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Korean Pines Demonstrate Cold Resilience through Non-Structural Carbohydrate Concentrations despite Light Deprivation during the Growing Season

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Abstract: Carbon is the fundamental element of plant life. Non-structural carbohydrates (NSC), synthesized using carbon dioxide through photosynthesis by plants, are essential for their growth and survival, which are also affected by light and temperature. However, few studies have investigated the effects of light conditions, season, and needle age together on field plants' carbohydrates. We measured total carbon, glucose, sucrose, fructose, and starch concentrations in current and 1-year-old needles of Korean pine (*Pinus koraiensis* Sieb. et Zucc) growing under two distinct light conditions (dense canopy and full light) from early summer to cold winter. Total carbon, glucose, fructose, SS (soluble sugar, sucrose + glucose + fructose), NSC (soluble sugar + starch), starch, and SS/NSC all significantly ($p < 0.05$) related to the DOY (day of the year, 2021). Total carbon reached the maximum in September when the fresh needles were mature. Glucose, NSC, SS, and SS/NSC reached the maximum at the last sampling time, which provided protection for the cells in cold winter. The season showed a bigger impact on total carbon and NSC (all parameters except total carbon) than light and needle age. Two different-aged needles under two light conditions all had similar patterns of variation in total carbon and NSC, but twigs showed a more significant ($p < 0.05$) difference in NSC concentrations between two light conditions on 25 October (DOY 249). Needles of Korean pines stored soluble sugars (mainly glucose) and consumed starch in winter. Moreover, we found that needles had more glucose, while twigs had more sucrose, which may be a result of their different functions and may be helpful for future spring growth. Although lacking light during the growing season, understory Korean pines still had enough cold tolerance, similar to full-light ones, which indicated that low light during the growing season has little effect on cold tolerance.

Keywords: Korean pine; needle; NSC; cold tolerance; shade

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1. Introduction

Photosynthesis is the process by which plants convert carbon dioxide into non-structural carbohydrates (NSC), including soluble sugars (mainly glucose, fructose, and sucrose) and starch [1]. It is primarily influenced by environmental components such as light and temperature. Light, in particular, plays a pivotal role in photosynthesis and interacts with seasonal temperature variations. Light intensity is a key determinant of photosynthesis. Under conditions of low light intensity, as experienced in shaded environments, plants adapt by increasing their light-capturing capacity. However, this adaptation comes at a carbon resource cost. Conversely, in high-light conditions, plants exhibit greater photosynthetic capacity and accumulate carbohydrates more efficiently [2]. Furthermore, light may influence a plant's response to cold temperatures [3].

Cold acclimation, a process that enhances a plant's tolerance to cold temperatures, is regulated by a combination of light and temperature. Research has demonstrated that a low light intensity inhibits the cold acclimation process when temperatures are low [4]. Moreover, studies have shown that both cold and light conditions affect freezing tolerance in plants, such as Arabidopsis [3] and peas [4]. Notably, Novák et al. (2021) found that a low photosynthetic photon flux density (PPFD) did not hinder the cold acclimation process. The interplay between light availability and temperature is of utmost importance for the physiological and biochemical processes in plants, and further exploration of this topic may help resolve existing controversies. It is worth noting that these experiments have been conducted under controlled laboratory conditions, and additional field-based comparisons are necessary to provide a comprehensive perspective. The early accumulation of sugar provides *Eucalyptus benthamii* with a higher capacity to acclimate to cold conditions [5]. As global warming and extreme weather events continue to pose challenges [6,7], temperature fluctuations can vary, potentially impacting a plant's ability to undergo cold acclimation. This process relies on a period of low but non-freezing temperatures for successful completion [4]. A better understanding of how plants respond to cold is crucial for assessing forests' adaptability under different climate change scenarios. It is important to note that the responses of tree growth to climate are often species-specific [8].

Therefore, comprehending the role of non-structural carbohydrates (NSC) in plants necessitates knowledge of both the overall quantity of carbon stored in plant tissues and the proportions in which NSC are distributed as sugars and starches [9].

NSC play essential roles during plant growth and cold acclimation [10]. Variations in light and temperature have a significant impact on carbon storage, allowing plants to support development in cases of temporal asynchrony between carbon supply and demand [11]. NSC generally represent the difference between photosynthetic production and respiratory processes, as well as allocation to new tissue growth in models [12]. Soluble sugars (SS) significantly influence a plant's physiological resistance to low temperatures, osmotic stress, and disease [13]. Starch, a type of storage polysaccharide in plants, serves as a temporary energy source for trees during periods of low springtime temperatures, supporting growth and metabolism [14–16]. To adapt to changing conditions, starch and soluble sugars can be transformed into one another [17].

