,21]. In the next step of research, we may explore the impact of carbon limitation on the hydraulic traits of *P. talassica* × *P. euphratica* seedlings, with the purpose of gaining a broader understanding of the comprehensive impacts of carbon limitation on tree growth.

Populus talassica (♀) × *Populus euphratica* (♂) [22] is a perennial deciduous tree with excellent characteristics, such as straight stem shape, excellent timber, flush stand, and fast growth, and it is widely planted in Northwest China [23]. In the previous field survey focusing on *P. talassica* × *P. euphratica* plantations and farmland shelterbelts in Xinjiang, it was determined that it is susceptible to carbon limitation caused by factors such as *Apocheima cinerarius* infestations, animal consumption, and anthropogenic interference, which reduces its popularization area and value. This research employed *P. talassica* × *P. euphratica*, the dominant species in ecological and timber forests, as the material and established different defoliation intensities to simulate carbon limitation, exploring the effects on morphology, biomass accumulation, and carbon allocation strategies of various organs. The aim was answer: (1) With the increase of defoliation intensities, what are the morphological changes of *P. talassica* × *P. euphratica* seedlings? (2) How is the carbon allocation strategies of *P. talassica* × *P. euphratica* seedlings under different defoliation intensities? This provided the groundwork for the thorough management of *P. talassica* × *P. euphratica* plantations and for understanding the mechanisms of tree self-sustainability and self-renewal.

2 Materials and Methods

2.1 Overview of the Study Site

This study was conducted in the plantation of *P. talassica* × *P. euphratica* in the 10th Regiment of First Division, Alar, Xinjiang (81°18′08″ E, 40°36′13″ N and altitude of 1014 m). Anually, the temperature, solar radiation, precipitation, sunshine hours, and evaporation average stand at 12.1°C, 133.7–146.3 Kcal cm^{-2}, 40.1–82.5 mm, 2556.3–2991.8 h, and 1876.6–2558.9 mm, respectively.

2.2 Plant Material and Experimental Treatments

The soil type was loam, The pH was 8.03. The contents of organic matter, alkali-hydrolyzed N, available K, and available P were 21.34 $g \cdot kg^{-1}$, 17.08, 143.07 and 22.05 $mg \cdot kg^{-1}$, in that order. Black plastic film was laid in early April 2023, and annual *P. talassica* × *P. euphratica* cuttings cultivated in the area were planted with the standard 30 cm × 30 cm spacing between plants. Cuttings are about 20 cm long and inserted into the underground part by about 10 cm. After 2.5 months of stable growth and sprouting, seedlings with good consistent growth, having no pests or diseases on their leaves, were separated into 4 groups of 50 each. Then, three groups were randomly selected for defoliation in a bottom-up order, with 25% defoliation (D_{25}) indicating cutting one of every four leaves, 50% defoliation (D_{50}) indicating cutting one of every two leaves, and 75% defoliation (D_{75}) indicating cutting three of every four leaves. It was necessary to cut off the whole leaf and only retain the petiole, without considering the leaf age. Additionally,, it was necessary to ensure that the terminal buds were not damaged to ensure the full development of the branches. Another group served as the control (CK), and tagged these groups properly.

2.3 Measurement of Growth Indexes

The day of the defoliation treatment was set as day 0. The initial plant height, ground diameter, and leaf number of the treated seedlings were measured. The leaf indexes, including length, width, and area, were determined using ImageJ analysis software (1.44 P, National Institutes of Health, Bethesda, MD, USA). The leaves were dried 105°C for quarter and then reached at constant weight at 80°C to determine their dry weights. These indexes were determined every 15 days, a total of five times.

Additionally, At the 30th and 60th d after treatment, root and biomass indicators were measured in 6 times repeatedly. The root indicators, including length, surface area, and volume, were measured by a dual-light color scanner (MICROTEK ScanMaker, i800 Plus) and a LA-S series analyzer system (Hangzhou Wanshen Testing Technology Co., Ltd., Hangzhou, China). The dry weights, including root, stem, and leaf, were measured separately. And the leaf shape index (LSI), specific leaf weight (SLW), and root/top ratio (R/T) were calculated.

2.4 Measurement of NSC Concentration

The NSC concentrations of different organs were determined using the anthrone-sulfuric acid method [24] on the 30th and 60th d after treatment. The dried tissue materials were first powdered using a pulverizer and passed through a 100-mesh sieve. In total, 0.1000 g of organ samples and 10 mL of distilled water were poured into a 15-mL test tube, which was then sealed. Samples were placed in a boiling water bath for half-hour with constantly shaking, and extracted three times. The supernatants were collected in a 25-mL volumetric flask. The test tubes were washed epeatedly, then made up to volume, which was used for the determination of soluble sugars.

