

Article

Root Traits Determine Variation in Nonstructural Carbohydrates (NSCs) under Different Drought Intensities and Soil Substrates in Three Temperate Tree Species

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Abstract: Nonstructural carbohydrates (NSCs) are a key factor in the physiological regulation of plants and can reflect buffering capacity of plants under diverse environmental conditions. The effects of diverse environmental conditions on plant NSCs and tissue or organ scales have been thoroughly studied, but their effects on fine root (root diameter < 2 mm) NSC concentrations are still not completely understood. Our aims were to explore the synergistic fluctuations in root traits and NSC concentrations under diverse environmental conditions. This study was conducted on two-year-old temperate seedling tree species (Juglans mandshurica Maxim., Fraxinus mandshurica Rupr., and Phellodendron amurense Rupr.) with different drought intensities and soil substrates. The specific root length (SRL) and specific root surface area (SRA) were significantly affected by drought intensities and soil substrates, while the root tissue density (RTD) and average diameter (AD) were not significantly affected by water intensities and soil substrates in all three species. The root C, N, and P concentration did not change according to drought stress but were significantly affected by the soil substrates in all three species. Similarly, the soluble sugar (SS) and starch (ST) concentrations were significantly affected by both the drought stress and the soil substrates in all three species. The AD explained 6.8% of the total variations in soluble sugar, while the SRL explains 32.1% of the total variation in starch. The root tip C, N, and P concentrations were not significantly correlated with NSCs under different treatments. The total variations in root tip morphology, chemistry, and NSC concentrations are greater among species than compared to different drought intensities and soil substrates. However, the root NSC concentrations were closely related to root morphological traits (SRL and AD) rather than chemical traits. On the basis of different soil resources, the species with thinner diameters have higher SS concentrations, while those of a thicker diameter have higher ST concentrations.

Keywords: root tip; nonstructural carbohydrates; morphological traits; drought; soil substrates

1. Introduction

Variations in climatic conditions, high temperatures, and low rainfall have caused most parts of the world to become dry, especially in temperate regions, resulting in tree death and forest ecosystem



declines [1–3]. The IPCC (Intergovernmental Panel on Climate Change) predicted that the most serious and frequent drought events will occur in the coming decades [4]. Nonstructural carbohydrates (NSCs, mainly composed of soluble sugar and starch) are a product of plant photosynthesis and the main substrate for plant respiration, which is responsible for the carbon budget balance [5,6]. NSC also reflects the level of substance for plant survival, growth, and their buffering capacity under environmental degradation [7] and NSCs are an important factor in the regulation of plant physiological phenomena under diverse environmental conditions. In recent years, most of the studies examining NSC dynamics at the tissue or organ scale have investigated the effects of water [1,8–10], CO₂ concentrations [11], shade [10,12], and other factors. However, these studies have rarely focused on root traits, especially the root tip morphology under different drought intensities. Some recent studies have documented that root tissue has the highest NSC concentrations, which can affect NSC storage and distribution in trees, but not in tree trunks [13,14]. Therefore, exploring the variation in the composition and distribution of fine roots under drought is important for better understanding how carbon balance and dynamics affect plant growth and survival [15].

Fine roots (root diameter < 2 mm) are the most important organs of plants and are used for water and nutrient uptake from soil. Moreover, their growth characteristics and conditions can directly affect the growth and development of individual plants. Carbohydrates in roots are mainly used for physiological activities, such as plant growth, respiration rate, nutrient absorption, and organic secretion [16]. Carbohydrate storage in plants can play an important role in plant metabolism, defense, and drought resistance, delaying or preventing plant death. Pregitzer et al. [17] found significant differences in the root morphology (such as diameter) and tissue nitrogen (N) content of the fine roots among nine North American tree species. In recent years, many researchers have observed that lower root order diameters (especially root tips) of plants that have a higher turnover rate, a shorter lifespan, a stronger absorption capacity for water and nutrients, and are more sensitive to diverse environmental conditions [18,19]. A subsequent study by Hartmann [8] found that drought stress can cause a decrease in NSC content in forest roots. However, we have little knowledge about the changes in the composition and level of NSCs in plant root tip tissue (first-order roots) under drought conditions. Several studies have found that there are large intra- and interspecific [20–22] variations in fine root diameter, especially in tropical species [23]. These differences in root diameter directly lead to changes in the absorption capacity, root lifespan, and turnover rate under different resource conditions [24]. Therefore, plant functional traits can be adjusted according to their environment, which could allow the same species to grow and survive under diverse environmental conditions [25]. By comparing the different root-order traits of different diameters of beech and Norway spruce, Weemstra et al. [26] found that tree species in poor soil changed their root architecture rather than their morphological traits; the authors also observed interspecies differences in plasticity reactions. The leaf intra- and interspecific plasticity for environmental resource availability has been widely reported [27,28], while only a the few studies were found on roots [29]. The response strategies of fine root of different species under different soil resources and drought intensities are especially scarce in the literature.