Korean pine (*Pinus koraiensis* Sieb. et Zucc.) is an evergreen coniferous species mainly located in northeast China [18]. Korean pine forests face restoration challenges due to their slow growth and suboptimal growth under full sunlight [19]. Nevertheless, Korean pine is the dominant species in the typical climax vegetation of Northeast China and exhibits strong environmental adaptability [20,21]. To restore this climax vegetation, an acknowledged restoration practice, namely reinforcing conifers while keeping broad-leaved trees, is widely adopted in managing secondary forests in Northeast China [22]. In this way, Korean pine must contend with both deep shade during the growing season and low winter temperatures. Given the ongoing and unpredictable nature of climate change, Korean pine is an ideal subject for studying shade and cold stress. Notably, we observed that Korean pine trees exhibited yellowing and withering under low light conditions in mid-spring, potentially linked to both low light exposure and winter damage. Therefore, we conducted a study to investigate the total carbon and non-structural carbohydrate (NSC) components (soluble sugars and starch) in current and 1-year-old needles and twigs of Korean pine trees under two distinct light conditions. Our study aimed to address the following questions. (a) Are there significant differences in the seasonal changes in total carbon, soluble sugars, NSC, and starch in Korean pine trees under low- and full-light conditions? (b) Can newly formed needles of understory Korean pine trees tolerate shade and cold stress as effectively as older needles? (c) Does shading during the growing season impact the cold tolerance of trees in winter? We hypothesized that (1) there are substantial changes in the total carbon and NSC concentrations in Korean pine needles from early summer to the cold winter under both low- and full-light conditions; (2) the cold tolerance of understory Pinus koraiensis may be influenced by prolonged exposure to low light during the growing season; and (3) there may be differences in how newly formed and 1-year-old needles of Korean pine trees tolerate cold stress.

2. Materials and Methods

2.1. Study Site

The study site is located at the Taiping working area in the Maoershan Experimental Forest Farm of Northeast Forestry University (45°21′–45°25′ N, 127°30′–127°34′ E; Heilongjiang, China) (Figure 1). The climate in this region is classified as a temperate continental monsoon climate with a growing season from May to September. The yearly average temperature in this region is 2.98 °C, while the monthly average temperature ranges from −19.7 °C in January to 22 °C in July. There are around 120–140 days that do not see frost on an annual basis, and the average amount of precipitation is between 600 and 800 mm, primarily (80%) occurring from May to September, with 2471.3 h of annual sunshine [8]. The soil at the location is Hap-Boric Luvisol, which is mainly dark brown in color and consists of loamy soil with a depth of 1–10 cm and sandy loam with a depth of 10–20 cm [23]. The pH of the soil is around 4.50. The soil's available nitrogen, available phosphorus, and available potassium concentrations are around 250 µg/g, 2 µg/g, and 200 µg/g, respectively [24]. Plantations and natural secondary forests are the two types of vegetation that are currently present there, which evolved as a result of recurrent human disturbances and are dominated by *Pinus koraiensis* and a variety of broadleaf tree species, including *Fraxinus mandshurica*, *Phelodendron amurense*, *Juglans mandshurica*, *Betula platyphylla*, *Quercus mongolica*, and *Tilia amurensis* [8].