In total, each of the above sediment was treated with 2 mL of distilled water, gelatinized for 15 min at boiling water and cooled. Afterwards, 2 mL of 9.2 mol·L^{-1} $HClO_4$ solution was introduced, and mixed for 15 min to ensure a full reaction. In total, 4 mL of distilled water was combined and blended thoroughly, followed by centrifuge at 3000 r·min^{-1} for 10 min. Supernatants were transferred into 50-mL volumetric flasks, followed by the addition of 2 mL of 4.6 mol·L^{-1} $HClO_4$ solution. Samples were shaken for 15 min. Then, 5 mL of distilled water was introduced, mixed and centrifuged for 10 min. Residues were combined. The test tubes were washed repeatedly with distilled water, then made up to volume. 620 nm was used for colorimetric determinations of soluble sugar and starch concentrations. adding the two values to obtain NSC concentration. Concentrations of soluble sugar and starch were determined as follows:

$$\mathrm{SSC}\left(\mathrm{mg}\cdot\mathrm{g}^{-1}\right) = \frac{C \times V_T \times n}{W \times V_S \times 10^3} \tag{1}$$

$$\mathrm{SC}\left(\mathrm{mg}\cdot\mathrm{g}^{-1}\right) = \frac{C \times V_T \times n \times 0.9}{W \times V_S \times 10^3} \tag{2}$$

where C represents the sugar concentration calculated from the glucose standard curve, V_T represents total volume of sample extraction solution, V_S represents sampling volume during measurement, W represents dry weight of samples, n represents dilution factor, and 0.9 represents the starch conversion coefficient.

2.5 Statistical Analyses

Statistical analyses were performed using SPSS 25.0. The data were analyzed using a one-way ANOVA with Duncan's method for multiple comparisons ($p < 0.05$). Differences in leaf indexes, root architecture, biomass, and concentration of non-structural carbohydrates between the 30th and 60th d of the same treatment were analyzed using independent sample *t*-tests ($p < 0.05$). Result were presented as means ± SEs. Charts were generated using Origin 2022.

3 Results

3.1 Effects of Defoliation on Plant Heights and Ground Diameters of P. talassica × P. euphratica Seedlings

As the defoliation intensity increased, plant heights and ground diameters showed trends from increasing to decreasing (Fig. 1). Under the D_{25} treatment, plant heights increased significantly from the 45th d in comparison with contrast, whereas ground diameters increased significantly only after the 15th d. Plant heights under the D_{75} treatment decreased significantly from the 15th d, and ground diameters decreased significantly from the 30th d. Over time, the increases in plant heights and ground diameters of each treatment group showed slow-fast-slow trends. The plant height changes reached a maximum at 15–30 d in the CK and D_{75} treatment groups, whereas those of the D_{25} and D_{50} treatment groups reached maximums at 30–45 d. The ground diameter changes were greater at 15–30 d and then gradually decreased.

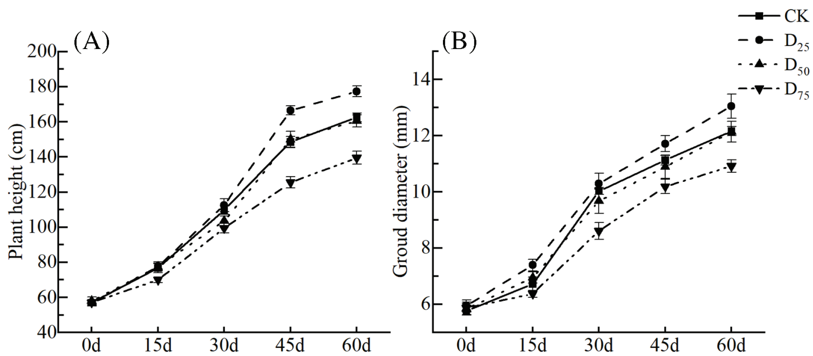

Figure 1: Effect of defoliation on plant heights (A) and stem diameters (B) of *P. talassica* × *P. euphratica* seedlings

Note: Data represent Mean ± SE, and lowercase letters indicate that different treatments for the same observed time are significantly different ($p < 0.05$).

3.2 Effects of Defoliation on Leaf Growth of P. talassica × P. euphratica Seedlings

The leaf traits under different defoliation intensities are shown in Table 1. At the 30th d after treatment, leaf number reduced as the defoliation intensity enhanced. Three defoliation treatments successively significantly reduced by 20.68%, 26.43%, and 43.28% in comparison with contrast. The difference in leaf area was not significant. LSI significantly decreased by 7.81% only under the D_{75} treatment in comparison with contrast. SLW showed a trend from increasing to decreasing with the increase of defoliation intensities, and the three defoliation intensities were significantly exceed control. At the 60th d, leaf number and area significantly decreased in comparison with contrast only under D_{75} treatment. The LSI has recovered to no significant change. Compared with other treatments, the SLW was significantly increased under D_{25} treatment. Over time, the leaf number and area gradually increased, while LSI gradually decreased. The SLW gradually increased under CK and D_{25} treatments, while there was no significant change in D_{50} and D_{75} treatments.