Juglans mandshurica Maxim. (JM), *Fraxinus mandshurica* Rupr. (FM), and *Phellodendron amurense* Rupr. (PA), known as the three most valuable hardwood trees, are important dominant species in broadleaf Korean pine forests in Northeast China [30]. However, the decline of these species in secondary forests is very common due to diverse environmental conditions [30,31]. Understanding the physiological and ecological phenomena of these tree species under diverse environmental conditions is important and could contribute to better forest management practices to successfully promote the succession of these species [32]. These species were also observed to have relatively large compound leaves with a fine root diameter gradient. Compound leaves are derived features in angiosperms that can develop independently in multiple lineages [33,34]. These species often appear in warm and water-restricted (arid or semi-arid) areas, indicating their ability to adapt to diverse environmental conditions [36,37]. It has also been observed that compound leaf species are more likely to be pioneers and early succession species [36,37]. They can produce relatively larger and cheaper petioles and grow

rapidly under favorable environmental conditions [38]. Conversely, they can also adapt to seasonal and unpredictable drought stress and can minimize their water loss by reducing their leaf size under drought conditions, thereby better protecting stems with a greater carbon cost from catastrophic hydraulic failure and withering than would be the case for typical hydraulic segmentation behavior [39,40]. However, the interspecific variation of fine root NSC levels in compound leaf species under different environmental conditions remains unclear, and the relationship between root morphological traits and NSCs has not been clarified.

Stands of JM, FM, and PA are normally distributed in the north–south latitude in Jilin Province, China. The soils in different areas are roughly divided into three types (humus, loam, and sandy-loam). In our study, these three species of temperate tree seedlings were experimentally grown under different drought intensities and soil substrates according to different soil textures. Our aim was to compare the effects of root tip NSCs with different fine root diameters under drought intensities and soil substrates on temperate tree seedlings. We hypothesized that (1) in mild and moderate drought intensities, the three species would have a higher specific root length (SRL), a higher specific root surface area (SRA), and a thinner average diameter (AD); (2) the root NSC concentrations would be significantly different among the three species, and the thinner root diameter would have a lower SS concentration; (3) the NSC concentrations of the plant tissue are hypothesized to be mainly affected by the carbon absorption and consumption of the plant, therefore the NSC concentrations are significantly correlated with the fine root chemistry.

2. Materials and Methods

2.1. Experimental Site and Sapling Preparation

A controlled pot experiment was conducted at the Xinli experimental site in Changchun, Jilin Province, China (43°33' N–44°41' N, 125°19' E–125°24' E). This area has a warm and continental monsoon climate with hot, wet summers and cold, dry winters. The study site has a frost-free period of 140 days and a mean annual rainfall of 600–800 mm, which mainly occurs from July to September; the mean annual temperature is 4.6 °C. The area features a natural distribution of *Juglans mandshurica*, *Fraxinus mandshurica*, and *Phellodendron amurense*.

The seeds of *Juglans mandshurica* (JM), *Fraxinus mandshurica* (FM), and *Phellodendron amurense* (PA) were collected by the Lushuihe Forestry Bureau in Jilin Province in 2015. In 2016, the sterilized and germinated seeds were sown into the nursery (humus soil), and the required amounts of water and fertilizer were applied during the entire growing season. The two-year-old seedlings of JM, FM, and PA were transplanted into brick-red plastic pots (24×20 cm) in April 2017, and were placed in plastic greenhouse cultivation before starting to bud. At the experimental site, the pots were placed in rows 50 cm apart from each other under full sunlight. All pots were equally irrigated prior to application of the drought treatments, and the gravimetric soil water content was initially maintained at field capacity. The soil surface was well ventilated throughout the experiment. The pots were filled with cultured soil with equal volumes of humus, loam, and sandy-loam, and the humus was collected in the natural coniferous and broadleaf forest around the experimental site. The soil type was classified according to the Food and Agricultural Organization soil classification system (FAO 1998) as Eum-Orthic Anthrosol.

2.2. Experimental Design

Three soil substrate samples were collected in July 2017 for the determination of total nitrogen, total phosphorus, available phosphorus, soil physical properties, water content, and field water holding capacity. The seedlings of JM, FM, and PA (with a uniform size in terms of their height and diameter) in each pot were randomly selected from three soil substrates to carry out the water control experiments. The collar diameter and plant height of each seedling were measured using a Vernier caliper with an accuracy of 0.01 mm and a measuring tape with an accuracy of 0.1 cm prior to the application of drought treatments in July 2017 (leaves fully expanded).