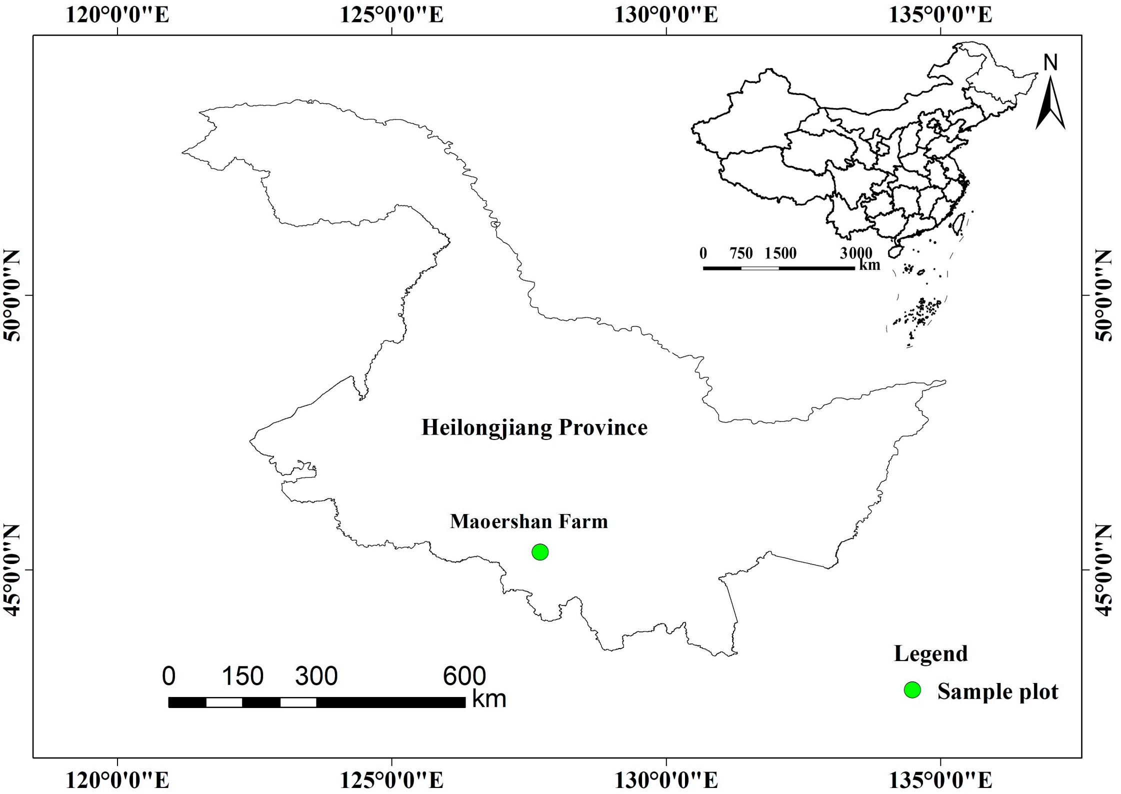

Figure 1. Map of the study area.

2.2. Experimental Design and Sampling

This study was conducted in 2021, in which current-year (flushed in 2021) and 1-year (flushed in 2020) needles of Korean pines (*Pinus koraiensis*) were examined under a dense canopy (low light) and a full light regime. For the two distinct light conditions, we measured the photosynthetic photon flux density (PPFD) and red/far-red ratio (R/FR) in July, when the canopy trees are fully foliated, as shown in Table 1. The PPFD was measured using a dual radiation meter (Spectrum Technologies, Inc., Aurora, IL, USA), and the R/FR was measured simultaneously using a R/FR light meter (Spectrum Technologies, Inc., Aurora, IL, USA) between 10:00 a.m. and 12:00 a.m. The current-year needles of these understory Korean pines were not fully grown and still had sheaths in June and July 2021, but we removed them throughout the experiment. The degree of canopy openness served as the controlled variable for regulating these trees, as described in Shen et al. [25]. Three biological replicates were sampled at five different times: 23 June, 29 July, 6 September, 25 October, and 1 December 2021, which corresponded to DOY (day of the year) 174, 210, 249, 298 and 335, respectively. In total, 6 *P. koraiensis* trees under two different light conditions were sampled at each sampling time. Several shoots were cut and placed in sterile polythene bags before being transported to the laboratory in a box with ice bags. Due to the incomplete experimental design and the slow growth of twigs, we did not choose to measure them at first. We wanted to focus more on winter cold tolerance, so we sampled and measured twigs at the second to last and last sampling times (DOY 298 and 335).

Table 1. Comparisons of PPFD and R/FR measured in July.

	PPFD (μmol/m^2/s)	**R/FR**
Under the canopy	6.83 ± 0.75	0.28 ± 0.02
Under full light	1440 ± 167.31	1 ± 0.08

Data represent the means of six replicates ± SD.

2.3. Measurement of Carbohydrate Concentrations

In this research, needle and twig samples of Korean pines were oven-dried at 70 °C until a constant weight was reached, and then milled using a grinding miller. Due to the small sample sizes, three biological replicates were mixed and milled together. After that, the total carbon concentration was determined by using an elemental analyzer (Elementar, VARIOMacro, Langenselbold, Germany). On the other hand, soluble sugar (glucose, sucrose, and fructose), and starch concentrations were determined using 50 mg of the dried samples according to the anthrone method [26].

2.4. Data Analysis

Soluble sugar (SS) was calculated by adding glucose, sucrose, and fructose together. Non-structural carbohydrates (NSC) were calculated by adding SS and ST. NSC refer to all components of NSC (SS, glucose, sucrose, fructose, starch, NSC, and SS/NSC). All the data analysis and graphs were made by using R 4.2.2 software. Pearson's correlations matrix was used to find the correlation coefficients (r) of all pairwise combinations of carbon and NSC in needles and twigs.

