

Article

Spatial Patterns of Non-Structural Carbohydrates in *Eucalyptus urophylla* × *E. grandis* under Dry-Season Irrigation with Fertilization

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Abstract: Non-structural carbohydrates (NSC) affect tree growth and survival when photosynthesis is impacted by climate change, such as seasonal drought and extreme precipitation. Nevertheless, it is still unclear whether *Eucalyptus* suffers growth limitation under natural conditions and if trees recover under artificial cultivation. In present study, we conducted a field control experiment to compare the NSC storage in *Eucalyptus urophylla* × *Eucalyptus grandis* trees on fertilization and dry-season irrigation to determine the variations of NSC under drought stress. The results indicated total soluble sugar (TSS) was the primary existing form of NSC. In spatial patterns, NSC concentration showed gradient differences from source organ to sink organ, and finally accumulated in root. The TSS concentration showed a decreased trend with height except leaf, while the trend of starch concentration was contrast. Surprisingly, fertilization and dry-season irrigation had not changed the carbon distribution among all tissues but reduced the TSS concentration in most organs. The fast-growing *E. urophylla* × *E. grandis* will consume the assimilates and carbohydrates of storage organs, but maintains the NSC concentration at a certain threshold. Our results help to comprehend the NSC allocation and improve the productivity of *E. urophylla* × *E. grandis* plantations in seasonal arid areas.

Keywords: *Eucalyptus urophylla* × *E. grandis*; dry-season irrigation; fertilization; non-structural carbohydrates



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1. Introduction

In the context of global climate change, the precipitation patterns around the world are changing. According to the research of Spinoni [1], drought events are becoming more frequent and severe worldwide. Under drought stress, vegetation decreases transpiration and photosynthesis rate due to less available water, resulting in declines of the physiological and morphological traits, ultimately decreasing forest productivity [2–4]. In *Eucalyptus* plantations, drought stress also reduced physiological traits such as photosynthesis and transpiration, and morphological traits such as leaf area and growth [5,6]. In addition, drought reduced nutrients and carbon storage in the soil, and microbial activity [7–9], which would reduce ecosystem stability and further limit forest productivity. Irrigation can eliminate the negative effects of drought and increase forest productivity [10]. Although rainfall in South China is comparatively plenty, tree growth is always limited by drought under the uneven spatial and temporal distribution of precipitation [11]. According to the data of Guangzhou Meteorological Observatory from 1969 to 2010, the precipitation between October and March is less than 20% of the whole year. As one of the three fast-growing tree species in the world, *Eucalyptus* plays an important role in the forestry of China, with a planted area of 4.5 Mhm² [12]. In recent years, seasonal drought has reduced

the water consumption and productivity of *Eucalyptus* in southern China [13]. Therefore, it is necessary to comprehend the response metabolism of *Eucalyptus* to drought stress for managing *Eucalyptus* plantations under climate change.

Non-structural carbohydrates (NSC), important compounds involved in plant physiological metabolism, greatly affect the growth and even survival of trees under drought stress. NSC include two components: soluble sugar and starch. Soluble sugars are the important substances directly involved in physiological metabolism, while starch is mainly used as energy storage. Soluble sugar and starch are interconvertible, providing energy and carbon substrates for physiological metabolism [14]. The NSC consumed for growing mainly come from recent assimilates or the storage organ by redistribution when the assimilates fail to meet growth requirements [15,16]. The growth would be slowed down under drought [17,18] and activate accumulation of soluble sugar converted by starch to keep cell turgor and maintain metabolism (e.g., osmoregulation) and repair tissue damage [19]. Although the organs need abundant NSC to maintain metabolism and repair the damage, carbon depletion is an infrequent phenomenon on account due to the active maintenance of NSC pool [19] and the NSC transport limitation caused by the damage of hydraulic and phloem [14], but these would adjust the allocation to resist the stress [20]. Thus, better understanding of NSC distribution is vital to realize the performance and productivity of trees under drought stress.

As a vital mechanism of plant adaptation to the changing environment, the distribution of NSC among organs (leaf, stem, root) is extremely complicated. Research on the NSC distribution in plantations has mainly focused on seasonal and height change [21–23]. However, we know little about NSC distribution in *Eucalyptus* due to the metabolic differences caused by experiment condition, species and intraspecific variation [24]. In most cases, leaves are the source organs with high NSC concentrations, while root and stem are the sink organs with low NSC concentrations [14]. High NSC concentration in leaves is beneficial to maintain photosynthesis and transport assimilates [25,26]. Carbohydrates stored in stem and root are important for tree survival. Tree restoration is supported by underground and aboveground NSC reserves after low levels of stress. While under stress, the restoration depends on the underground NSC reserves [14]. Therefore, carbohydrates are usually stored in root to improve survival rates and restoration [27,28]. On the whole, the distribution of NSC from source organ to sink organ follows the principle of proximity [10] and demand priority [29]. However, the NSC allocation mechanism varies (e.g., primary storage substance, storage organ and carbon redistribution) after the environment changed [24]. Under drought stress, due to carbon assimilation lower than carbon requirements, NSC would be remobilized and converted to osmotically active compounds for metabolism and respiration [30]. For the synthesis effects of growth consumption, carbon translocation interrupted by higher pathway resistance and viscosity of the phloem sap [30,31], trees showed a response that NSC decreased in roots [30] and accumulated in the aboveground parts [32,33] under drought. Due to interspecific and intraspecific variations of NSC [34], the response mechanism of NSC with height has not been clarified, but it might be of biological significance related to water strategy [35]. Some research has explored the NSC trend with height, which showed significant differences in various species and growth stage [26,30,34]. For instance, as a fast-growing tree species, the response of *Eucalyptus* with height showed a decreased trend [36], while *Pinus* tends to increase carbohydrate concentration with height [21,37]. Furthermore, the addition of nutrients would cause slight and moderate drought and boost metabolism [38], which further complicates the carbon allocation mechanism, and especially under the seasonal drought stress. The increases of biomass and photosynthesis caused by fertilization would result in larger transpiration and drought stress, despite the addition of nutrients that would increase the water use efficiency [38]. Nitrogen addition aggrandized carbon allocation to growth and respiration, of which the increase aboveground was stronger than that of underground [39]. Notably, common nitrogen fertilization did not affect foliar NSC concentration [30], while nitrogen enrichment decreased foliar NSC [39]. Due to the role of potassium in maintaining osmotic

adjustment [40] and stomal regulation [41], potassium is generally fertilized to increase water use efficiency and resist drought stress, which decreased the part of carbon allocated underground [42,43]. Under the combination of potassium fertilization and drought stress, drought stress would counteract the effects of potassium and increase the belowground carbon allocation [6,44]. However, the combined effects of irrigation and fertilization on NSC distribution have been rarely reported. Due to the different response mechanism of carbohydrates to fertilization and drought stress [44,45], the variations of NSC distribution are difficult to predict based on past studies. Thus, exploring the whole tree NSC carbon distribution under fertilization and drought stress can furnish a theoretical basis for carbon resources management with seasonal drought.