A two-factor complete orthogonal design was conducted with three tree species, three soil substrates, and four drought intensities. Three soil substrates were set with four drought intensities (80%, 60%, 40%, and 20%) for the field water holding capacity (representing a normal water supply CK, mild drought T1, moderate drought T2, and severe drought T3) with a total of 12 treatments (Figure S1). Each treatment consisted of three blocks, and 40 pots per tree species were selected in each block (with a uniform size in terms of their height and diameter). For the water control experiments, the whole pot weighing method was used during the experiment. Based on the previously measured water content and field water holding capacity, the total weight of the soil, pots, and seedlings was calculated under different water conditions, and the pots were weighed every two days using an industrial grade balance with an accuracy of 5 g. Subsequently, each pot was irrigated to maintain the desired pot mass, and this watering regime was continued until the 10 September (abscission period). Since the seedling's own weight was constantly changing during the whole drought regime, the seedling weight was measured again every 20 d (select five sampling, three times in total until September 10), and the theoretical total weight was corrected before maintaining the desired pot mass.

2.3. Sampling and Measurements

2.3.1. Plant Sample Collection

In each experimental block, 10 seedlings per treatment were randomly selected, and the root samples were collected after 60 days of continuous drought stress. The root samples were sorted carefully from the soil and washed with tap water to remove soil particles. They were then placed in a labeled pocket and stored (2–3 °C). Then, the root samples were divided into two parts: one for root morphological traits and the other for chemical traits. All root samples were transferred to the laboratory on the same day and stored (–20 °C); the live root samples were then analyzed, and the dead roots were picked and discarded.

2.3.2. Soil Physical and Chemical Properties

The physicochemical properties of the three soil substrates were determined before the application of drought stress (Table 1). The soil total nitrogen was determined by Kjeldahl titration. The soil total phosphorus was measured by the sulfuric acid-perchloric acid-molybdenum anti-colorimetric method [41]. Soil available phosphorus was extracted by double acid extraction, and soil water content and bulk density were surveyed by the ring knife method. Methods for the determination of total soil porosity, soil aeration, water absorption multiple, water seepage rate, and evaporation rate were used as previously described [42].

Soil Substrate Physicochemical Property	Humus	Loam	Sandy-Loam		
Bulk density (g⋅cm ⁻³)	$1.18 \pm 0.02b$	$1.35 \pm 0.02a$	$1.32 \pm 0.02a$		
Total porosity (%)	$53.19 \pm 1.12b$	$58.90 \pm 0.91a$	$36.72 \pm 0.95c$		
Aeration porosity (%)	$25.61 \pm 0.48a$	$23.52 \pm 0.53a$	$13.01 \pm 0.99b$		
Water absorption capacity	$0.23 \pm 0.01b$	$0.26 \pm 0.01a$	$0.18 \pm 0.01c$		
Penetrate rate $(g \cdot min^{-1})$	$4.49 \pm 0.04a$	$4.01 \pm 0.12b$	$3.04 \pm 0.18c$		
Evaporation rate $(g \cdot h^{-1})$	$0.59 \pm 0.01a$	$0.50 \pm 0.01 b$	$0.37 \pm 0.01c$		
Total nitrogen (mg·g ⁻¹)	$7.09 \pm 0.78a$	$3.11 \pm 0.05b$	$1.20 \pm 0.03c$		
Total phosphorus ($mg \cdot g^{-1}$)	$0.71 \pm 0.04a$	$0.34 \pm 0.09b$	$0.32 \pm 0.01b$		
Available phosphorus (mg·kg ⁻¹)	$13.10 \pm 0.82a$	$5.04 \pm 0.21b$	$12.75 \pm 0.69a$		

Table 1. Physicochemical properties of three soil substrates.

Note: the different letters in the same line indicate significant difference among the different treatments (p < 0.05); The same below (LSD test); all data were mean ± standard error (mean ± SE).

2.3.3. Root Tip Morphological and Chemical Traits

In the laboratory, the root samples for morphological trait analysis were carefully dissected with forceps based on their branching order, following the procedure described in [17] and [20], with the distal nonwoody roots regarded as first-order roots. Then, the root samples of each species were scanned with an Expression 10000XL 1.0 scanner at the Northeast Forestry University (Epson Telford Ltd., Telford, UK). The mean diameter, total length, and volume of the root tips (first-order roots) were determined using Root System Analyzer software (WinRhizo 2004b, Regent Instruments, Inc., Québec, Canada). Next, these root samples were oven-dried (65 °C, 48 h), weighed (\pm 0.0001 g), and their specific root length (SRL), root surface area (SRA), average diameter (AD), and root tissue density (RTD) were calculated.

For the root chemical analyses, as mentioned above, the fine root sample (after scanning) was dried (65 °C, 48 h) and weighed (±0.0001 g). The dried fine root sample was ground and homogenized by using a ball mill instrument (RETSCH MM 400, GmbH, Haan, Germany), and 1 g of the dry powder sample was weighed and pressed with a FYD-20 electric tableting machine (Nuoleixinda Technology Co., Ltd., Tianjin, China) to form a plate that was 6 mm thick with a diameter of approximately 13 mm. For the pellet sample, the tableting conditions were adjusted according to the actual situation, and the test parameters were maintained (16 MPa, 3 min). The carbon (C), nitrogen (N), and phosphorus (P) elements in the sample were measured after tableting using a J200 Tandem laser spectroscopic element analyzer (Applied Spectra, Inc., Fremont, CA, USA).

