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Effects of Environmental Factors on the Nonstructural Carbohydrates in *Larix principis-rupprechtii*

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Abstract: Nonstructural carbohydrates (NSCs) reflect the carbon balance in trees and play a central role in their responses to environmental stresses. However, our understanding of tree NSC storage dynamics under environmental stress is unclear. Here, we quantified the deciduous conifer *Larix principis-rupprechtii* at five altitudes on Luya Mountain, North-Central China. We measured NSCs in different organs and simultaneously monitored environmental variables during the growing seasons in 2018 and 2019. The results showed that temperature was the main environmental factor affecting tree NSCs. Furthermore, as the soil–atmosphere temperature gradient decreased, the concentrations of soluble sugars in the needles increased while their starch decreased, as well as the concentrations of starch in the shoots, stems, and roots increased. These results may indicate that temperature gradients may assist carbohydrate allocation within individual trees. The NSCs, especially starch, in the needles increased with lower soil moisture, and the soluble sugars and starch in the needles and shoots increased with a higher vapor pressure deficit. The soluble sugars in the stems were significantly affected by soil moisture, and the NSCs in the roots were mainly affected by temperature. These results provide new insights to improve our understanding of the environmental stress effects on future forest C balances.

Keywords: larch; nonstructural carbohydrates; temperature; soil moisture; vapor pressure deficit

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1. Introduction

Nonstructural carbohydrates (NSCs) are crucial to multiple functions in trees, including metabolism, transport, and osmoregulation [1]. NSCs have distinct ecological functions and are largely composed of soluble sugars and starch [2]. Soluble sugars have a variety of direct functions in addition to supporting new growth and the need for respiration and defense [3–6]. Starch is the main storage material in trees, which can be converted into soluble sugars under environmental stresses [5,7]. Thus, NSCs play a central role in tree responses to environmental stresses, especially under temperature and drought stress conditions [8,9]. Moreover, the ability of starch to convert to sugar and back again is an important consideration for survival under environmental changes [10,11].

Trees have an altitudinal range in mountains, and their growth and distribution are strongly influenced by the environment. In addition, tree NSCs play an important role in the response to environmental stress [1,11–14]. Many studies have found that trees will enhance their cold tolerance by increasing their content of soluble sugars, and this adaptation strategy is important for their survival in harsh environments [10,15,16]. Previous studies have found higher concentrations of NSC in tree organs exposed to growth-limiting low temperatures than those exposed to higher temperatures, regardless of the altitudinal origin of the tested trees [15]. Xu and Huang [17] proposed that the NSC reduction in roots was more severe than that in shoots under high temperatures. Furthermore, soil temperature was more important than air temperature in regulating photosynthesis, whole plant or root respiration, and carbohydrate accumulation and distribution. In temperate climate zones, temperatures change daily and seasonally, with soil and air showing measurable thermal differences that rarely converge in timing or magnitude [18]. Trees growing in temperate regions across both soil and air environments often experience internal thermal gradients. These gradients vary seasonally and affect NSC metabolism by changing carbohydrate demand, transport, and synthase rates [19]. A study showed that sugars are transported to the roots when the mean soil temperature is higher than the air temperature and transported to the canopy when the mean soil temperature is lower than the air temperature [19]. Therefore, it is difficult to examine the influence of temperature on tree NSCs without distinguishing between the air and soil temperature.

NSCs in trees also play an important role in mitigating water deficits or drought stress [4,12]. During drought stress, trees reduce carbon assimilation rates by reducing stomatal conductance [20], but their respiration rates remain at a certain level [21]. Consequently, stored carbon reserves could be depleted to maintain normal respiration [1]. However, the influence of drought stress on NSCs remains uncertain. Some studies have found that NSCs increase [12,22], whereas other studies have found that NSCs decrease [23,24] or remain constant [25,26] under drought. These differences can be attributed, in part, to the severity of the drought or the potential differential responses of different organs to drought [27].

When facing environmental stress, trees will change NSC allocation among different organs [1,28]. For example, dryness either dramatically raises or maintains the NSC content in aboveground organs but reduces it in sapling roots [29]. In addition, the NSCs in different organs have different response strategies to environmental factors [1,27]. A recent study found that *Picea meyeri* needle soluble sugars were more sensitive to air temperature than those in other organs. Additionally, the shoot NSCs and stem NSCs were mainly affected by both soil moisture and atmospheric factors, and the root NSCs were sensitive to soil moisture [12]. In addition, trees may benefit from more NSC allocation by reducing their growth to ensure long-term survival during stressful periods [4,7,30]. Understanding the dynamics of NSCs under environmental stresses has become an important means for investigating tree adaptation strategies [1]. However, previous research has focused more on aboveground organs and neglected the role of belowground organs [1]. Therefore, a whole-tree approach in natural settings that considers many tree organs and environmental conditions is required to comprehend how environmental stresses affect carbon change [31,32].