Table 1: Effect of defoliation on leaf growth of *P. talassica* × *P. euphratica* seedlings

Time/d	Treatment	Leaf number	Leaf area/cm^2	LSI	SLW/($g \cdot cm^{-2}$)
30	CK	250.5 ± 1.9 aB	23.9 ± 1.1 aB	6.4 ± 0.2 aA	0.007 ± 0.0003 bB
	D_{25}	198.7 ± 11.1 bB	21.1 ± 0.9 aB	6.5 ± 0.1 aA	0.010 ± 0.0004 aB
	D_{50}	184.3 ± 6.8 bB	21.4 ± 1.2 aB	6.5 ± 0.1 aA	0.009 ± 0.0002 aA
	D_{75}	142.3 ± 6.2 cB	21.4 ± 1.0 aB	5.9 ± 0.2 bA	0.009 ± 0.0003 aA
60	CK	380.9 ± 17.7 aA	32.7 ± 0.6 aA	4.9 ± 0.2 aB	0.010 ± 0.0005 bA
	D_{25}	378.0 ± 18.2 aA	35.1 ± 1.2 aA	4.7 ± 0.2 aB	0.013 ± 0.0006 aA
	D_{50}	401.7 ± 11.8 aA	35.1 ± 1.5 aA	5.0 ± 0.2 aB	0.009 ± 0.0003 bA
	D_{75}	324.3 ± 15.0 bA	29.0 ± 0.5 bA	5.0 ± 0.3 aB	0.010 ± 0.0004 bA

Note: Data represent Mean ± SE. Different capital letters in the same column indicate that different observed times for the same treatment are significantly different ($p < 0.05$), and lowercase letters indicate that different treatments for the same observed time are significantly different ($p < 0.05$).

3.3 Effects of Defoliation on the Root Architecture of P. talassica × P. euphratica Seedlings

Fig. 2 illustrates the changes in root architecture under different defoliation intensities. The total root lengths, root surface areas, and root volumes showed increasing and then decreasing trends along with the increase in defoliation intensity, except for the total root length at the 60th d and root volume at the 30th d. At the 30th d after treatment, the total root length and root surface area were the greatest under the D_{50} treatment, at 660.83 cm and 226.86 cm^2, respectively, which were significantly exceed CK by 55.07% and 42.39%, respectively. The total root length was significantly exceed D_{25} by 45.81%, but this was not significantly different compared with the D_{75} treatment. By the 60th d, the total root lengths were no longer significantly different, and the root surface area was 463.21 cm^2 under the D_{25} treatment, which was only significantly greater than that of the CK (Fig. 2A,B). There was not significant different in the root volume by the 30th d. However, by the 60th d, the root volume was 59.49 cm^3 under the D_{25} treatment, which was a significant increase of 31.95% compared with the CK. There were no significant changes between the others and CK (Fig. 2C).

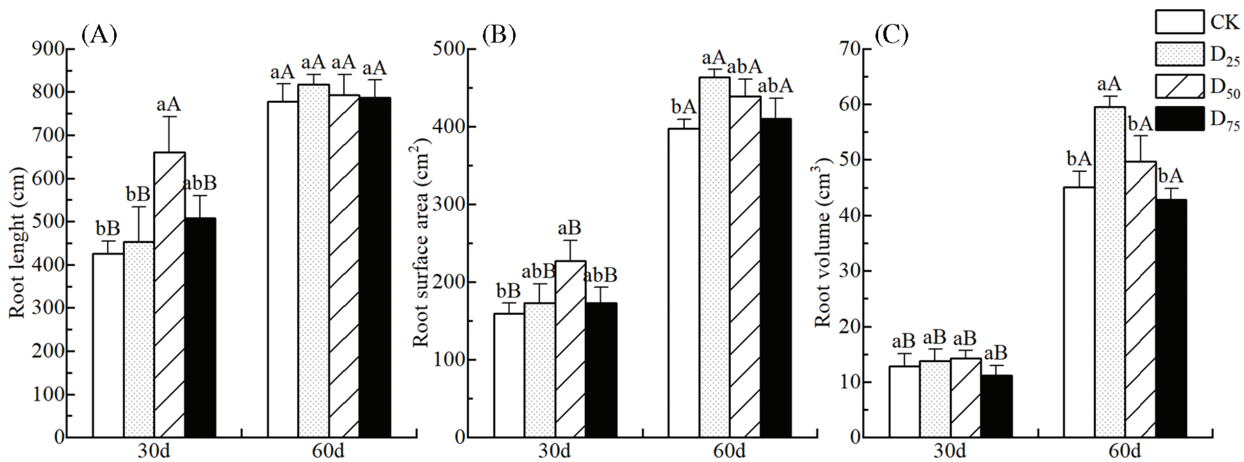

Figure 2: Effect of defoliation on the root length (A), root surface area (B), and root volume (C) of *P. talassica* × *P. euphratica* seedlings

Note: Data represent Mean ± SE. Different capital letters in the same column indicate that different observed times for the same treatment are significantly different ($p < 0.05$), and lowercase letters indicate that different treatments for the same observed time are significantly different ($p < 0.05$).