2.3.4. NSC Concentration

The NSC concentration was defined as the sum of soluble sugar (SS) and starch (ST) concentrations that were measured using the anthrone method [43]. The root sample (0.1000 g) was placed into a 10 mL centrifuge tube with the addition of 2 mL of 80% ethanol. The mixture was incubated at 80 °C in a shaking water bath for 30 min and centrifuged at 4000 rpm for 5 min. Next, two extractions from the pellets were carried out with 80% ethanol. The supernatant was retained, combined, and stored at -20 °C for soluble sugar determination.

Starch was extracted from the ethanol-insoluble pellet following removal of the ethanol by evaporation. The starch in the residue was then released by boiling in 2 mL distilled water for 15 min. After cooling at room temperature, 2 mL 9.2 M HClO₄ was added, and the mixture was shaken for 15 min. Next, 4 mL distilled water was added, and the mixture was centrifuged at 4000 rpm for 5 min. A further extraction was carried out with 2 mL 4.6 M HClO₄. The supernatant was also retained, combined, and stored at -20 °C for starch determination.

Sugar and starch determination was performed based on the absorbance at 625 nm using the same anthrone reagent in a spectrophotometer [43]. Sugar concentration was calculated using the regression equations based on the standard glucose solutions and starch concentrations by multiplying the glucose concentration with a conversion factor of 0.9 [44].

2.4. Data Analysis

A multiway ANOVA of root tip morphological and chemical traits was performed using SPSS 19.0 (IBM Co., Armonk, NY, USA) to examine the differences in the root tip traits of seedlings between different treatments (LSD, $\alpha = 0.05$). Principal component analysis (PCA) and redundancy analysis (RDA) were performed on the root tip traits of seedlings under different treatments using the Canoco software for Windows (Version 4.56, Biometris Plant Research International Wageningen, The Netherlands). A Monte Carlo replacement test was performed on the parameters in the RDA analysis using vegan package in R language [45]. Regression analysis was used to determine the relationship between the specific root length, mean diameter, and NSCs. All data were represented as the mean \pm standard error (mean \pm SE).

3. Results

3.1. Root Tip Morphology

All root tip morphological traits were significantly different among the three tree species. *J. mandshurica* (JM) had a comparatively larger SRL and SRA but a lower RTD and a smaller AD than *F. mandshurica* (FM) and *P. amurense* (PA) (Figure 1A,C,E,G; p < 0.01). In JM and FM, the SRL and SRA significantly increased with an increase in the intensity of drought treatments (p < 0.05). Compared with CK, the SRL and SRA of the three species were significantly increased in T3, with an average range for the soil substrates of 17.22% and 14.77%, while in JM and FM, the RTD and AD did not change significantly under drought treatment (p > 0.05). The SRL of JM, FM, and PA, respectively, increased by 13.23%, 26.08%, and 33.46% in sandy-loam soil compared to the values in humus soil (Figure 1B). The RTD of JM, FM, and PA, respectively, increased by 18.53%, 22.89%, and 19.15% in humus soil compared to the values in sandy-loam soil (Figure 1H). Variations in the SRA and AD of the three tree species according to different substrates were not considered significantly change the root morphological traits.



Figure 1. Cont.



Figure 1. Root tip morphological traits ((**A**,**B**) Specific root length; (**C**,**D**) Specific root surface area; (**E**,**F**) Average diameter; (**G**,**H**) Root tissue density) in different drought and soil substrates. The histogram represents the average values across four drought treatments. The error bars represent SEM. H, humus; L, loam; SL, sandy-loam; JM, *Juglans mandshurica* Maxim.; FM, *Fraxinus mandshurica* Rupr.; PA, *Phellodendron amurense* Rupr. CK, control; T1, mild drought; T2, moderate drought; T3, severe drought; n.s. denotes not significant; D, drought stress; S, soil substrates; Spp., species; *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001.

3.2. Root Tip Carbon, Nitrogen, and Phosphorus

Variations in the root tip N concentration were significantly explained by species (Table 2, p = 007). The root N concentration for JM was higher in humus soil under T2 and T3 treatments than that in CK. The root N concentration for FM was 11.89% higher in sandy-loam soil in T3 than that in CK (Table 3, p < 0.05). Compared to CK, PA had a lower N concentration in humus soil in T1 and T2. Drought did not significantly affect the root C and N concentrations in the three tree species, while the soil substrate significantly changed the root C, N, and P concentrations (Table 2, $p_C < 0.001$; $p_N < 0.001$; $p_P = 0.003$). However, the root C, N, and P concentrations significantly differed considering the soil–species and drought–soil–species interactions (Table 2, p < 0.01).

Table 2. Results of three-way (drought intensity \times soil substrates \times species) factorial ANOVA of chemical traits of the root tip. Values in bold type indicate significant effects.