This experiment selected the species *E. urophylla* × *E. grandis*, generally planted in South China, and studied the NSC distribution with height among organs under fertilization and dry-season irrigation. *Eucalyptus* plantations were variously treated with fertilization to increase productivity, especially in southern China where the soil fertility is poor [46]. Fertilization, which accelerates the consumption of groundwater resources [6], has been shown in many studies to promote the growth of *Eucalyptus* [47–49]. However, fertilization has a dual effect on the water status of *Eucalyptus*. Excessive use of groundwater exacerbates carbon supply constraints and hydraulic damage in extreme droughts, which is detrimental to survival of *Eucalyptus* [14,50,51]. In contrast, potassium and sodium fertilization increased the water use efficiency, which alleviates water deficit of *Eucalyptus grandis* [52,53]. In general, fertilization increased the productivity of *Eucalyptus*, but also increased water requirements so as to exacerbate water stress during drought. Management of *Eucalyptus* plantations under climate change requires reconsideration of fertilization regimes and water supply in order to improve tree water status and growth. Thus, the following hypothesis is proposed in this study: (1) the trend of soluble sugar with height is increased to overcome hydraulic limitation; (2) NSC are primarily stored in roots to promote growth and water uptake; (3) dry-season irrigation can alleviate drought stress and decrease soluble sugar concentration; (4) fertilization can increase NSC concentration to resist drought stress, especially the soluble sugar concentration in roots. The purpose of this study is to explore the response of non-structural carbohydrates at organ and height level of *E. urophylla* × *E. grandis* trees under dry-season irrigation with fertilization.

2. Materials and Methods

2.1. Study Site and Plant Material

The study was conducted at Zengcheng Teaching and Research Bases of South China Agricultural University, Guangzhou (23°14'48 N, 113°38'20 E), which belongs to subtropical monsoon climate with an annual average temperature of 21.9 °C. Mean annual precipitation is ca. 2004.5 mm, of which about 80% occurs in the rainy season (April to September). According to the meteorological data of Guangzhou, the monthly precipitation at Zengcheng district of Guangzhou, was from 0.8 to 304.3 mm in the dry season of 2017–2019. The mean precipitation of the dry season was only 50.2 mm (Figure 1).

An orthogonal water and fertilizer experimental design set up in April 2017 with high productivity genotypes (*Eucalyptus urophylla* × *E. grandis* seedlings). The details were described in Yu's study [54]. There were four treatments replicated in five blocks, which were divided by the horizontal terraced land preparation method. The four treatments were (1) non-irrigation and non-fertilization (CK), (2) irrigation and non-fertilization (W), (3) non-irrigation and fertilization (F), (4) irrigation and fertilization (WF). Irrigation was carried out only in the dry season (October to December). Irrigation was given 8 h/week at 4 L/h to ensure the soil water concentration at 40 cm depth and 40 cm away from the tree reached 90% of the field water holding capacity. The fertilizer application amount referred to *Eucalyptus* production. The base fertilizer (400 g) was applied to each tree in March 2017. The fertilizer contained N: 24 g, P: 72 g and K: 24 g. In July 2018, the top dressing (300 g/plant) was applied to F and WF treatments, containing N: 45 g, P: 21 g and K: 24 g. The *E. urophylla* × *E. grandis* seedlings with a height of 20–35 cm tall were

carefully planted in April 2017. The planting density was 3×2 m (1667 plants/hm²) and the total area was about 0.57 hm². In the early stage, young trees invest most assimilates into growth, which consume a mass of carbohydrates, so they are more likely to exhibit carbon starvation than mature trees [55]. In addition, trees require a large amount of soluble sugar to maintain osmotic adjustment under drought stress, which is not conducive to the growth of *Eucalyptus* [51]. Thus, studying the influence of dry-season irrigation on young trees can provide data support to maintain the rapid growth during the early stage of *Eucalyptus* plantations under seasonal drought stress.

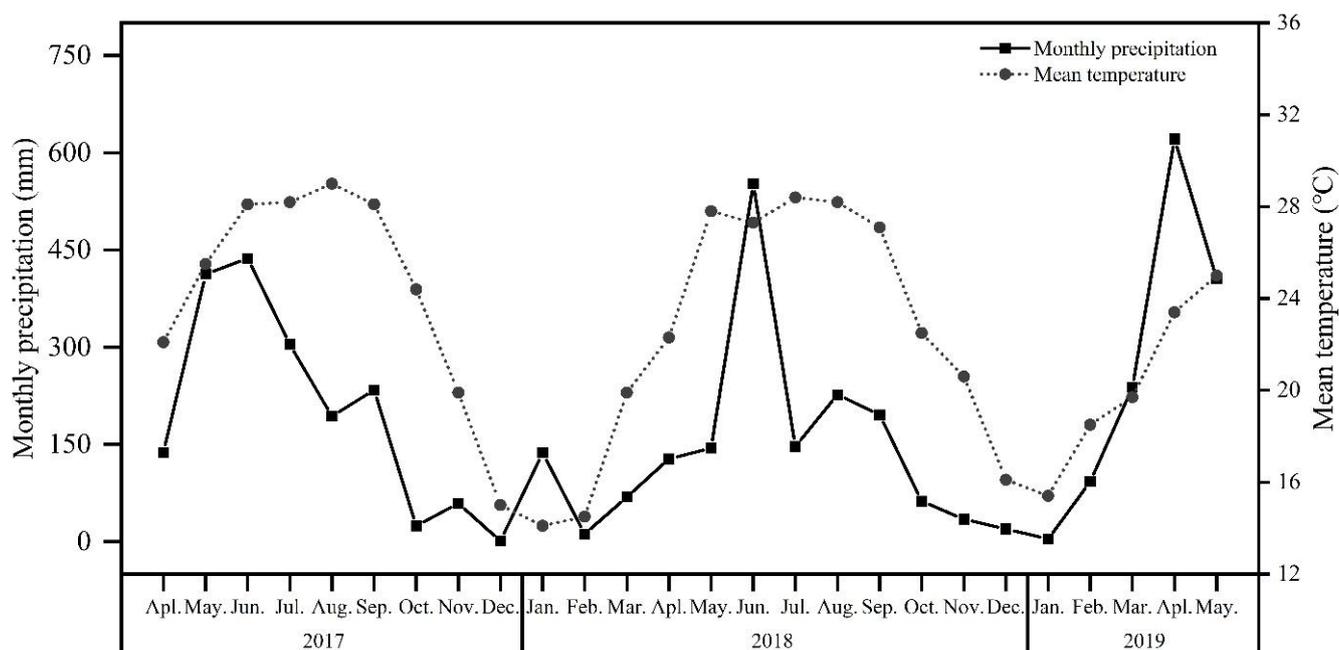


Figure 1. Monthly precipitation and mean temperature from April 2017 to May 2019 before standard tree cutting. The square pattern with the solid line indicates the monthly precipitation and the circle pattern with imaginary line represents the mean temperature.

To define the soil moisture under fertilization and dry-season irrigation, the soil water content (SWC) was measured at the end of the month with 3 repeats in each block (total 15 repeats each treatment). In each block, the soil samples from three random points (40 cm away from the trees and 40 cm depth) were packed into a small aluminum box. All boxes were weighed and then baked in a 105 °C oven to a constant weight, where $SWC (\%) = (\text{fresh weight} - \text{dry weight}) / (\text{dry weight} - \text{aluminum box weight}) \times 100\%$. The field moisture capacity was measured once a year with the ring-knife method. The relative soil water content ($SWCr = SWC / \text{field moisture capacity}$). The $SWCr$ from May 2017 to May 2019 is shown in Table 1.

Table 1. The relative soil water content (%) from April 2017 to May 2019, before sampling.