Larix principis-rupprechtii is a dominant species in cold coniferous forests (1800–2800 m a.s.l.) in North-Central China [33,34]. It is an important tree in these mountainous regions and plays a central role in regional carbon storage due to its large biomass [12,33]. However, little is known about the effects of environmental factors on NSC dynamics in *L. principis-rupprechtii*. Therefore, in this study, the NSCs in the needles, shoots, stems, and roots were measured, as well as the environmental factors were also monitored during the growing seasons in 2018 and 2019. We aimed to determine: (1) the effects of environmental factors on the NSCs in *L. principis-rupprechtii* and (2) the differences among organs responding to environmental factors.

2. Materials and Methods

2.1. Sites and Sample Trees

Luya Mountain (38°35′–38°45′ N, 111°50′–112°5′ E), in the Shanxi Province, China, was chosen as the study area. The mean annual air temperature was 5.29 °C and the average annual precipitation was 478 mm from 1957 to 2019 according to the meteorological station in Wuzhai (38°33′ N, 111°29′ E, 1401 m a.s.l.). The annual mean temperature, mean annual precipitation, and annual maximum daily vapor pressure deficit (VPD) have shown an increasing trend since the 1990s [33]. The soil of the forest is sandy loam (depths of 40-95 cm) overlying the fractured parent material. *L.principisrupprechtii* in this area mainly distributes from 2000 m to 2800 m a.s.l. Moreover, five sites along the altitudinal gradient (2040 m, 2260 m, 2440 m, 2600 m, and 2740 m a.s.l.) were established in Luya Mountain. In this study area, the fluctuations in soil temperature were relatively stable and did not always maintain the same characteristics of change as air temperature, and the soil temperature fluctuation lagged behind that of air temperature, being higher than in the air in winter and lower during summer. The soil temperature was lower than the air temperature from April to mid-August, and the soil temperature was close to the air temperature from mid-August to mid-October. Daily VPD values and variations at all sites were high in spring and early summer, and soil moisture peaked after snowmelt. Sites and tree properties at the five measured sites are shown in Figure 1 and Table 1.