3.4 Effects of Defoliation on the Biomasses of P. talassica × P. euphratica Seedlings

The effects in biomass of root, stem, leaf, total, and R/T under different defoliation intensities are shown in Table 2. At the 30th d after treatment, the stem, leaf, and total biomasses decreased as the defoliation intensity increased. Compared with the CK, they were significantly reduced by 12.06%, 11.31%, and 10.28% under the D_{50} treatment, respectively, and by 32.32%, 31.43%, and 31.66% under the D_{75} treatment, respectively. The root biomass and R/T showed trends from increasing to decreasing as the defoliation intensity increased. The root biomass was significantly reduced by 28.72% in D_{75}, and the R/T successively significantly increased by 12.35% and 20.99% in the D_{25} and D_{50} treatments in comparison with contrast. At the 60th d, with the increase in defoliation intensities, the biomasses of root, stem, leaf, total, and R/T, showed trends from increasing to decreasing. Compared with CK, stem, leaf, and total biomasses significantly decreased by 21.81%, 11.48%, and 16.83% under the D_{75} treatment, respectively, whereas the root biomass and R/T of each defoliation treatment group showed no significant changes compared with the CK.

Table 2: Effect of defoliation on the biomasses of *P. talassica* × *P. euphratica* seedlings

Time/d	Treatment	Root biomass/g	Stem biomass/g	Leaf biomass/g	R/T	Total biomass/g
30	CK	2.8 ± 0.0 aB	17.9 ± 0.3 aB	16.4 ± 0.2 aB	0.081 ± 0.002 cB	37.1 ± 0.3 aB
	D_{25}	3.0 ± 0.1 aB	17.3 ± 0.2 aB	15.9 ± 0.3 aB	0.091 ± 0.003 abB	36.2 ± 0.4 aB
	D_{50}	3.0 ± 0.1 aB	15.8 ± 0.2 bB	14.6 ± 0.2 bB	0.098 ± 0.004 aB	33.3 ± 0.5 bB
	D_{75}	2.0 ± 0.1 bB	12.1 ± 0.3 cB	11.3 ± 0.4 cB	0.084 ± 0.003 bcB	25.4 ± 0.8 bB
60	CK	7.2 ± 0.3 abA	37.5 ± 1.6 aA	22.5 ± 0.8 aA	0.121 ± 0.006 aA	67.2 ± 2.4 aA
	D_{25}	7.9 ± 0.3 aA	39.7 ± 1.0 aA	23.6 ± 0.6 aA	0.124 ± 0.004 aA	71.1 ± 1.0 aA
	D_{50}	8.3 ± 0.6 aA	35.9 ± 1.2 aA	24.1 ± 0.9 aA	0.137 ± 0.004 aA	68.3 ± 2.1 aA
	D_{75}	6.7 ± 0.5 bA	29.3 ± 1.2 bA	19.9 ± 0.6 bA	0.137 ± 0.012 aA	55.9 ± 1.3 bA

Note: Data represent Mean ± SE. Different capital letters in the same column indicate that different observed times for the same treatment are significantly different ($p < 0.05$), and lowercase letters indicate that different treatments for the same observed time are significantly different ($p < 0.05$).

3.5 Effects of Defoliation on the NSC Concentrations of P. talassica × *P. euphratica Seedlings*

Changes in the NSC concentrations of various organs under different defoliation intensities are shown in Fig. 3. At the 30th d, the NSC concentration in leaf increased along with defoliation intensity, and all the treatments achieved significant levels in comparison to CK. Among them, soluble sugar concentrations increased significantly in three treatment groups, whereas the starch concentrations decreased significantly by 24.63% and 27.49% under the D_{25} and D_{75} treatments, respectively (Fig. 3A–C). The NSC concentration in stem tended to increase and reached a significance level only under the D_{50} treatment. The soluble sugar concentration in stem increased significantly under the D_{50} treatment. Although the starch concentrations increased significantly under the D_{75} treatment, the soluble sugar concentrations tended to decrease (Fig. 3D–F). The NSC concentration trend in root was opposite to that in leaf, and soluble sugar and starch concentrations of three treated groups showed significantly decreasing trends (Fig. 3G–I). By the 60th d, the NSC concentration in leaf decreased as the defoliation intensity increased, reaching significance levels, decreasing by 9.82% and 14.17%, under the D_{50} and D_{75} treatments, respectively, compared with the CK. The soluble sugar concentration changed similarly, whereas the starch concentrations did not change significantly. When the defoliation intensity increased, the NSC concentrations in stem and root had a tendency of increasing at the beginning and decreasing in late, reaching significant levels and maximum values under the D_{25} treatment. The changes in soluble sugar concentrations were similar, whereas the starch concentrations showed significant decreasing trends. Over time, under the defoliation treatments, the soluble sugar and NSC concentrations in various organs gradually higher (except for the soluble sugar concentration in stem under D_{50} treatment), while the change was no significant at the starch concentration in leaf. The starch concentration in the stem gradually increased under D_{25} and D_{50} treatments. The starch concentration in root gradually increased under D_{25} treatment, while the opposite was observed under D_{50} and D_{75} treatments.