Season	Treatment	May 2017–May 2018	June 2018–May 2019
Dry season	CK	67.48 ± 0.73	77.11 ± 1.84
	W	90.56 ± 0.42	89.06 ± 1.29
	F	57.40 ± 0.51	68.06 ± 1.68
	WF	83.44 ± 0.43	84.06 ± 1.53
Rainy season	CK	85.82 ± 0.47	80.80 ± 1.27
	W	85.64 ± 0.47	85.74 ± 1.49
	F	81.95 ± 0.55	75.83 ± 1.60
	WF	83.62 ± 0.51	83.09 ± 2.03

Mean ± SE are shown. CK, non-irrigation and non-fertilization; W, irrigation and non-fertilization; F, non-irrigation and fertilization; WF, irrigation and fertilization.

2.2. Index Measurements

2.2.1. Field Sampling and Initial Sample Processing

Sampling was conducted shortly after ending the dry-season irrigation (May 2019). At each block, we selected a standard tree to cut down (total 5 trees per treatment). Each standard tree was in the middle of the treated plot to avoid the forest edge effect. The aboveground parts were electric-saw-felled and the underground parts were dug out by hand. After cutting down the tree, we measured its DBH, tree height and canopy length (Table 2). Considering the metabolism of organs at the same height varying greatly under different treatments, we referred to Smith's study [36] and divided the canopy and stem into five equal parts to collect leaves, branchlet, old branch and disk. The disk was taken from the middle stem of each segment and carefully whittled into xylem (including metaxylem and secondary xylem) and vascular cambium with a razor blade. The root was dug out after cutting down and divided into fine roots (FR) and coarse roots (CR), which were differentiated by the diameter of 2 mm, and rinsed with water and toothbrush to remove soil from the surface. Each tissue was randomly sampled over 100 g from the divided material in the field (dead branch and leaves excluded). Non-structural carbohydrate samples were immediately dried at 105 °C for 3 h, oven-dried to constant weight at 65 °C, and then ground into powder in a mill with a 0.5 mm sieve.

Table 2. The diameter at breast height (DBH), tree height and under-crown height of standard trees felled in May 2019 with different treatments.

Treatment	DBH (cm)	Tree Height (m)	Under-Crown Height (m)
CK	5.52 ± 0.30	7.58 ± 0.25	3.66 ± 0.28
W	6.52 ± 0.11	9.00 ± 0.15	6.28 ± 1.07
F	10.14 ± 0.10	12.78 ± 0.24	4.92 ± 0.24
WF	10.37 ± 0.06	12.94 ± 0.24	4.96 ± 0.81

Mean ± SE were shown. CK, non-irrigation and non-fertilization; W, irrigation and non-fertilization; F, non-irrigation and fertilization; WF, irrigation and fertilization.

2.2.2. Chemical Analysis

We measured two basis components of NSC: soluble sugar (the sum of glucose, sucrose and fructose) and starch. Soluble sugar and starch analysis followed anthrone methods [56], which are confirmed to be true and highly correlated with other analysis approaches [57]. To measure soluble sugar concentration, a 0.2 g powdered sample was extracted using 80% hot ethanol (80 °C) for 30 min and rinsed twice followed by colorimetric analysis with ethyl anthrone acetate reagent. The resulting soluble sugar extract was read at 620 nm on a microplate reader. To determine the starch concentration, the remainder of soluble sugar was solubilized in perchloric acid and then colorimetric analysis conducted with ethyl anthrone acetate reagent at 620 nm. Soluble sugar and starch concentration were calibrated by a standard curve for a known concentration made at the same time.

2.3. Statistical Analysis

For multivariate general linear model, we used Duncan's multiple comparison analysis and correlation analysis; all measured data were square-root transformation to improve normality and heteroscedasticity. Data means of various heights were used as input parameters to conduct PCA.

Excel 2021 was used for data consolidation; SPSS 22.0 and Origin 2021 software were used for the multivariate general linear model (MGLM), Duncan's multiple comparison analysis, correlation analysis and principal component analysis (PCA) of TSS, starch, NSC and ratio of TSS to starch. For MGLM and Duncan's multiple comparison analysis, all measured data were square-root transformation to improve normality and heteroscedasticity. The MGLM including three fixed factors (height, dry-season irrigation and fertilization) along with one covariate (block) was used to explain the variations of NSC concentrations and tested the effects of height/classification and treatment using a factorial ANOVA with a nested block structure (block/tree/height). Data means of various heights were used as input parameters to conduct PCA.

3. Results

3.1. Variation of Aboveground NSC Concentrations under Different Treatments

In the aboveground part of 2-year-old *Eucalyptus*, the TSS, starch and NSC concentrations formed gradient differences from source organ to sink organ (Figures 2 and 3), ranking as leaf > branchlet > old branch > stem xylem > stem cambium. In addition, the TSS showed a decreased trend with height in most tissues (except leaves), while the starch showed a contrary trend in leaves, branchlet and old branch.

Duncan's multiple comparison analysis revealed that dry-season irrigation and fertilization decreased the TSS concentrations of the aboveground part (Figures 2 and 3), but barely affected the NSC concentrations (except leaf in 2/5C, stem xylem in 1/5H and stem cambium in 5/5H). Even though the differences of various treatments in most tissues of canopy were not significant, W and F decreased the TSS concentrations of branchlet and old branch, and only the TSS concentration of branchlet in 5/5C was decreased by F (42%, Figure 2). In addition, compared to CK, WF significantly decreased the foliar TSS concentration in 2/5C (−23%, Figure 2(a-1)). Variations of different treatments in starch concentration were much less than that of TSS concentration. Significant decrease of foliar starch concentration induced by W and F was only observed in 5/5H (−8% and −14%), while decrease caused by WF was only detected in 4/5H (−18%, Figure 2(a-2)). In addition, due to the increased starch concentration of branchlet in 5/5H, the starch concentration in 5/5H under WF was significantly lower than that under W (−70%, Figure 2(b-2)). In the stem, dry-season irrigation and fertilization significantly decreased the TSS concentrations of stem xylem and stem cambium. In stem cambium, the TSS of stem cambium under CK was significantly higher (106%, 152%, 120% and 59%) than that under WF in 1/5H, 2/5H, 3/5H and 5/5H (Figure 3(b-1)). In stem xylem, F significantly decreased the TSS concentration in 1/5H (−54%, Figure 3(a-1)).

On the whole, even though dry-season irrigation and fertilization significantly affected TSS concentration of various tissues, only WF significantly decreased the NSC concentration of leaf in 2/5C (−19%, Figure 2(a-3)) and stem xylem in 1/5H (−46%, Figure 3(a-3)). The axial patterns in ratio were consistent with TSS, while the radial patterns were not. In particular, W and F treatments had more influence on stem cambium than other tissues, and specifically, W only significantly decreased the ratio of branchlet in 5/5H (−37%, Figure 2(b-4)) and F significantly decreased the ratio of stem xylem in 1/5H (−62%, Figure 3(a-4)). In stem cambium, W and F significantly decreased the ratio in 1/5H (−42% and −60%). Compared to W, the ratio of cambium in 4/5H under WF significantly decreased about 48%, while the ratio in 3/5H under WF was significantly lower than that under CK (−69%, Figure 2(b-4)).