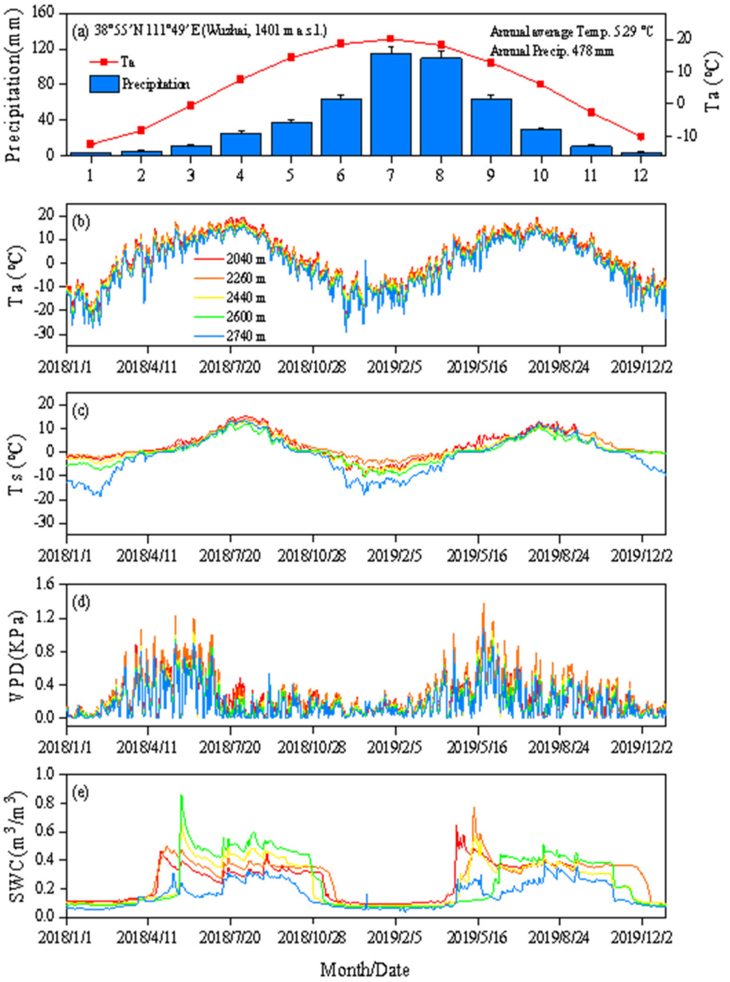

Figure 1. Seasonal changes in the mean monthly air temperatures and the mean monthly precipitation of 1957–2019 (mean ± SE) (**a**), daily mean of (**b**) air temperature (Ta), (**c**) soil temperature (Ts), (**d**) vapor pressure deficit (VPD), and (**e**) soil water content (SWC) at each site in 2018 and 2019.

Table 1. Tree properties at the five monitoring sites (mean ± SD). DBH: diameter at breast height.

Altitude (m a.s.l.)	Aspect (°)	Slope (°)	Soil Depth (cm)	Age (a)	Height (m)	DBH (cm)
2040	NE25	20	63	50 ± 5	8.2 ± 0.9	25.2 ± 2.8
2260	NE12	10	95	62 ± 8	13.1 ± 1.1	37.9 ± 2.8
2440	NE30	10	85	75 ± 6	12.7 ± 0.9	31.9 ± 4.8
2600	NE20	15	45	73 ± 5	8.5 ± 0.8	30.9 ± 2.8
2740	NE5	20	40	69 ± 9	7.24 ± 0.5	29.2 ± 2.8

2.2. Nonstructural Carbohydrate Sampling and Analyses

L. principis-rupprechtii is the dominant tree species in the coniferous forest belt of North China [34]. This species begins to sprout and grow around the middle of May, reaches its peak growth in the middle of July, and starts to defoliate in the middle of September. As needles were still immature in May, our sampling began in mid-June. Previous studies have found that trees are more responsive to environmental factors at the beginning and end of the growing season [35–37], so the sampling frequency increased during this period. Specifically, five mature trees were selected at each site (with heights of 2040 m, 2260 m, 2440 m, 2600 m, and 2740 m) on 15 June, 26 June, 5 July, 14 July, 15 August, 14 September, and 27 September 2018 and on 15 June, 23 June, 4 July, 15 July, 15 August, 17 September, and 26 September 2019. Five mature trees that were at least 20 m apart from each other and had a well-developed canopy with no obvious wounds were chosen from each sample plot. All samples were collected during midday hours only (10:00–15:00 local time) on a sunny day, which can greatly reduce the impact of diurnal NSC changes [12]. From each sampled tree, we collected sun-exposed needles and shoots from 1- to 2-year-old branch segments. At a height of 1.3 m from the stem base, we collected 2 cm-long stem cores (excluding bark) using a standard 5 mm increment borer (Haglöf, Långsele, Sweden). At the same time, we also collected root samples (1-3 mm in diameter) at about 10 cm soil depth. Immediately after sampling, all samples were kept cool using the water ice (approximately 5 °C) in the field. Additionally, samples were microwaved for 90 s at 700 W to halt enzymatic activity within 6 h (usually less than 4 h). In the laboratory, samples were dried at 65 °C for 72 h and ground into a fine powder using a rotary mill (Fritsch PULVERISETTE 14, Germany). In this analysis, NSCs were calculated as the total of all soluble sugars plus starch. The anthrone–sulfuric acid method was used to measure the concentrations of soluble sugars and starch, and the detailed experimental procedures can be found in Wang's papers [38,39].

2.3. Environmental Factors

Each data logger was deployed every 1 h at a height of 2 m above ground to measure the air temperature (Ta, °C) and relative humidity (RH, %) (Hobo Pro v2-U23-002; onset; Bourne, MA, USA). VPD (kPa) was calculated based on air temperature and relative humidity [40]. The calculation formula is as follows:

$$VPD = 0.611 \times exp((17.27 \times Ta)/(Ta + 237.3)) \times (1 - RH/100) \quad (1)$$

At each site, a GP1 data logger (Delta-T, Cambridge, England) continuously recorded the soil volumetric moisture content (m^3/m^3) and soil temperature (Ts, °C) and saved the data every hour. A probe (Theta Probes Type ML2x, Delta-T, Cambridge, UK) was placed into the soil at a 10 cm depth with the instruments enclosed in waterproof boxes.