3.6 Correlation Analysis between Growth Indicators and Non-Structural Carbohydrates of P. talassica × *P. euphratica Seedlings*

At the 60th d, pearson correlation analysis of growth indicators and NSCs in various organs are shown in Fig. 4. The soluble sugar concentration in leaf was significant negative correlated with leaf biomass in CK, including the NSC concentration in leaf and stem biomass, and the starch concentration and volume in root. The starch concentration in stem was significantly positively correlated with root biomass. In addition, the relationship between the starch concentration in root and stem biomass, total biomass were also like this. Under D_{25} treatment, the soluble sugar concentration in leaf was markedly or highly markedly negatively correlated with root volume and total biomass, while there was a notable positive correlation relationship between the starch concentration in leaf and LSI. The soluble sugar concentration in stem showed greatly positively correlated with root length, but exhibited opposite trends with root biomass and R/T. The NSC concentration in stem showed a significant positive correlation with root surface area, but opposite to leaf number, root biomass, and R/T. Under D_{50} treatment, Growth indicators and nonstructural carbohydrates in leaf and stem mostly showed significant negative correlations, e.g., the soluble sugar concentrations in leaf and stem biomass, the starch concentration in leaf and leaf area, the NSC concentrations in stem and root surface area. There was a significant positive correlation between the soluble sugar concentration in root and root length, along with the starch concentration in root and leaf area, and the NSC concentration in root and root biomass. Under D_{75} treatment, there was a highly significant positive correlation between the soluble sugar concentration in leaf and R/T, along with the NSC concentration in stem and root biomass. Significant positive correlation between NSC concentration in stem and root volume, while the NSC concentration in root was opposite to R/T.

4 Discussion

4.1 Effects of Defoliation on the Aboveground of P. talassica × P. euphratica Seedlings

Defoliation affects the growth of trees by decreasing the branch growth rate, and the incremental changes in stem diameter and plant height. It affects the radial growth of trees more than the longitudinal growth [25–27], and it decreases the new leaf number, decreases the leaf area, and increases the specific leaf area [28,29]. Growth indicators (plant height, ground diameter, and leaf number) are reflections of growth status and reveal changes in plant growth adaptations. Barry et al. [30] suggested that the plant height and ground diameter of *Eucalyptus globulus* and *Eucalyptus nitens* decreased under the 40% defoliation treatment. In this study, the increases in plant heights and ground diameters of *P. talassica* × *P. euphratica* became less and less as the defoliation intensity enhanced. Over time, 25% defoliation treatment promoted plant height and ground diameter, resulting in taller plants and robust growth, whereas the 75% defoliation treatment suppressed the plant height and ground diameter. This is probably because *P. talassica* × *P. euphratica* has self-compensating mechanisms [31]. These self-repair mechanisms may not be able to compensate for defoliation greater than 50%, leading to higher stress responses, resulting in stunted plant heights and ground diameters.

Leaves have strong environmental sensitivity and plasticity [32], and changes in their morphological structures will directly affect the photosynthetic efficiency of the plant. This leads to changes in the distribution and utilization of photosynthetic products, which affects plant growth. Previous study showed that the new leaf number and the leaf area are related to defoliation intensity and harvest time, nevertheless, the adverse impact of high-intensity defoliation on these indicators intensifies with time [33]. As a dominant species in plantations, *P. talassica* × *P. euphratica* has many leaves and branches, and leaves are spirally and interactively attached to the branches. In this study, with the defoliation intensity increased, the new leaf number increased initially, followed by an decrease. Compared with CK, the leaf number under 75% defoliation treatment was always significantly reduced, the leaf area was significantly reduced at the 60th d, and the LSI was significantly reduced only at the 30th d. This may indicate an adjustment in leaf morphology in response to nutrient deficiencies caused by leaf area loss. The SLW significantly increased under the defoliation treatments at the 30th d. A change in SLW is related to the trade-off between photosynthetically relevant tissue structures and thinning tissues [34], but it cannot simply be assumed that SLW shows a positive correlation with photosynthetic rate, because the latter is also related to the species and growth environment [35,36]. Owing to the compensatory photosynthesis [37] could not supply the requirements for normal growth of *P. talassica* × *P. euphratica* seedlings, resulting in a shortage of photosynthetic products, a decrease in leaf biomass accumulation, and a decreasing trend in SLW.