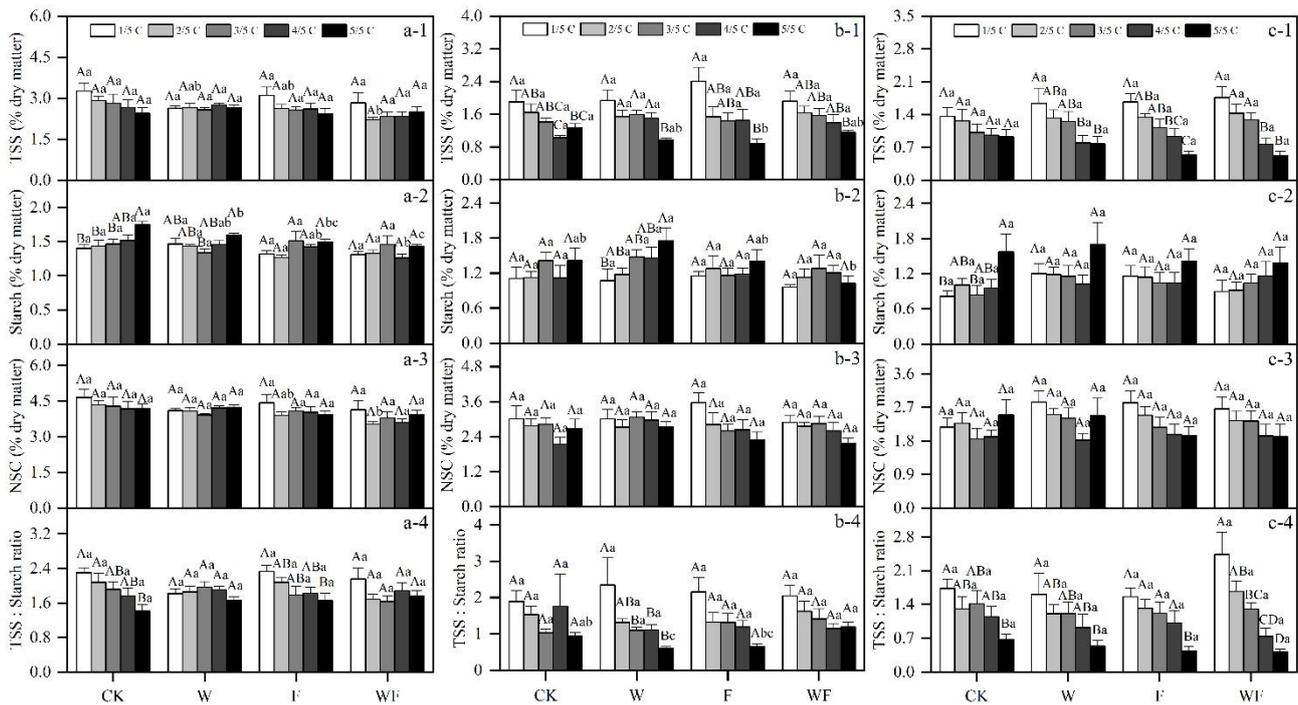


Figure 2. Changes in TSS, starch, NSC concentration and ratio of TSS to starch in the canopy of *E. urophylla* × *E. grandis* under different treatments. The average value and standard error of each time are shown ($n = 5$). The (a–c) represent the leaf, branchlet and old branch. Numbers 1–4 indicate the concentrations of TSS, starch, NSC and the ratio of TSS to starch, respectively. The letters over the bars represent the results of Duncan’s multiple comparison analysis. Different capital letters indicate significant differences between different canopy heights (C) under the same treatment ($p < 0.05$). Different lower-case letters indicate significant differences between different treatments for the same tissues ($p < 0.05$).

3.2. Variation of Underground NSC Concentrations under Different Treatment

Underground, TSS concentrations were still obviously higher than starch concentration and the ratios of FR and CR were distinctly higher than aboveground. In spite of source organ (leaf) as the primary sink organ, roots had the greatest concentrations of TSS (2.2%, Figure 4), which were notably higher than that of branchlet, old branch, stem xylem and stem cambium (0.3–1.5%, Figures 2 and 3). Compared to the aboveground part, different treatments had a little influence on the concentrations of FR and CR, for which only the TSS concentration in coarse root under WF was significantly higher (55.5%) than that under F (Figure 4a).

3.3. Multivariate General Linear Model (MGLM) of the NSC Concentrations in Various Tissues

According to the MGLM, the NSC concentrations of various tissues were mostly affected by height and block, but not the treatments (Tables 3 and 4). Dry-season irrigation mainly influenced the TSS and NSC concentrations of stem xylem and cambium, while fertilization primarily affected NSC concentrations of leaf and stem cambium. The mixed effects of dry-season irrigation, fertilization and height completely had no significant influences on the NCS concentrations in all tissues (Table 3). The variation of block accounted for 5.6%–65.6% of the NSC concentrations in all tissues.