2.4. Statistical Analyses

Tukey's multiple range test was used to analyze the differences in soluble sugars, starch, NSCs, and sugar–starch ratios among different altitudes and different organs. All data were standardized, and min–max normalization was performed. Relations between the NSCs and their components in different organs vs. the daily average of the environmental variables of 10 days before the date of sampling were calculated using a linear mixed model (LME; lme4 packages, "lmerTest"). Altitudes and organs were used as random effects, and environmental factors were used as fixed effects. The relative importance of the fixed factors to NSCs and their components in different organs was determined by the "GLMM.hp" package in R [41]. The figures were plotted using Origin 2021 (Origin Lab Corp., Northampton, MA, USA), and the statistical analysis was carried out using R-4.2.2 (R Core Team, 2022).

3. Results

3.1. Altitudinal Characteristics of NSCs and Their Concentration Differences among Different Organs

The NSCs and their components (soluble sugars and starch) in all four organs of *L. principis-rupprechtii* had higher concentrations at the upper and lower altitudes, and other altitudes had similar concentrations in our study area (Figure 2). The NSCs in the needles were higher at 2740 m than those at the other altitudes ($p < 0.05$). The NSCs in the roots and the soluble sugar–starch ratios in the shoots, stems, and roots were not significantly different among the five altitudes in the study period ($p > 0.05$; Figure 2 and Table 2), while the soluble sugar–starch ratios in the needles were higher at the lower altitudes than at the higher altitudes.

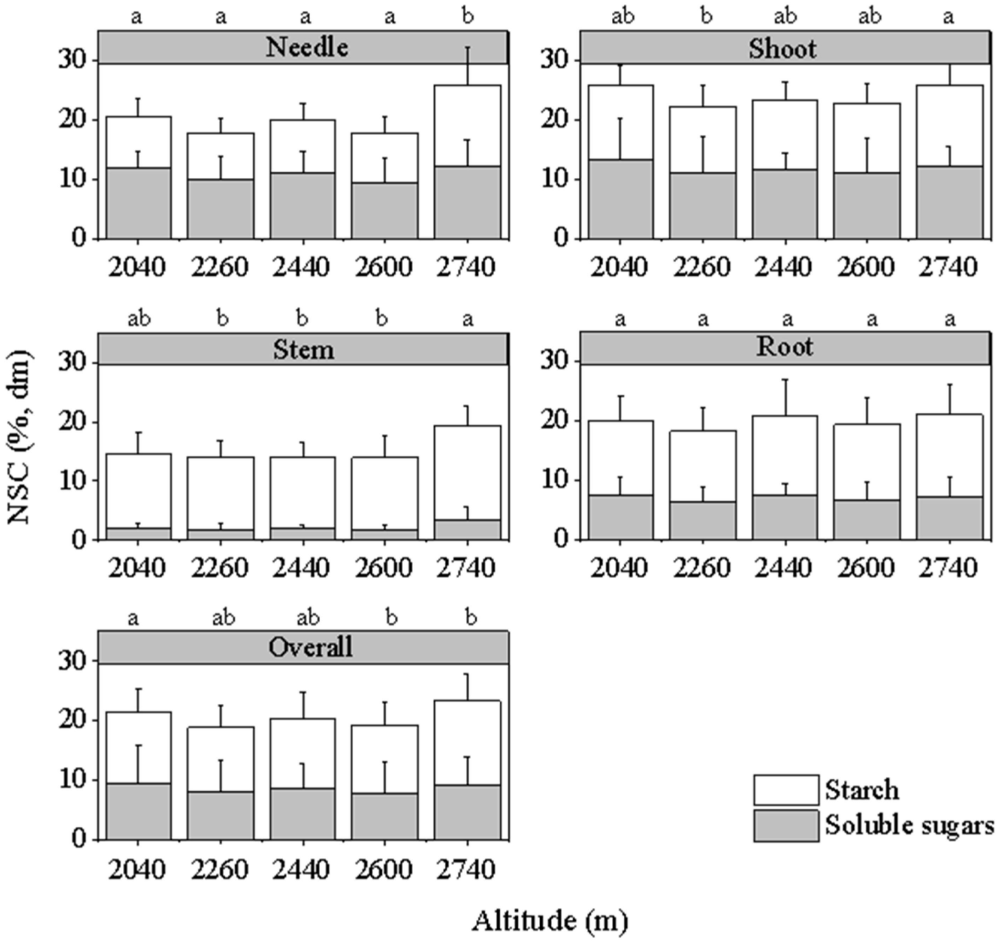

Figure 2. Comparison of the concentrations of soluble sugars (SS, % dm), starch (% dm), and NSC (% dm) in the needles, shoots, stems, and roots of *L. principis-rupprechtii* among the different sites (mean ± SD). Different lowercase letters indicate significant differences among NSC at the 0.05 level.