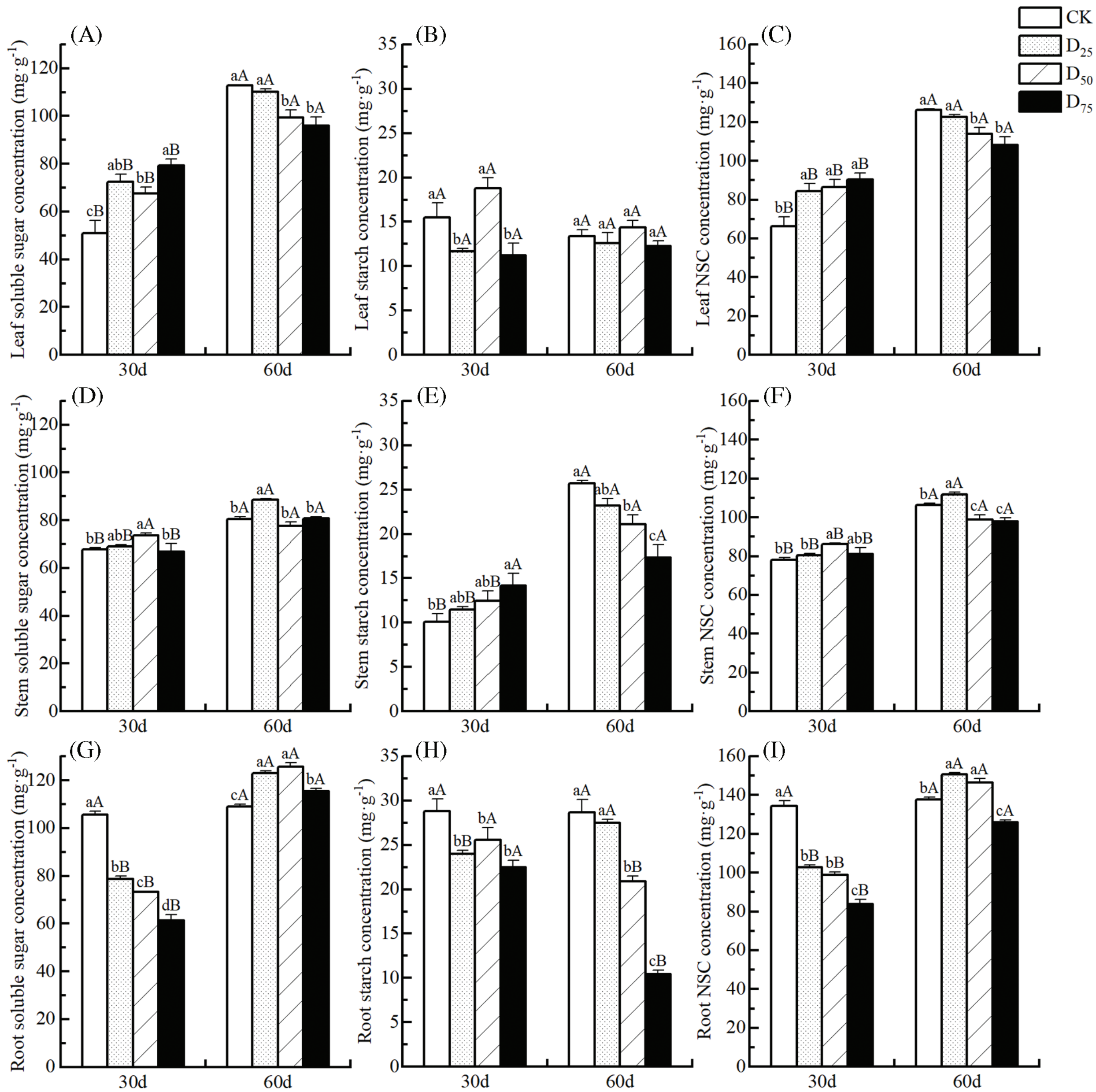

Figure 3: Effect of defoliation on the soluble sugar, starch, and NSC concentrations in various organs of *P. talassica* × *P. euphratica* seedlings. (A)–(C): leaf; (D)–(F): stem; (G)–(I): root

Note: Data represent Mean ± SE. Different capital letters in the same column indicate that different observed times for the same treatment are significantly different ($p < 0.05$), and lowercase letters indicate that different treatments for the same observed time are significantly different ($p < 0.05$).

4.2 Effects of Defoliation on the Root Architecture and Biomass of P. talassica × P. euphratica Seedlings

As an vital 'sink' organ for tree growth, root development requires the consumption of large amounts of photosynthetic products. Defoliation leads to reductions in the leaf area and carbon sources, and the allocation of carbohydrates to various organs changes, which may lead to a carbon imbalance, resulting in weakened root development or even death [38,39]. Defoliation alters root growth, including attenuating or stagnating lateral root growth, which makes plant recovery difficult [40]. In this study, at the 30th d after treatment, as the defoliation intensity increased, the total root lengths and surface areas showed increasing and then decreasing trends. Under 50% defoliation, root length and surface area were greatest, but there was no significant change in root volume. This indicates that the moderate defoliation intensity is favorable to the growth of root systems within a certain period of time. By the 60th d, the total root length did not change significantly, but root surface area and volume showed increasing and then decreasing trends, with only the 25% defoliation treatment showing significant increases in root surface area and volume. Thus, defoliation treatments changed the carbon source allocation strategy in the plant, The light and moderate defoliation intensities, stimulated root growth, which increased the water and nutrient uptake levels [41,42].