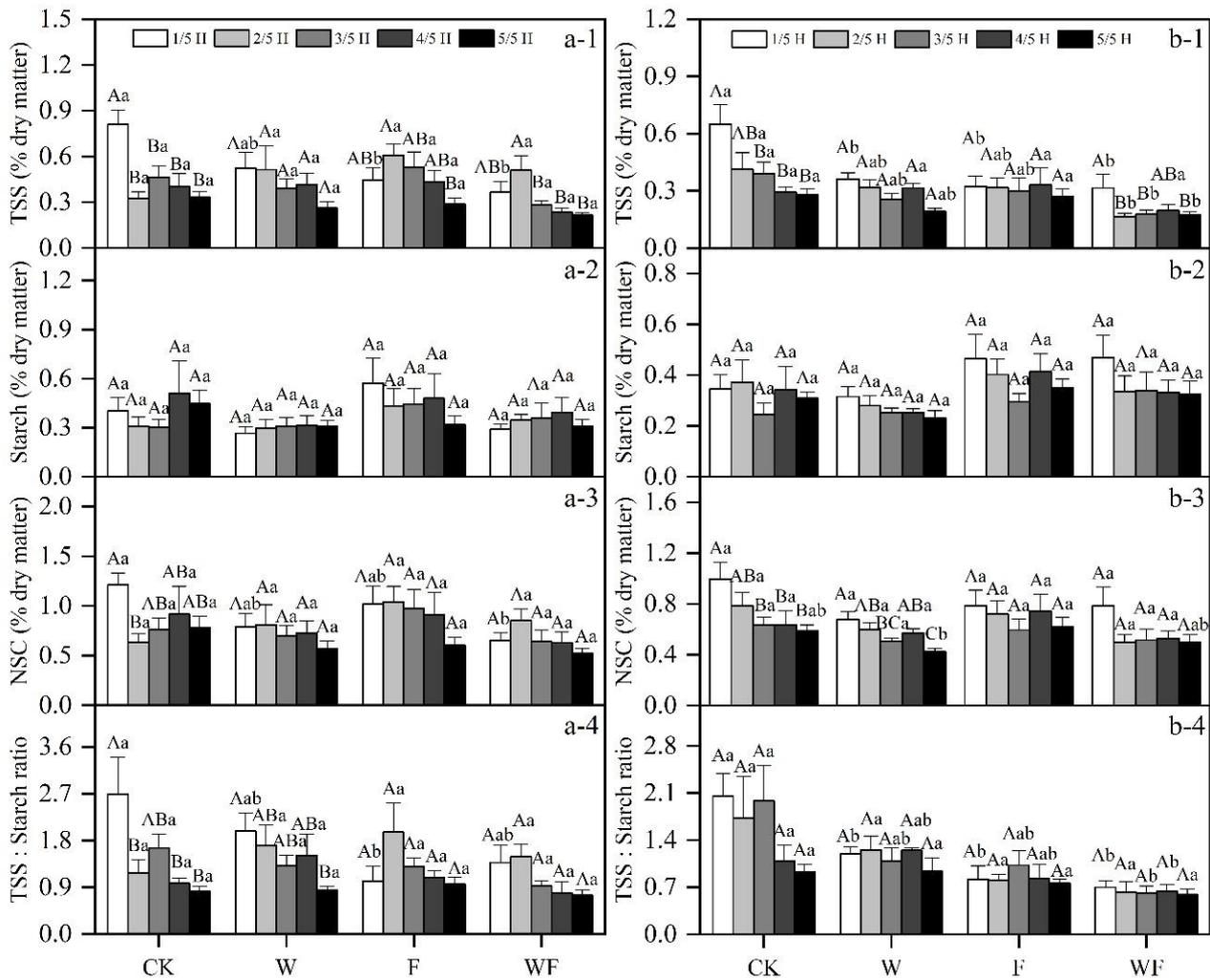


Figure 3. Changes in TSS, starch, NSC concentration and ratio of TSS to starch in the stem of *E. urophylla* × *E. grandis* under different treatments. The average value and standard error of each time are shown ($n = 5$). The (a,b) represent the stem xylem and stem cambium. Numbers 1–4 indicate the concentrations of TSS, starch, NSC and the ratio of TSS to starch, respectively. The letters over the bars represent the results of Duncan’s multiple comparison analysis. Different capital letters indicate significant differences between different tree heights under the same treatment ($p < 0.05$). Different lower-case letters indicate significant differences between different treatments for the same tissues ($p < 0.05$).

Table 3. The multivariate general linear model (MGLM) of the aboveground NSC concentrations with treatment, height, and block.

Tissue	Variables	Fixed Factors						Covariates		R ²
		Height	Dry-Season Irrigation (W)	Fertilization (F)	Height × W	Height × F	W × F	Height × W × F	Block	
Leaf	TSS	0.050 *	0.060	0.057	0.319	0.772	0.600	0.859	0.002 **	0.310
	Starch	0.002 **	0.139	0.002 **	0.467	0.104	0.942	0.817	0.481	0.356
	NSC	0.239	0.025 *	0.007 **	0.706	0.757	0.686	0.871	0.002 **	0.299
	Ratio	0.001 **	0.351	0.924	0.093	0.373	0.595	0.833	0.013 *	0.352
Branchlet	TSS	0.000 **	0.594	0.719	0.663	0.812	0.907	0.228	0.090	0.421
	Starch	0.118	0.802	0.170	0.757	0.523	0.100	0.708	0.022 *	0.241
	NSC	0.064	0.625	0.571	0.594	0.551	0.271	0.789	0.941	0.191
	Ratio	0.000 **	0.857	0.521	0.960	0.905	0.206	0.434	0.002 **	0.420
Old branch	TSS	0.000 **	0.807	0.937	0.465	0.135	0.966	0.982	0.000 **	0.585
	Starch	0.016 *	0.486	0.829	0.967	0.909	0.111	0.790	0.007 **	0.256
	NSC	0.041 *	0.480	0.883	0.833	0.582	0.295	0.913	0.508	0.185
	Ratio	0.000 **	0.759	0.875	0.511	0.297	0.069	0.711	0.000 **	0.656

Table 3. Cont.

Tissue	Variables	Fixed Factors					Covariates			R ²
		Height	Dry-Season Irrigation (W)	Fertilization (F)	Height × W	Height × F	W × F	Height × W × F	Block	
Stem xylem	TSS	0.000 **	0.008 **	0.179	0.458	0.028 *	0.179	0.344	0.095	0.522
	Starch	0.909	0.036 *	0.273	0.751	0.729	0.933	0.844	0.181	0.150
	NSC	0.112	0.008 **	0.942	0.584	0.361	0.472	0.802	0.853	0.238
	Ratio	0.000 **	0.530	0.024 *	0.823	0.030 *	0.297	0.218	0.001 **	0.432
Stem cambium	TSS	0.000 **	0.000 **	0.000 **	0.827	0.328	0.825	0.154	0.001 **	0.517
	Starch	0.132	0.173	0.006 **	0.723	0.954	0.692	0.997	0.004 **	0.569
	NSC	0.002 **	0.001 **	0.679	0.975	0.827	0.704	0.648	0.985	0.304
	Ratio	0.122	0.004 **	0.000 **	0.300	0.381	0.884	0.478	0.000 **	0.580

The *p* value is shown. Ratio: ratio of TSS to starch. The TSS, starch, NSC and ratio were square-root transformed to improve normality and homoscedasticity; * *p* < 0.05, ** *p* < 0.01.

Table 4. The multivariate general linear model (MGLM) of the underground NSC concentrations with treatment, classification and block.

Tissue	Variables	Fixed Factors					Covariates			R ²
		Classification (C)	Dry-Season Irrigation (W)	Fertilization (F)	C × W	C × F	W × F	C × W × F	Block	
Root	TSS	0.776	0.661	0.531	0.026 *	0.798	0.257	0.382	0.441	0.224
	Starch	0.770	0.276	0.181	0.041 *	0.855	0.754	0.466	0.081	0.273
	NSC	0.900	0.649	0.682	0.510	0.836	0.428	0.845	0.614	0.056
	Ratio	0.880	0.423	0.230	0.023 *	0.771	0.465	0.520	0.162	0.263

The *p* value is shown. Ratio: ratio of TSS to starch. The TSS, starch, NSC and ratio were square root transformed to improve normality and homoscedasticity; * *p* < 0.05.

3.4. Relationship between Nonstructural Carbohydrates and Growth

In general, NSC concentration in most tissues had no correlation with DBH and height (Figure 5), while the Pearson analysis showed significantly negative correlation with growth indexes in NSC concentration of both stem cambium and xylem (*p* < 0.01 and *p* < 0.05). However, the relationship between them and growth indexes was different. The TSS of stem cambium was significantly negatively correlated with height and DBH (Figure 5(d-1)), resulting in the same correlation of the ratio of TSS to starch (Figure 5(d-4)). The starch of xylem also showed a negative correlation with height and DBH (Figure 5(e-2)). In particular, xylem only showed negative correlation with height in NSC (Figure 5(e-3)). Nevertheless, there was no correlation between the ratio of TSS to starch and growth indexes in xylem.

3.5. Effects of Different Treatments on NSC

Fertilization and dry-season irrigation induced the modification of *E. urophylla* × *E. grandis* in NSC that reflected in changes of the six tissues (leaf, branchlet, old branch, stem xylem, stem cambium and root). We used variance analysis and Pearson correlation analysis to identify parameters that are most sensitive for fertilization and dry-season irrigation. However, each parameter was not completely independent because the NSC distribution of plants was different under various environments. Therefore, PCA was used to comprehensively evaluate the changes of experimental data, which could effectively analyze the variation of different treatments on NSC. The result of PCA shown in Figure 6a–d represent PC1 (42.4%, 36.9%, 37.1%, 37.4%), PC2 (22.7%, 21.4%, 18.7%, 17.8%) and PC3 (12.9%, 17.5%, 16.9%, 15.5%) of the total variance in TSS, starch, NSC and ratio, respectively. In TSS, the sample distribution within PC1/PC2/PC3 three-dimensional space is not homogeneous. The scatter of various treatments was clearly divided into four regions (Figure 6a). In Figure 6a, the four elliptical regions overlap each other partly ($1/4-1/3$), except that the ellipse of WF is completely separated from F. Compared to TSS, the four ellipses in starch, NSC and ratio have a higher degree of overlap (Figure 6b–d). In particular, the WF ellipse in starch completely overlaps with F ellipse, while the WF ellipse is perpendicular to W ellipse with minimal overlap (Figure 6a–b). It means that

fertilization and dry-season irrigation have various effects on TSS and starch. Dry-season irrigation has a great effect on TSS and fertilization has a significant effect on starch.