Table 2. Comparison of the soluble sugar–starch ratio (SSR) in the needles, shoots, stems, and roots of *L. principis-rupprechtii* among the different sites (mean ± SD). Different lowercase letters indicate significant differences at the 0.05 level.

Altitude (m a.s.l.)	Needle	Shoot	Stem	Root	Overall
2040	1.54 ± 0.75^{a}	1.08 ± 0.40^{a}	0.17 ± 0.07^{a}	0.67 ± 0.36^{a}	0.90 ± 0.63^{a}
2260	1.36 ± 0.61^{ab}	1.04 ± 0.50^{a}	0.14 ± 0.07^{a}	0.58 ± 0.26^{a}	0.81 ± 0.59^{ab}
2440	1.38 ± 0.56^{ab}	1.12 ± 0.50^{a}	0.16 ± 0.06^{a}	0.63 ± 0.27^{a}	0.85 ± 0.59^{ab}
2600	1.11 ± 0.51^{b}	0.98 ± 0.43^{a}	0.16 ± 0.06^{a}	0.53 ± 0.17^{a}	0.72 ± 0.48^{b}
2740	1.11 ± 0.83^{b}	0.90 ± 0.26^{a}	0.23 ± 0.14^{a}	0.64 ± 0.69^{a}	0.74 ± 0.61^{b}

Our results showed that NSCs and its components of *L. principis-rupprechtii* differed among organs (Table 3). Specifically, the concentrations of soluble sugars in the shoots (11.91 ± 5.29%) and needles (10.91 ± 3.94%) were substantially higher than those in the roots (7.04 ± 2.81%) and stems (2.21 ± 1.39%), while the concentrations of starch were similar in the shoots (12.19 ± 3.49%), stems (13.03 ± 3.48%), and roots (12.86 ± 4.84%), but not in the needles (9.56 ± 4.27%). The concentrations of NSCs in the shoots (24.10 ± 7.41%) and needles (20.47 ± 6.24%) were higher than those in the roots (19.90 ± 6.35%) and stems (15.25 ± 4.21%). The highest soluble sugar–starch ratio was found in the needles (1.30 ± 0.68), followed by the shoots (1.02 ± 0.43) and roots (0.61 ± 0.39), and the lowest value was found in the stems (0.17 ± 0.09) (Table 3).

Table 3. Comparison of the concentrations of soluble sugars (% dm), starch (% dm), NSC (% dm), and the soluble sugar–starch ratio (SSR) in the needles, shoots, stems, and roots of *L. principis-rupprechtii* (mean ± SD). Different lowercase letters indicate significant differences at the 0.05 level.

Organs	Soluble Sugars	Starch	NSC	SSR
Needle	10.91 ± 3.94^{b}	9.56 ± 4.27^{b}	20.47 ± 6.24^{b}	1.30 ± 0.68^{a}
Shoot	11.91 ± 5.29^{a}	12.19 ± 3.49^{a}	24.10 ± 7.41^{a}	1.02 ± 0.43^{b}
Stem	2.21 ± 1.39^{d}	13.03 ± 3.48^{a}	15.25 ± 4.21^{c}	0.17 ± 0.09^{d}
Root	7.04 ± 2.81^{c}	12.86 ± 4.84^{a}	19.90 ± 6.35^{b}	0.61 ± 0.39^{c}

3.2. Effects of Environmental Factors on the NSCs

In our study, higher concentrations of NSC and its components *in L. principis-rupprechtii* were found under low soil moisture and high VPD conditions, as well as under low air temperature conditions ($p < 0.001$, Figure 3 and Table S1). Additionally, in contrast with the response to air temperature, lower concentrations of NSC and its components were found in *L. principis-rupprechtii* under lower soil temperature conditions (Figure 3a–d and Table S1). Moreover, we found that the needles' soluble sugars had a significant negative relationship ($p < 0.001$, Figure 3e, and Table S2), and their starch had a significant positive relationship ($p = 0.006$, Figure 3e, and Table S2) with the soil–atmosphere temperature gradient, while a negative relationship was found between the starch in the shoots, stems, and roots and the soil–atmosphere temperature gradient ($p = 0.000$, $p = 0.006$, and $p = 0.000$, respectively, Figure 3e and Table S2).