Source of Variation	df _	С		Ν		Р	
Source of variation		F	р	F	р	F	р
D	3	0.399	0.754	0.188	0.904	3.001	0.036
S	2	22.109	< 0.001	11.669	< 0.001	6.191	0.003
Spp.	Spp. 2 D × S 6		0.092	5.369	0.007	2.03	0.139
$D \times S$			0.123	0.895	0.503	2.405	0.036
$D \times Spp.$	6	1.897	0.093	2.226	0.050	4.567	0.001
$S \times Spp.$	4	19.142	< 0.001	16.257	< 0.001	13.802	< 0.001
$D \times S \times Spp.$	12	3.801	<0.001	2.871	0.003	2.245	0.018

D, drought stress; S, soil substrates; Spp., species.

Soil Substrates	Drought Stress	JM			FM			PA		
		C (%)	N (%)	p (%)	C (%)	N (%)	p (%)	C (%)	N (%)	p (%)
Н	CK	$41.35 \pm 0.12a$	$2.12 \pm 0.01b$	$0.23 \pm 0.01a$	$41.05 \pm 0.03a$	$2.06 \pm 0.02a$	$0.25 \pm 0.01a$	$41.17 \pm 0.08a$	$2.10 \pm 0.03a$	$0.25 \pm 0.01a$
	T1	$41.30 \pm 0.12a$	$2.12 \pm 0.02a$	$0.24 \pm 0.01a$	$41.05 \pm 0.05a$	$2.06 \pm 0.03a$	$0.25 \pm 0.01a$	$41.04 \pm 0.01a$	$2.03\pm0.01\mathrm{b}$	$0.25 \pm 0.01a$
	T2	$41.41 \pm 0.16a$	2.16 ± 0.06 ab	$0.23 \pm 0.01a$	$41.09 \pm 0.05a$	$2.08 \pm 0.03a$	$0.25 \pm 0.01a$	$41.16 \pm 0.02a$	$2.07 \pm 0.01 ab$	$0.24 \pm 0.01a$
	T3	$41.51 \pm 0.11a$	$2.20\pm0.04a$	$0.23 \pm 0.01a$	$41.01 \pm 0.04a$	2.03 ±0.02a	$0.25 \pm 0.01a$	$41.04\pm0.03a$	$2.03\pm0.01\mathrm{b}$	$0.25 \pm 0.01a$
L	CK	$41.12 \pm 0.08a$	$2.06 \pm 0.03a$	$0.25 \pm 0.01a$	$41.02 \pm 0.06b$	$2.04 \pm 0.03a$	$0.25 \pm 0.01a$	$41.00 \pm 0.15a$	$2.01 \pm 0.06a$	$0.25 \pm 0.01a$
	T1	40.98 ± 0.02 ab	$2.00 \pm 0.01 bc$	0.25 ± 0.01 bc	$41.05\pm0.08\mathrm{b}$	$2.07 \pm 0.04a$	$0.25 \pm 0.01a$	$41.00 \pm 0.04a$	$2.01 \pm 0.02a$	$0.25 \pm 0.01a$
	T2	$40.87 \pm 0.03b$	$1.96 \pm 0.01c$	$0.26 \pm 0.01c$	$41.01\pm0.04\mathrm{b}$	$2.04 \pm 0.02a$	$0.25 \pm 0.01a$	$41.11 \pm 0.06a$	$2.06 \pm 0.03a$	$0.25 \pm 0.01a$
	T3	$40.92 \pm 0.03a$	2.02 ± 0.01 ab	0.25 ± 0.01 ab	$41.44 \pm 0.10a$	$2.02 \pm 0.04a$	$0.22 \pm 0.01b$	$40.93 \pm 0.10a$	$2.05 \pm 0.01a$	$0.25 \pm 0.01a$
SL	CK	$41.03 \pm 0.01a$	$2.02 \pm 0.01a$	$0.25 \pm 0.01a$	$41.00 \pm 0.13a$	$2.02 \pm 0.06b$	$0.25 \pm 0.01a$	$41.08 \pm 0.04a$	$2.05 \pm 0.02a$	$0.25 \pm 0.01a$
	T1	40.97 ± 0.02 ab	$2.00\pm0.01\mathrm{b}$	$0.25 \pm 0.01b$	$41.24 \pm 0.09a$	$2.16 \pm 0.05 ab$	$0.24 \pm 0.01a$	$41.05 \pm 0.04a$	$2.03 \pm 0.02a$	$0.25 \pm 0.01a$
	T2	$40.93 \pm 0.01b$	1.98 ± 0.01 bc	0.25 ± 0.01 bc	$41.15 \pm 0.07a$	2.11 ± 0.03 ab	$0.25 \pm 0.01a$	$41.09 \pm 0.07a$	$2.05 \pm 0.03a$	$0.25 \pm 0.01a$
	T3	$40.92 \pm 0.03b$	$1.98 \pm 0.01c$	$0.25 \pm 0.01c$	$41.44 \pm 0.10a$	$2.26 \pm 0.05a$	$0.24 \pm 0.01a$	$40.93 \pm 0.10a$	$1.98 \pm 0.04a$	$0.25 \pm 0.01a$

Table 3. Root tip carbon, nitrogen, and phosphorus in different drought, soil substrates, and species.