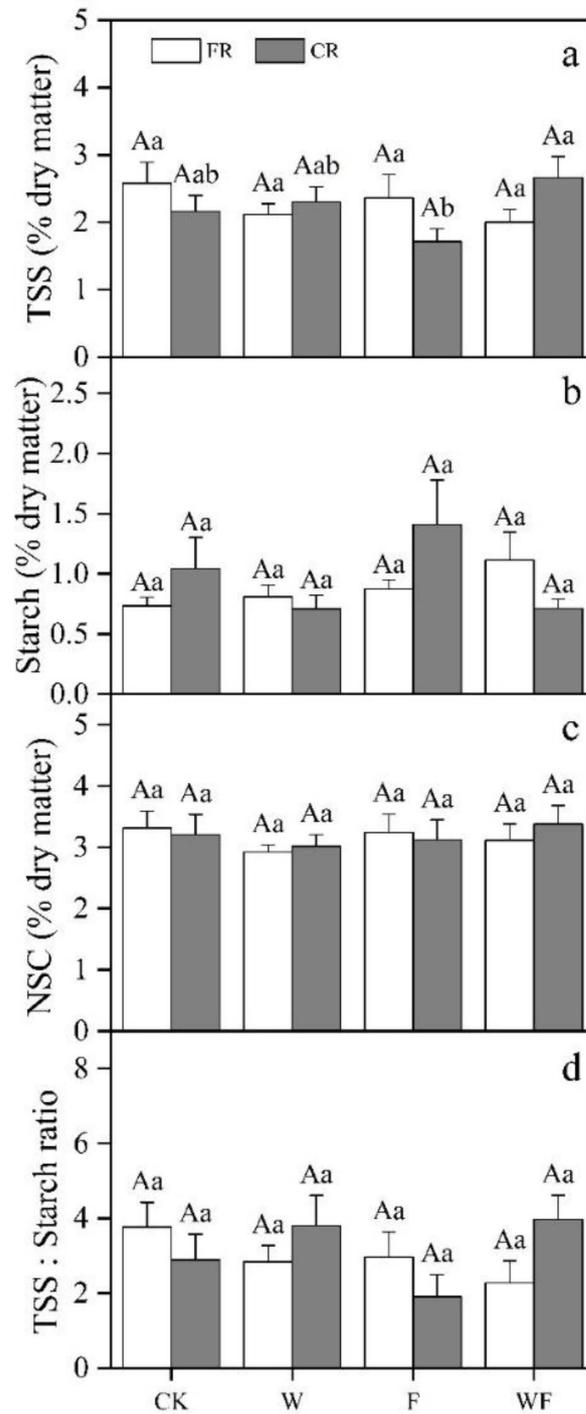


Figure 4. Changes in TSS, starch, NSC concentration and ratio of TSS to starch in the roots of *E. urophylla* × *E. grandis* under different treatments. The average value and standard error of each time are shown ($n = 5$). The letters (a–d) indicate the concentrations of TSS, starch, NSC and the ratio of TSS to starch, respectively. The letters over the bars represent the results of Duncan’s multiple comparison analysis. Different capital letters indicate significant differences between fine root (FR) and coarse root (CR) under the same treatment ($p < 0.05$). Different lower-case letters indicate significant differences between different treatments for the same tissues ($p < 0.05$).

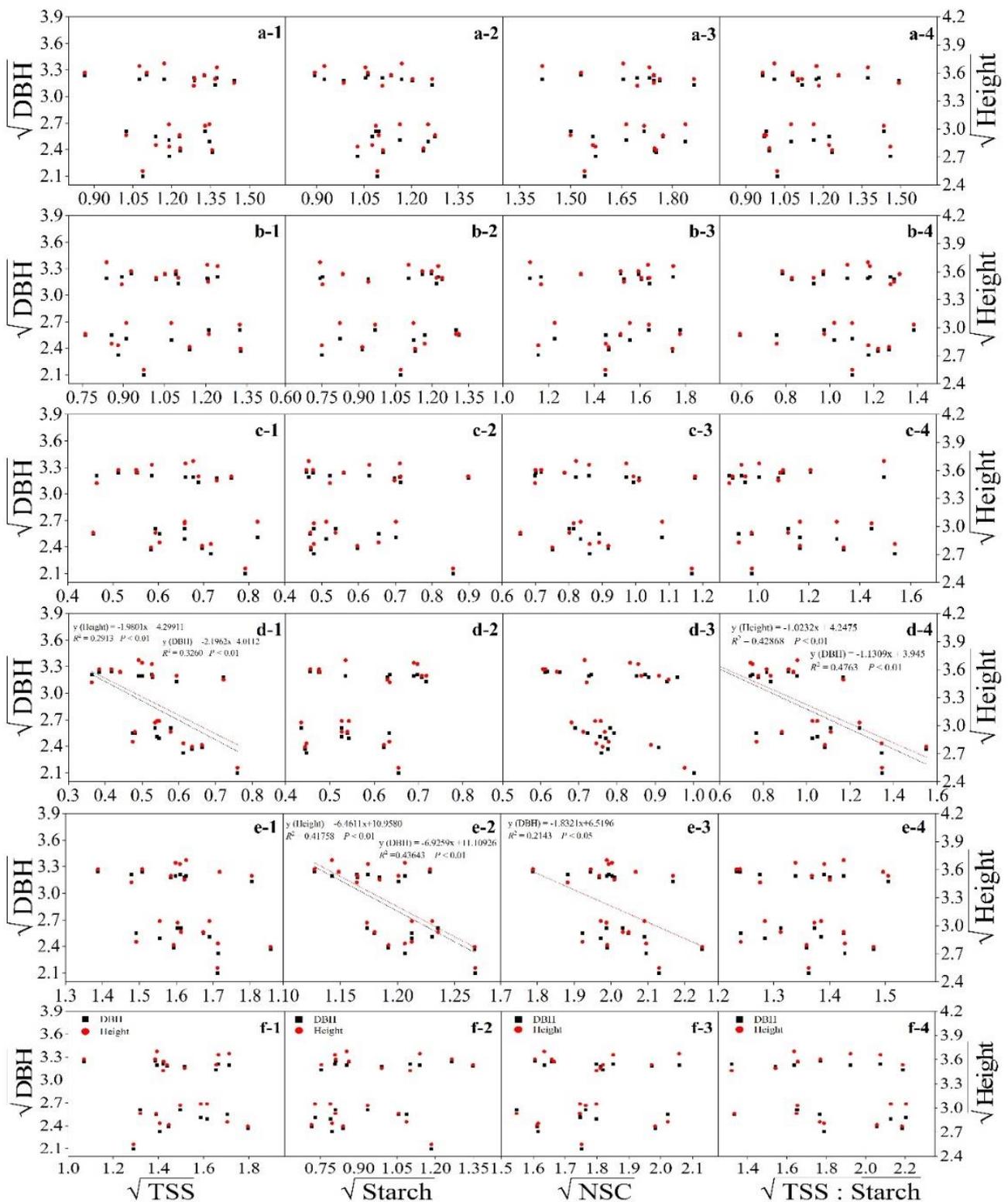


Figure 5. The correlations of NSC and growth indexes. Relationships of (a) branchlet, (b) old branch, (c) stem xylem, (d) stem cambium, (e) leaf and (f) root with (1) total soluble sugar (TSS), (2) starch, (3) non-structural carbon (NSC) and (4) ratio of TSS to starch. All data were square root transformed to improve normality and homoscedasticity before linear regression analysis and Pearson correlation analyses were used to obtain regressions and correlations (R^2 and p values).