In general, the soluble sugars of *L. principis-rupprechtii* were significantly affected by soil temperature ($p = 0.000$) and VPD ($p < 0.05$, Figure 3 and Table S1). Furthermore, the starch in *L. principis-rupprechtii* was significantly affected by air temperature, soil temperature, VPD, and soil moisture ($p < 0.001$, Figure 3 and Table S1). The NSCs of *L. principis-rupprechtii* were significantly affected by air temperature, soil temperature, VPD, and soil moisture ($p < 0.001$, Figure 3 and Table S1), and the effects of environmental factors on NSCs and starch in different organs were similar. The soluble sugar–starch ratio was significantly affected by air temperature ($p = 0.000$) and soil moisture ($p = 0.041$).

Furthermore, we analyzed the interpretation rate of environmental factors on the concentration of NSCs and their components in four organs of *L. principis-rupprechtii.* Our results revealed that temperature (including air temperature and soil temperature) was the major factor influencing the changes in NSC storage (Figure 4). In addition, the effects of environmental factors on NSCs varied for different organs. Specifically, the concentrations of NSC and its components in the needles and stems were mainly affected by soil moisture and temperature, while the shoots and roots were mainly influenced by temperature (Figure 4).

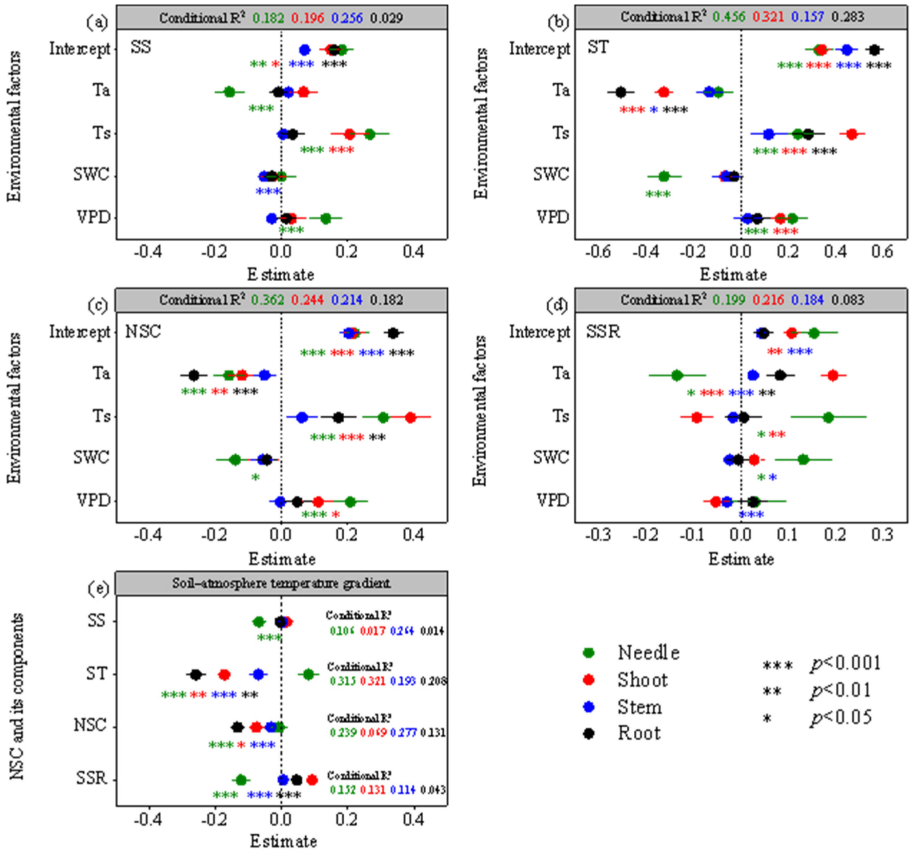

Figure 3. Estimated effects of Ta (air temperature), Ts (soil temperature), SWC (soil water content), and VPD (vapor pressure deficit) on the (**a**) soluble sugars (SS), (**b**) starch (ST), (**c**) NSC, and (**d**) soluble sugar–starch ratios (SSR) in the needles, shoots, stems, and roots. Moreover, the estimated effects of (**e**) soil–atmosphere temperature gradient (ΔT = Ta − Ts) on the soluble sugars (SS), starch (ST), NSC, and soluble sugar–starch ratios (SSR) in the needles, shoots, stems, and roots. Conditional R^2: induced by both fixed effects and random effects R^2.