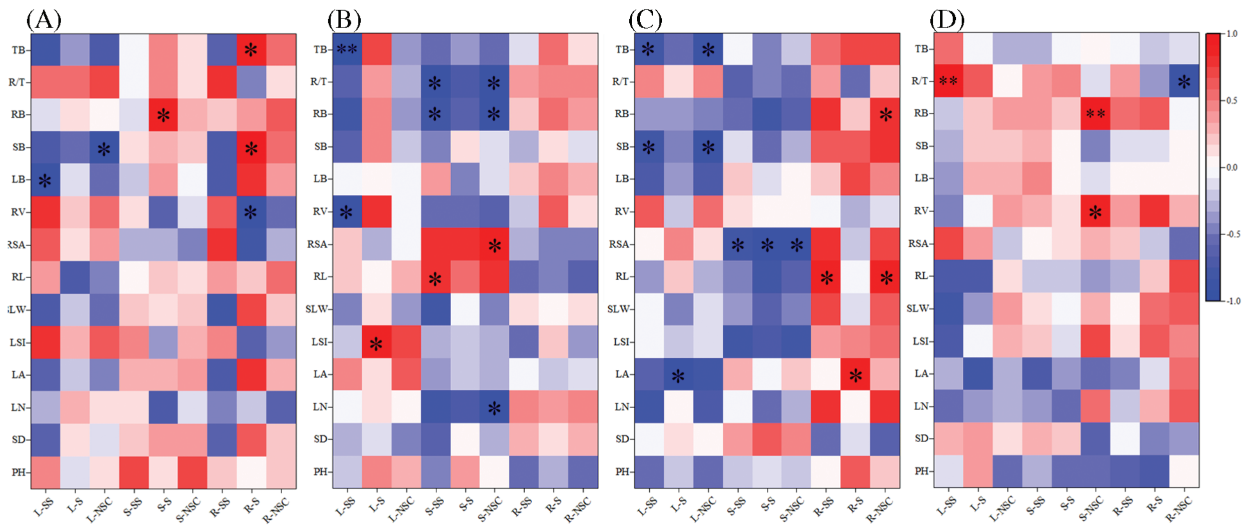

Figure 4: Correlation analysis between growth indicators and non-structural carbohydrates of *P. talassica* × *P. euphratica* seedlings. (A) CK; (B) D_{25} treatment; (C) D_{50} treatment; (D) D_{75} treatment. Abscissa: L-SS: soluble sugar concentration in leaf; L-S: starch concentration in leaf; L-NSC: NSC concentration in leaf; S-SS: soluble sugar concentration in stem; S-S: starch concentration in stem; S-NSC: NSC concentration in stem; R-SS: soluble sugar concentration in root; R-S: starch concentration in root; R-NSC: NSC concentration in root. Ordinate: PH: plant height; SD: stem diameter; LN: leaf number; LA: leaf area; RL: root lenght; RSA: root surface area; RV: root volume; LB: leaf biomass; SB: stem biomass; RB: root biomass; TB: total biomass

As a basic biological characteristic and functional trait, biomass reflects the carbon storage and also the accumulations of acquired substances and energy [43]. The defoliation treatments directly affected the plant carbon acquisition ability, which in turn affected the accumulation of plant biomass. The biomass allocation of each organ is not only affected by the plant species, environmental factors, and defoliation intensity, it is also closely related to the harvest time [44]. Helbig et al. [45] applied three consecutive defoliation treatments to five species of *Populus* and three species of *Salix*. They significantly reduced the plant heights and the fresh weights of the aboveground biomasses and had greater effects on *Populus* than on *Salix* species. In this study, defoliation treatments affected biomass allocation in *P. talassica* × *P. euphratica*. At the 30th d, there was no significant change in root biomass, significant decreases in stem and leaf biomasses, and a significant increase in the R/T under 50% defoliation. By the 60th d, the indexes were no longer significantly different compared with the CK. This suggests that in the short term, under a moderate defoliation intensity, *P. talassica* × *P. euphratica* seedlings preferentially reduced stem and root biomass accumulations to compensate for carbon supply limitations, but they were able to recover growth within a certain time period. However, a higher defoliation intensity instead promotes the accumulation of biomass [46]. However, in this study, the biomass of each organ was always significantly reduced under the 75% defoliation treatment, which shows that the heavy defoliation intensity severely inhibited the growth of seedlings. This may have been due to insufficient carbon storage in the organs of the *P. talassica* × *P. euphratica* seedlings to cope with the limited carbon supply caused by heavy defoliation. This resulted in stress that negatively affected the its growth.