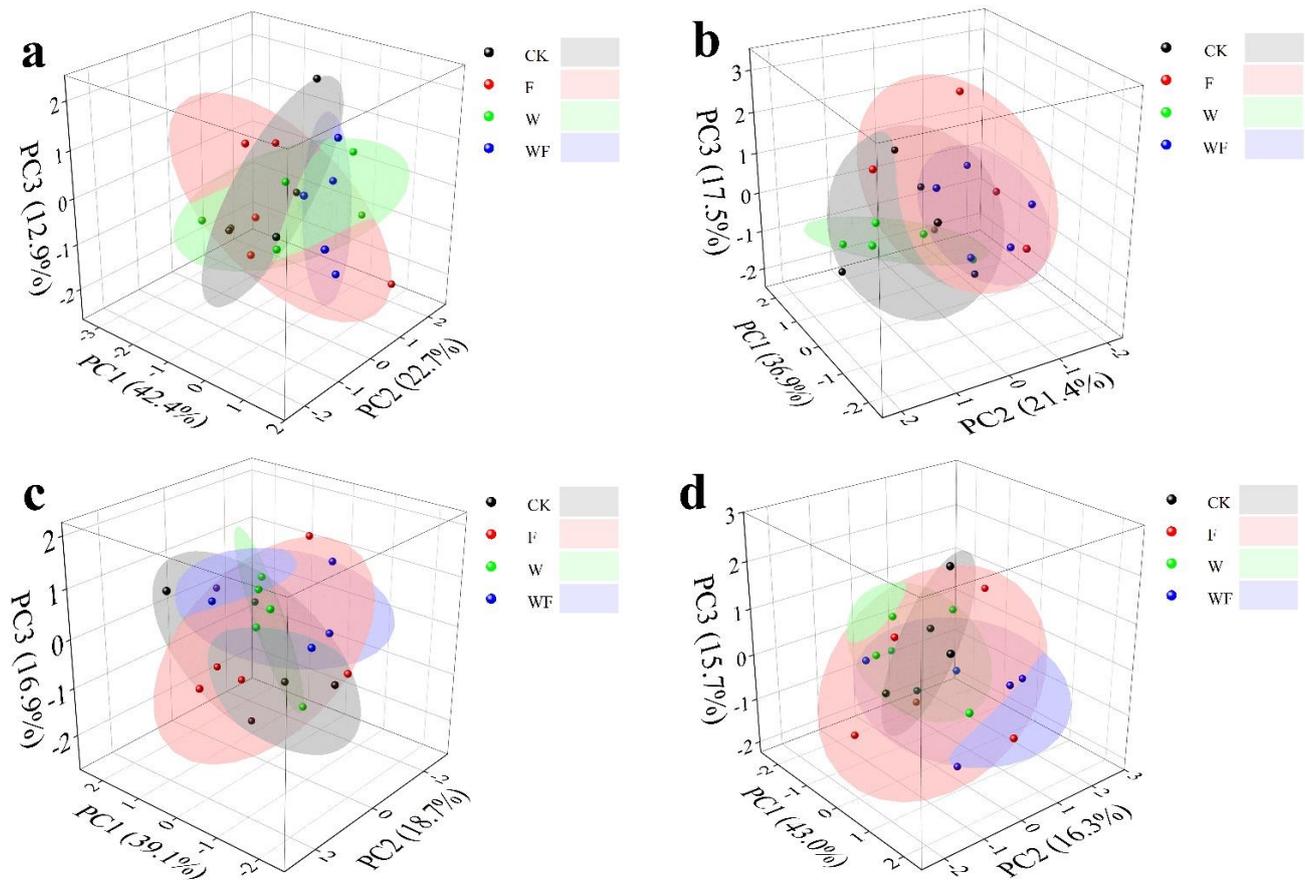


Figure 6. Principal component analysis of TSS (a), starch (b), NSC (c) and ratio of TSS to starch (d) under different treatment. The gray, red, green and blue ellipsoids represent CK, F, W and WF treatment, respectively. The data used for PCA were averaged ($n = 5$).

4. Discussion

4.1. Soluble Sugar as the Primarily Existing Form of NSC

The ratio of TSS to starch was greater than one in most tissues, which proved that soluble sugar is the primary existing form of NSC, consistent with other *Eucalyptus* species [36,51,58,59]. However, our study showed lower NSC concentration in stem and higher TSS concentrations in root, which may be caused by the difference of metabolic intensity under various growth stages [30,60] and interspecific and intraspecific variations [22]. TSS concentration varied significantly among tissues indicating that TSS was not distributed uniformly across all tissues but preferentially stored in specific storage pool. In contrast, starch concentrations were similar among all organs but maintained at a lower level, indicating that starch was not accumulated. As an anisohydric strategy species, high TSS concentration was beneficial to maintain a high metabolic intensity to ensure growth throughout the year, which was not conducive to store NSC to resist adversity [61].

Fertilization and dry-season irrigation reduced the TSS concentration of most tissues, while having little effect on starch and NSC. TSS performs immediate functions (e.g., osmoregulation) and provides energy for metabolism and repairing tissue damage, while starch is used as a carbon storage pool with inactive osmosis [62]. When trees suffer drought stress, a water deficit would lead to overproduction of reactive oxygen species (ROS) in leaves, causing damage to proteins, lipids and nucleic acids [63]. High TSS concentration is conducive to enhance osmotic adjustment, protect macromolecules (such as protein) and provide energy for antioxidant defense system used to scavenge ROS [64,65]. In addition, high TSS concentration could promote the repair of xylem embolism caused by drought stress [66]. Affected by the strategies of anisohydric, *Eucalyptus* relies on

osmotic adjustment to control the osmotic potential of living cells in leaves and stems under drought stress. The substances involved in the osmotic regulation of *Eucalyptus* are mainly composed of soluble sugar, cyclitols and potassium ions [41,67], among which the soluble sugar plays an important role [68]. Thus, the decrease in TSS induced by dry-season irrigation proved the presence of drought stress in *Eucalyptus* under control and fertilization treatments. However, the decrease of TSS caused by fertilization is inconsistent with the observation that fertilization stimulated trees accumulation of soluble sugar under drought stress [69,70]. These soluble sugars are converted to starch under drought stress (Figures 2–4), even though fertilization has no effect on the starch concentration of most organs. In another drought experiment of *E. urophylla*, Chen [51] found that starch was converted by over 35% to soluble sugar to maintain osmotic adjustment and repair the xylem embolism, which is contrary to our results. Fertilization decreasing soluble sugar concentration in our results may be caused by one or a combination of several elements: (i) application of potassium enhanced osmotic regulation, causing K^+ partly to replace the role of soluble sugar [53] or (ii) rapid growth characteristics result in the consumption of large amounts of soluble sugar.