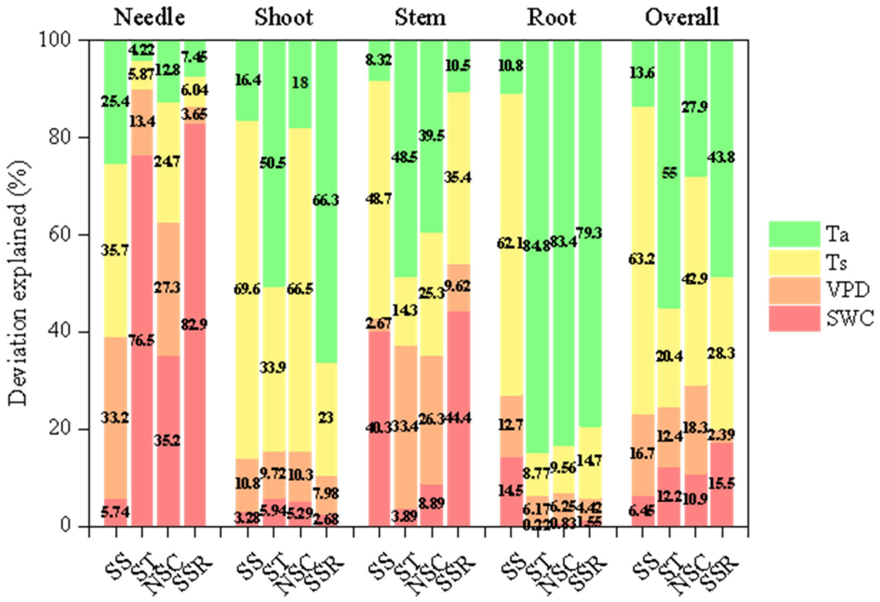

Figure 4. Interpretation rate of the environmental variables to soluble sugars (SS), starch (ST), NSC, and soluble sugar–starch ratios (SSR) in different organs based on a linear mixed model.

4. Discussion

4.1. Temperature Gradients May Assist Carbohydrate Allocation within Tree Individual

In this study, we found that the concentrations of NSCs were mainly affected by temperature (air temperature and soil temperature), which was in line with the results of previous studies [15,18,42,43], indicating that temperature played a key role in the NSC dynamics of *L. principis-rupprechtii* during the growing season.

Interestingly, lower concentrations of soluble sugars and starch in all four organs of *L. principis-rupprechtii* were found under higher air temperatures, whereas they showed lower concentrations under lower soil temperatures (Figure 3). These results suggested that air and soil temperatures may affect on NSCs in trees in various ways, and we speculate that this may be related to the driving temperature gradient between air temperature and soil temperature. Our results also demonstrate that the air temperature was higher than the soil temperature most of the time throughout the growing season, and the soil–atmosphere temperature gradient showed a maximum from the beginning of the growing season (Figure S1). Therefore, we conducted further analysis and found that the needle soluble sugars had a significant negative relationship and its starch had a significant positive relationship with the soil–atmosphere temperature gradient, while negative relationships were found between the starch in the shoots, stems, and roots and the soil–atmosphere temperature gradient (Figure 3e and Table S2). These results may indicate that the temperature gradient between the root system and the canopy facilitates xylem transport of these soluble sugars upwards, which may be further supported by the transpiration of the warm canopy, leading to the accumulation of carbohydrates in the canopy to provide rapid growth, especially at the beginning of the growing season when needles were not photosynthetically active [1,18]. Previous studies have shown that the phloem transport allows NSCs to further diffuse to the apoplast and may transport carbohydrates through the xylem, and that the soil–atmosphere temperature gradient may promote carbohydrate distribution from cold roots to the warm canopy [18,19].