Note: the different letters indicate significant difference among the drought treatments in same soil substrates (*p* < 0.05); The same below. H, humus; L, loam; SL, sandy-loam; JM, *Juglans mandshurica* Maxim.; FM, *Fraxinus mandshurica* Rupr.; PA, *Phellodendron amurense* Rupr. CK, control; T1, light drought; T2, moderate stress; T3, severe stress; all data were mean ± standard error (mean ± SE).

3.3. Root Tip NSC Concentration

Root tip soluble sugars (SS), starch, and NSCs significantly decreased under drought treatments in the three tree species (Figure 2). With an increase of drought intensity, the root SS, ST, and total NSC concentrations were significantly affected. The root SS, ST, and total NSC concentrations for JM in T3 significantly decreased, respectively, by 40.20%, 79.12%, and 72.80% on average for the soil substrates compared to CK (Figure 2A–C). Compared to CK, FM and PA had lower SS, ST, and NSC concentrations in T3 for the three soil substrates. In general, with the exception of ST in FM, the SS and ST concentrations in humus soil were higher than those in the loam and sandy-loam soil. The root SS and ST concentrations for JM in humus soil were, respectively, 29.69% and 120.53% higher than those in the sandy-loam soil. The SS concentrations for FM and PA in the humus soil were, respectively, 181.63% and 97.26% higher than those in the sandy-loam soil (Figure 2B,D, p < 0.01). Soil substrate, drought, and species had no significant effect on SS/ST (Figure 2G,H). The water–soil and soil–species interactions significantly affected the root ST and NSC concentrations, while no significant change was observed in the SS and ST concentrations under each treatment (Figure 2C–F).



Figure 2. Cont.



Figure 2. Root tip nonstructural carbohydrate ((**A**,**B**) Soluble sugar; (**C**,**D**) Starch; (**E**,**F**) NSC; (**G**,**H**) SS/ST) in different drought and soil substrates. The histogram represents the average values across four drought treatments. The error bars represent SEM. H, humus; L, loam; SL, sandy-loam; JM, *Juglans mandshurica*; FM, *Fraxinus mandshurica*; PA, *Phellodendron amurense*. CK, control; T1, mild drought; T2, moderate drought; T3, severe drought; n.s. denotes not significant; D, drought stress; S, soil substrates; Spp., species; *, p < 0.05; **, p < 0.01; ***, p < 0.001.

3.4. Relationship between Root Tip Morphology and NSC

By analyzing the root morphological and chemical traits under different water and soil conditions in the three temperate tree species, the first two axes of the redundancy analysis (RDA) explained approximately 90% of the total variations between all treatments (Figure 3). The degrees of separation of different tree species were better than those of water and soil. However, the root morphological and chemical traits under all treatments were subjected to a Monte Carlo test, while the root ST and NSC concentrations were the main influential factor of the first and second sorting axis, respectively (ST: $R^2 = 0.451$, p < 0.001; NSC: $R^2 = 0.637$, p < 0.001). Therefore, the root SS and ST concentrations were positively and negatively correlated with SRL and negatively and positively correlated with AD.



Figure 3. Redundancy analysis of root tip morphological and chemical traits in different drought, soil substrates and species. Red, humus; blue, loam; black, sandy-loam; circle, JM; square; FM; triangle, PA; SRL, specific root length; SRA, specific root surface area; AD, average diameter; RTD, root tissue density; SS, soluble sugar; ST, starch; C, carbon; N, nitrogen; P, phosphorus.

The root SS concentration was significantly affected by the treatments in all three species, but the treatments had no significant effect on the SRA and RTD, while the average diameter explained up to 6.8% of the total variation in soluble sugar (Figure 4A,B; p < 0.001). A significant correlation was observed between root ST concentrations and SRA, RTD, SRL, and AD; the SRL and AD, respectively, explain up to 32.1% and 28.4% (48.4% and 38.9% without 'ln' conversion, respectively) of the total variations in starch concentrations (Figure 4A,B; p < 0.001). There was no significant correlation between root C, N, P, and NSC concentrations under different treatments (data not shown).



Figure 4. Relationships of the SS and ST concentrations with SRL (**A**) and AD (**B**) of root tip in the three specie. SS, soluble sugar (black symbol); ST, starch (white symbol). Data for starch concentration data had undergone ln conversion.