4.2. NSC Concentrations of Tissues under Dry-Season Irrigation and Fertilization

At the tissue level, soluble sugar and starch were not distributed uniformly among all tissues, forming a gradient difference from source organ to sink organ. NSC were primarily stored in roots, which were conducive to uptake water and survive in drought stress [71–73], with small part stocks located in the branches, which can be consumed by bud break and leaf expansion for growth [74]. The significantly different distribution of carbohydrates between stem and root means that NSC storage is spatially proximate to root. It is clear that the coordination between NSC storage and root is an optimized storage strategy that evolved with underground growth. In contrast, the root system is a virtual component of the forest ecosystem: water and nutrient uptake, mechanical support and carbon and nutrients supply to the rhizosphere [75]. On the other hand, roots can act as soil environmental sensors, sending signals to the canopy [76]. Higher NSC concentrations are beneficial to keep cell turgor [77] and promote root growth [78] to optimize water uptake, especially *Eucalyptus*, a species with great transpiration [79]. However, different treatments had little influence on root NSC concentrations when drought stress existed in CK and F treatment (Table 1). In most cases, drought increased root NSC concentration to obtain water [28,62], but decreased them over time due to carbon consumption larger than supply [61]. Although there some research observed that root NSC concentrations did not change under drought stress, the response mechanism has been rarely reported [80].

In present study, we observed that the NSC concentration of roots is about 2–6 times greater than that of stems, which is different from previous studies [58,59]. During the early growth stages in *Eucalyptus*, excessive growth rate of stems consumes a large amount of NSC, especially 2 years after planting [81]. The negative correlations of growth and the NSC concentrations in stem xylem and cambium indicate that investment in DBH and height are the main reason for the lowest NSC concentrations among all tissues. As the growth rate of stems slow with age, stems accumulate NSC to slightly lower than that of roots [36,59,60]. However, low stem NSC concentration was adverse to the repair of xylem embolism caused by drought stress, which further enhanced the water deficit in *Eucalyptus* [66]. Compared to significant differences in height and DBH, stem xylem and cambium NSC under different treatments are maintained at similar levels, which further indicates that *E. grandis* × *E. urophylla* as an anisohydric species put all the assimilates increased by fertilization and dry-season irrigation into growth [36,49]. In particular, fertilization and dry-season irrigation also had little effect on the soluble sugar and starch concentration of leaves and branches, while having significant influences on stem cambium. This finding is contrary to the widely accepted conclusion that soluble sugar concentration increased under drought stress, and in addition, the presence of drought stress had been proved in our study during the dry season and under fertilization (Table 1) [36]. The soluble sugar decline in stem cambium

under dry-season irrigation further indicates that drought stress had affected the metabolic activity [82]. In the drought experiment of *Eucalyptus*, the response to drought stress varies greatly at the organ level, even though the level of whole tree soluble sugar concentration increased [51,59]. Fertilization decreased TSS concentration contrary to our hypothesis that fertilization can increase TSS concentration to resist drought stress. Compared to the starch concentration, fertilization promoted the soluble sugar to convert into starch in stem cambium, which is contrary to the result of Li [39] and Huang [69]. This might be caused by the formation of a gradient difference in carbon distribution [14] and consumption of rapid growth [59]. In particular, the effects of additional irrigation were not obvious under fertilization, which seems that irrigation had not alleviated the drought stress caused by fertilization. Those findings are contrary to another hypothesis that fertilization had positive effects on NSC, which can be affected by dry-season irrigation [30,69]. In fact, compared to irrigation treatment, the increase of canopy biomass under fertilization diluted the effects of dry-season irrigation to a great extent.

On the whole, fertilization and dry-season irrigation had little effect on NSC concentration except stem cambium. Drought stress induced by fertilization and climate in our study site decreased physiological traits such as photosynthesis and transpiration, and morphological traits such as leaf area and growth [38,51]. The reaction of NSC is detrimental to survival under severe drought. However, we currently lack the data of prolonged and severe droughts to test whether NSC insensitivity is associated with growth and survival. If so, fast-growing plantations need to strengthen water management under mid-drought to reduce the risk of severe droughts.

4.3. TSS Concentrations Decreased with Height

Most tissues indicated a decreasing trend of NSC concentration with height, which was mainly caused by the change of soluble sugar concentration. However, such change was not significant, except in stem xylem and cambium. There was also a decreased trend with height for soluble sugar, whereas starch concentration did not vary with height in most tissues, which is consistent with Smith's finding [36] but contrary to our hypothesis. The decreased trend indicates that carbon demand at the top of trees is larger than supply. Due to the apical dominance [83], upper parts of trees grow faster than other parts, resulting in a large amount of NSC consumption. In addition, foliar photosynthesis decreased with height because of enhancing hydraulic limitations [84]. When newly photosynthetic assimilates could not meet the demand, the NSC in storage organs would be remobilized to meet the metabolic requirement [15,17,85]. However, the remobilization could damage the phloem under drought stress. According to the Münch model [86], the allocation of NSC was driven by hydrostatic pressure gradient from source organ to sink organ. Notably, water movement from xylem to phloem could be limited by the high xylem tensions in upper xylem and apoplast, which results in increase of the phloem sap concentration and limit of assimilate transport [31]. The lower soluble sugar concentrations increase the osmotic potential, which leads to hydraulic limitation and restriction of water influx from xylem [87,88], and eventually resulting in phloem damage by long-distance transport and excessive sap concentration [14,31,51]. In the source organ, sugar is loaded into the phloem in two ways, either energetically against gradient difference with consuming energy or moving along the gradient difference passively [27]. The decreased trend of TSS with height helped *E. urophylla* × *E. grandis* transport TSS from below to the above. However, because of few studies of *Eucalyptus*, it is unclear that the long-distance transport of sugar from bottom to top results in extreme phloem damage [31,53]. We were surprised to find that fertilization and dry-season irrigation had little effect on NSC concentrations in horizontal and vertical direction, which further proved that the strategy of anisohydric species coping with drought stress is aggressive. Although it is well known that root growth greatly responds to drought and fertilization [42,43,78], past research has not adequately studied the NSC distribution underground. In order to realize NSC distribution of the entire tree

under different growth patterns, further research will need to identify the NSC distribution in the underground part of *Eucalyptus* and its response to fertilization and irrigation.

5. Conclusions

In a subtropical zone in southern China with seasonal drought, our results indicated soluble sugar was the primary existing form of NSC and mainly stored in roots. Throughout the tree, *E. urophylla* × *E. grandis* formed gradient differences from source organ to sink organ and from bottom to top in aboveground parts, which were not changed by fertilization and dry-season irrigation. However, the mechanism of how trees form the gradient differences need further study. Using the isotope label method can make this carbon-derived dynamic more apparent. There are still many factors affecting carbohydrate distribution, which directly correlate to nutrient concentration [88] and cyclitol concentration [67]. Under drought stress, NSC concentration changes without significant differences in most tissues of *E. urophylla* × *E. grandis*, but the TSS concentrations were reduced by fertilization and dry-season irrigation. The reaction of NSC is detrimental to survival under severe drought. *Eucalyptus* can uptake deep groundwater to alleviate drought stress [6]. There may be a gradient difference of NSC concentration in the underground part as similar with that of the aboveground part, which needs further investigation. In conclusion, this research helps us better understand the strategy between carbon storage and growth under seasonal drought, further realizing the spatial pattern of carbon in a fast-growing tree species.

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