4.2. Needle Starch and Stem Soluble Sugars May Be Important for Trees to Respond to Water Stress

In addition to the effect of temperature, the needle NSCs were also affected by soil and atmospheric moisture conditions. In this study, the NSCs, especially starch, in the needles had a significant negative relationship with soil moisture (Figure 3), as well as their soluble sugars and starch also had a significant positive relationship with VPD (Figure 3). These results indicated that needles significantly increased both soluble sugar and starch concentrations under lower soil moisture and dry atmospheric conditions. Thus, the accumulation of NSC in the needles, especially starch, may play an important role in coping with water deficits.

Needles, as the main organ of photosynthesis, maintain normal cellular turgor by allocating more soluble sugars, thus maintaining stomatal opening and normal transpiration [12]. Simultaneously, starch storage in the needles may be beneficial for trees in terms of maintaining stable soluble sugars to relieve water deficits and could be used for the needs of respiration and defense for needles under more severe water stress, ensuring energy balance when the stoma is closed [5,7,44,45]. Our results are consistent with a previous study that reported starch allocation to be an important drought resistance strategy in *Pinus sylvestris var. mongolica* and *Betula platyphylla* [22]. In addition, some studies have shown that the accumulated NSC in trees during a moderate drought might be consumed during severe or long-term drought [27,46]. Compared to the long-term records, climate conditions in 2018 and 2019 were wetter and warmer in this study region. This indicates that water stress may not have been severe during our study period, resulting in NSCs accumulating in trees.

In this study, we also found that soil moisture significantly influenced the stem soluble sugars, indicating that stems were more sensitive to soil moisture than those in the other organs (Figure 3). Stems, as the main channel organs of water transport in trees, which increase the concentrations of soluble sugars under lower soil moisture conditions, are conducive to lowering osmotic pressure, reducing water potential, and thus improving the efficiency of water transport in trees [4,7,22,47]. Our results are consistent with previous studies which showed that stem soluble sugars are important for trees to maintain osmotic regulation and may enhance the ability to recover from an embolism in cold and dry conditions [5,6,11,48,49].

Interestingly, lower stem soluble sugars were found under higher VPD conditions, while higher soluble sugars were found in other organs under the same conditions, especially in the needles (p = 0.006, Figure 3). Our findings are consistent with a study that reported the soluble sugars in the xylem sap of *Prunus dulcis* decreased from 1.7 to 0.7 mg mL^{-1} after sunrise, implying that soluble sugars would flow to leaves via transpiration [50]. On the one hand, our results indicate that the soluble sugars in xylem sap may also flow back to the canopy with transpiration, resulting in a decrease in stem soluble sugars with rising VPD [12,49,51]. On the other hand, we cannot exclude the effect of photosynthetic product input, and the increase in NSC concentration in the needles and shoots may also be partially affected by the products of photosynthesis; however, the transport of these products from the needles down to the stem may be limited under higher VPD conditions [50]. Therefore, stem soluble sugars may play an important role in a tree's response to water stress. However, it should be pointed out that we cannot distinguish stem soluble sugars derived from the remobilization of the underground NSC or new assimilates due to the lack of information on the sources of soluble sugars in our study. Therefore, more research is needed on this topic in the future.

5. Conclusions

Our results indicated that temperature was found to be the most important environmental factor influencing NSC dynamics in *L. principis-rupprechtii*, with the soluble sugars in needles having a significant negative relationship and the needles' starch content also having a significant positive relationship with the soil-atmosphere temperature gradient. Starch in the shoots, stems, and roots had a significant negative relationship with the soil-atmosphere temperature gradient. These results may indicate that temperature gradients between roots and the canopy facilitate the xylem transport of these soluble sugars upward, resulting in carbohydrates accumulating in the canopy to provide rapid canopy growth, especially at the beginning of the growing season. Furthermore, starch storage may be an effective strategy for needles to cope with water deficits, and soluble sugars in the stem may be important for trees in their response to water stress. Overall, carbon storage plays a key role in the survival of trees under environmental stress, and this study provides new insight for better understanding the effects of environmental stress on future forest carbon balances. These results are also helpful to estimate the possible growth decline or tree mortality of forests in a future warmer and drier world, which provides a theoretical basis for forest management and sustainable development under global change scenarios.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14020345/s1, Figure S1: Temperature gradients (ΔT = Ta − Ts) for Ts (soil temperature) to Ta (air temperature) during the growing season; Table S1: Model fitted for the NSC and its components in different organs vs. environmental factors; Table S2: Model fitted for the NSC and its components in different organs vs. ΔT (ΔT = Ta − Ts).

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Conflicts of Interest: The authors declare no conflict of interest.

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