4. Discussion

4.1. Response of Root Morphological Traits to Drought and Soil Substrates

According to our first hypothesis, the root morphological traits of all three species, especially the SRL, significantly changed under drought stress and different soil substrates. With increased drought intensity, the roots showed a larger SRL, SRA, a finer AD, and a higher RTD. Through their meta-analysis, Ostonen et al. [46] documented that SRL can be used as an indicator for plant drought stress tolerance. Olmo et al. [47] studied 10 different tree and shrub species and revealed that very fine root (root diameter < 0.5 mm) biomass significantly decreased under drought conditions, while very

fine root biomass and SRL increased, but AD and tissue N concentrations decreased. According to the traditional resource economic hypothesis, plants grown on poor soils should maintain their fine roots by adjusting their morphological plasticity, thereby maintaining a lower SRL (thicker and longer lifespan) to retain scarce resources (conservative strategy). Similarly, the plants grown on soil with sufficient nutrient availability are more likely to have a higher SRL to obtain soil resources efficiently and quickly (acquisition strategy) [48–50]. Conversely, some researchers have suggested that poor soils can cause plant fine roots to have a higher SRL (i.e., thinner with a shorter lifespan) because these roots can obtain nutrients faster and more effectively [51–53]. This trade-off between resource conservation and acquisition shows that fine root traits may also play a critical role within species. Our results are consistent with the latter hypothesis. However, in sandy-loam soil with relatively poor soil conditions, the root tip has a larger SRL, SRA, and a lower RTD. The SRL of JM, FM, and PA were increased by 13.23%, 26.08%, and 33.46% in sandy-loam soil compared to the values in humus soil, respectively. These results indicate that the variations in fine root morphology are mainly affected by the availability of soil resources. Weemstra et al. [26] revealed that the fine root SRL and RTD of beech and spruce do not significantly differ for clay vs. sandy soils. These fluctuations in fine roots suggest that their research sites were in field plots rather than in greenhouse pots, as the biotic and abiotic factors under field conditions are relatively complex.

The minute changes in fine root AD, SRL, and SRA could result in dramatic changes [54], and increases in root length mean that the absorption range of the root system in the soil has expanded [55]. Soil nitrogen deficiency usually promotes the growth of the primary root, particularly lateral root elongation but not lateral root initiation [56], while the formation of lateral roots is almost completely absent under severe N shortage [57]. In a nutrient-rich environment, the RTD was significantly higher than that in nutrient-poor environments. Previous studies have shown that RTD is positively corelated with root dry matter [58] and also with the root respiration rate [59] as plants can invest more photosynthetic products in maintaining root lifespan, resulting in a lower relative growth rate of the aboveground plants [60]. Therefore, there is a trade-off relationship between root carbohydrate input and aboveground productivity [61]. Plants usually increase their ability to capture nutrients through root plasticity under nutrient-deficient soil [62], the specific performance of which expands the absorption range or improves the absorption capacity in situ. Accordingly, a higher root density, branching density, root length, SRL, AD, and root volume were observed in nutrient-deficient soil compared to those under higher nutrient soil [54]. Thus, our results indicate that the seedlings significantly increased main root growth, lateral root elongation, and branching intensity of their fine roots, thereby expanding the distribution of roots in the soil and improving their ability to absorb soil resources. The seedlings with higher SRL, SRA, and number of root tips had significantly increased ability to absorb and utilize soil resources in situ [27] due to the establishment of a dense root network system [63]. This system also consumes more photosynthetic products due to accelerated fine root turnover. A higher root diameter and volume (PA) means that there were more coarse roots, which can be helpful in improving plant resistance to stress and the rapid growth of aerial parts.

4.2. Effect of Drought and Soil Substrates on Fine Root NSC Concentration

Nonstructural carbohydrates (NSCs) are an important component of plant carbon budgets. The content of soluble sugar in plants varies greatly over the entire plant growth and development, and is closely related to changes in environmental factors. Further, NSC adjustment is an important mechanism for adapting to environmental fluctuations [64]. Some previous studies have demonstrated that the degree of drought stress in trees is reflected by NSC concentrations. Therefore, NSCs could be used by plants to maintain root respiration, metabolism, and production, while severe drought stress can lead to a decline in NSC concentrations [65,66]. Zang et al. [67] documented that drought reduced the photosynthetic and root respiration rates in *Fagus sylvatica* L. leaves, but water deficit did not show such a relationship between drought, fine root starch, and NSC concentrations, except for fructose. A further study by Galvez et al. [68] revealed that *Populus tremuloides* Michx. can adapt to drought

by transforming its growth into root reserves with a two times higher starch concentration. Under severe drought intensity, Norway spruce, two eucalyptus species, and radiata pine showed lower concentrations of starch and sugar [9,69]. In our current study, the concentrations of soluble sugar and starch in roots in all three species gradually decreased with increasing drought intensity, and lower concentrations were observed in sandy-loam soil. However, in accordance with our second hypothesis, JM and FM (with a thinner root diameter) had higher SS concentrations compared to PA, while the starch concentrations were higher in trees with thinner root diameters (JM and FM) and lower in those with larger root diameters (PA). Thicker roots have relatively larger total cross-sectional areas of stele, and generally have a longer lifespan and lower turnover rate; thus, these roots can resist environmental stress, mechanical damage, and the feeding of soil animals [70]. Similarly, thicker roots have a relatively smaller cortical area, lower N content, and a slower metabolic rate [19,71,72]. Fort et al. [73] documented that thinner fine roots have a fast resource acquisition strategy to absorb larger amounts of water in a shorter time period and can consume larger amounts of water under water deficit conditions. Conversely, species with more conservative strategies have thicker roots and absorb less water during peak growth seasons while retaining their water uptake over long periods of time and consuming less water during periods of low water availability. We conclude that the JM roots with smaller diameters under drought stress have a larger SRL and can maintain their own growth (per unit of root length) by investing less carbohydrates and maintaining more nutrients; in return, they consume less SS to withstand stress conditions. Fort et al. [73] and Padilla et al. [74] found that drought-to-fast-resources have less of an impact on species than conservation strategies through short-term drought simulation experiments; the latter survive more efficiently in long-term droughts [49]. Plants with thicker roots generally have longer lifespans and greater transportive capabilities [75,76]. In our study, compared with JM and FM, PA (thicker root) contained higher starch and NSC concentrations, which could ensure long-term survival under adverse environmental conditions.