4.3 Effects of Defoliation on the NSC Concentrations of P. talassica × P. euphratica Seedlings

The regeneration capacity of trees is crucial for preserving vegetative diversity in forest ecosystems [47], whereas the allocation of NSCs to various organs significantly influences the growth and development of trees. It also reflects the adaptive capacity of trees in response to changes or disturbances in the external environment [48]. Alabarce et al. [49] demonstrated that *Araucariaceae angustifolia* is tolerant to branch and leaf damage, and it can respond to damage by increasing starch content. It also increases flavonoid concentrations in the leaf and stem, which protects it from animal consumption-related damage by weighing the allocation strategy between defense and tolerance during individual growth and development. In this study, defoliation significantly increased the NSC concentration in leaf at the 30th d, whereas the NSC concentration in root decreased. The overall performance is the consumption of NSCs in root. It may be that after defoliation, *P. talassica* × *P. euphratica* seedlings preferentially promote new branches grow, and photosynthate mainly accumulated in the leaves. However, with an insufficient carbon supply, the conversion of starch to soluble sugar is promoted and carbon utilization increases. Because photosynthetic products cannot be transported to the root system in time, *P. talassica* × *P. euphratica* seedlings may preferentially consume NSCs from the root system to maintain root development. This involves increasing growth of fine root and surface area, as well as the ability to absorb water and nutrients from the soil, in accordance with the proximity principle [50,51]. By the 60th d, with the increase in the defoliation intensity, the NSC concentration of each organ gradually reduced, indicating that NSCs in leaf and stem began to accumulate from the root under light and moderate defoliation intensities. Additionally, the consumption of the starch concentration in stem was dominant. This may be why defoliation inhibits the longitudinal growth of trees [52]. However, under the 75% defoliation treatment, the NSC concentration significantly decreased due to the lower starch concentrations in root and stem. This indicates that under long-term heavy defoliation, carbon storage in the stem and root can no longer compensate for the insufficient allocation of photosynthesis products owing to the reduction in leaf area; therefore, plant growth and development are limited. Long-term exposure to such an environment may even lead to the death of *P. talassica* × *P. euphratica* seedlings owing to carbon starvation. Thus, the starch content (carbon storage) is instrumental in forest growth and development, including physiological defenses and other processes [53].

4.4 Correlation between Growth Indicators and Non-Structural Carbohydrates of P. talassica × P. euphratica Seedlings

The contents and distribution of NSC in different plant tissues can reflect differences in the response of plant organs to carbon supply status and adaptation to environmental changes [54]. This research examined the correlation between growth indicators and non-structural carbohydrates of *P. talassica* × *P. euphratica* seedlings subjected to different defoliation methods. The results showed that defoliation affected the NSC concentration of its organs and regulated growth. Under different defoliation intensities, the correlation between the concentrations of NSCs and growth indicators varies in various organs. At the 60th d, with the decrease of defoliation intensities, the soluble sugar and NSC concentrations in leaf gradually decreased, and a decreasing trend of negative correlation with total biomass. This may be due to the fact that as the defoliation intensity increases, the leaf number and total area reduced, and the accumulation of leaf NSCs decreased, resulting in a reduced impact on the total biomass. Under D_{50} treatment, the NSC concentrations in leaf and stem showed a negative correlation with most growth indicators, while the NSC concentrations in roots showed a positive correlation with most growth indicators. These results indicate that under moderate defoliation intensity, NSCs in root contributes significantly to plant growth, i.e., the NSC concentration in root is crucial for plants to respond to environmental changes [55]. As a fast-growing tree species, *P. talassica* × *P. euphratica* requires more photosynthate to supply its growth during the seedling stage. Heavy defoliation directly leads to the slowing down of plant growth, the reduction of biomass, and the decrease of NSC concentrations in various organs, which disrupts carbon balance [56]. At this time, non-structural carbohydrates in stem showed mostly positive trends with root morphology and biomass, while the NSC concentrations in leaf and root are not strongly correlated with most growth indicators. This may be because the carbon storage in the root is greatly consumed, and newly synthesized carbon is not transported to the root in time, requiring a longer recovery time [57].

5 Conclusions

Defoliation affected the plant height, ground diameter, leaf number, root architecture, biomass accumulation, and NSC allocation strategies of *P. talassica* × *P. euphratica* seedlings, and they changed dynamically over time. Within a time period, the damage caused by light and moderate defoliation on plant root architecture and biomass could be recovered by adjusting the allocation of NSCs, and this could even promote plant height, ground diameter, and leaf number. Severe defoliation significantly reduced plant height, ground diameter, leaf number, single-leaf area, organ biomass accumulation, and NSC concentration. In particular, it depleted the starch concentrations in the stem and root, limiting plant growth. The main reason for the energy imbalance and under-compensated plant growth under severe defoliation may depend on the insufficient root NSC concentration and newly synthesized carbon. This provided the groundwork for the thorough management of *P. talassica* × *P. euphratica* plantations and is a reference for the study of carbon allocation strategies in forests under carbon-limited conditions. In the future, in accordance with the geographical characteristics of Xinjiang, we will carry out multi-factor interaction experiments by combining defoliation with drought, light, and other environmental factors, to explore the impacts of carbon limitation and carbon regulatory mechanisms on forest growth.

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