4.3. Relationship between Root SRL, Diameter, and NSC Concentration

Root diameter and SRL are the most common parameters of fine root (<2 mm) morphology and have been extensively studied as indicators under diverse environmental conditions, such as soil nutrient availability and drought stress [52]. The three compound leaf species in this study were clearly separated by two morphological axes, reflecting the different response mechanisms of different diameter gradients facing environmental fluctuations (Figure 3). The RDA1 axis was closely related to the SRL and SRA and was recognized as an acquisition strategy axis (Figure 3). The JM distributed on the right side of the axis among the three compound leaf species indicating its high capability to acquire soil resources (per root mass with a higher root length). Species of this strategy generally have shorter lifespans and more branches under diverse conditions, and mostly occur in moist soils [77]. Conversely, the species that are typically distributed around the RDA2 axis may be more inclined to employ a conservative strategy, indicating that the species can invest more biomass into their roots and maintain a longer lifespan under drought; these trees are distributed in cooler and drier areas [77]. Starch and NSC concentrations were negatively correlated to the ability to acquire resources (the higher the starch and NSC, the lower the SRL and SRA, such as in PA). The root lifespan of such species was relatively longer under a conservative strategy, maintaining a higher starch concentration and a low level of soluble sugar in order to avoid animal feeding, mechanical damage, etc. [78]. Guo et al. [79] documented that NSC concentrations are maintained by the construction and maintenance of fine roots with a diameter of 0.35 mm and are much lower than those in fine roots with a diameter of 0.80 mm. A correlation analysis between the root morphology and NSC concentrations of the three compound leaf species showed that SRL and starch are negatively correlated with each other, which could explain up to 32.1% of the total variation. However, the AD was negatively correlated with soluble sugar and positively correlated with starch and can explain 6.8% and 28.4% of the total variation, respectively. Some previous studies have demonstrated that under constant root tissue density, the SRL is closely correlated to the reciprocal of the AD square in temperate forest [24,75,80–82], subtropical forest [83–85],

and boreal forest [52]. Meanwhile, the SRL demonstrates high correlation with root respiration [86]. NSC is an important substrate for root respiration and can stimulate the root respiration rate [87,88]. Previous studies reporting on the relationship between fine root diameter and respiration found that shallow roots with a thinner diameter have a higher respiratory rate [89–91]. This further suggests that fine roots are very physiologically active [17,92], and their relatively larger SRL provides greater surface area (based on the mass per unit root) for more efficient water and nutrient absorption in heterogeneous soil, which results in a larger respiratory rate. Accordingly, for JM root with relatively larger SRL, shorter root lifespan and higher turnover rates are used to reduce its own losses and, thus, maintain a lower NSC level (acquisition strategy). By contrast, for PA with relatively thicker roots, a longer lifespan and lower turnover rate contribute to maintaining a higher NSC level (a conservative strategy) in order to maintain growth.

5. Conclusions

Our study showed that the root morphological traits and NSC concentrations in three temperate tree species are significantly different under drought stress and different soil substrates, but the differences between species were found to be larger than those observed under different drought and soil substrate conditions. The root NSC concentration (soluble sugar and starch) was closely related to the root morphological traits (SRL and AD) but did not correlate with the root C and N concentrations. Under a state of different soil resources, the root tip with the thinner diameter had higher SS concentrations, while the root tip with the thicker diameter had higher ST concentrations. Therefore, it was concluded that the three temperate tree species could construct a root system in the most "economical" way under different soil substrates; these species also presented the most beneficial (nutrient acquisition) and cost-effective (root structure and maintenance) relationship. This rationally demonstrates a trade-off in the root resource allocation model and determines the ecological countermeasures for plant adaptation to diverse environmental conditions. However, our research mainly focused on the root traits and NSC concentrations in temperate deciduous tree species. Few previous studies have focused on root anatomical traits under different drought intensities and soil substrates based on the economic spectrum of roots. Therefore, it is necessary to further explore the relationship between the morphological and anatomical root traits of temperate tree species in different climatic zones and taxonomic levels, especially regarding the mechanism underlying NSC levels and fine root lifespan and turnover.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/4/415/s1, Figure S1: A schematic map of the field experimental treatments.

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