3. Results

3.1. Variations in Total Carbon and NSC in Needles

In general, variations in total carbon and NSC concentrations in needles followed almost the same pattern, irrespective of the light conditions and the needles' age. Fructose, NSC, and SS all decreased first and then increased (Figure 2d,f,g). Glucose and SS/NSC increased throughout time (Figure 2b,h), while starch decreased (Figure 2e). Total carbon concentration experienced a bimodal pattern and reached the maximum on DOY 249 (Figure 2a, Table S1). Sucrose initially decreased and then increased to a maximum on DOY 298, then decreased again (Figure 2c). Except for the starch and sucrose concentrations, the others all positively and significantly ($p < 0.05$) related to DOY (Table 2), while the starch concentration was significantly ($p < 0.05$) and negatively related to DOY.

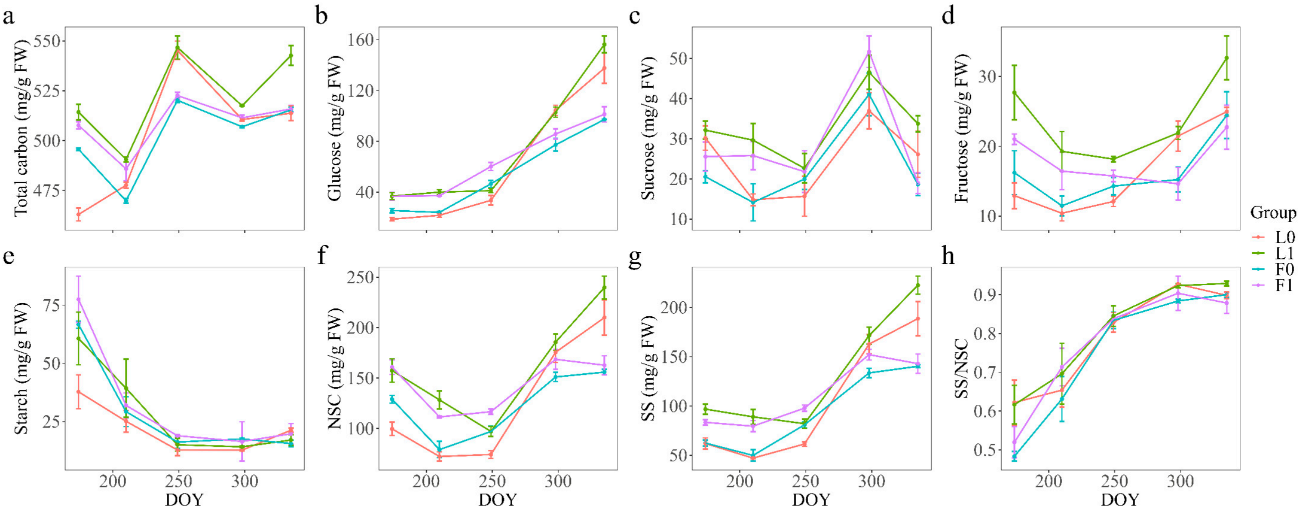

Figure 2. Total carbon (**a**), glucose (**b**), sucrose (**c**), fructose (**d**), starch (**e**), NSC ((**f**), calculated by adding sucrose, fructose, glucose and starch), SS ((**g**), calculated by adding sucrose, glucose and frucrose) and SS/NSC (**h**) in current- and 1-year-old needles under low and full light. SS, soluble sugar; 0, current-year needles; 1, 1-year needles; L, low light; F, full light; DOY, day of the year 2021.

Table 2. Correlation matrix, based on Pearson correlations, including the correlation coefficients (r) of all pairwise combinations of carbon and NSC in needles. Non-significant correlations are omitted (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$). Negative correlations are shown in white color, and positive correlations are in red; the color gradient shows the strength of the correlation. SS, soluble sugar; glucose + sucrose + fructose; NSC, glucose + sucrose + fructose + starch; DOY, day of the year 2021.

	Total Carbon	Glucose	Sucrose	Fructose	Starch	NSC	SS	SS/NSC
DOY	0.52 ***	0.89 ***		0.42 *	−0.72 ***	0.6 ***	0.83 ***	0.9 ***
Total carbon		0.48 **		0.44 *			0.46 **	0.58 ***
Glucose			0.41 *	0.68 ***	−0.48 **	0.87 ***	0.98 ***	0.76 ***
Sucrose						0.58 ***	0.57 ***	
Fructose						0.82 ***	0.73 ***	
Starch							−0.40 *	−0.88 ***
NSC							0.92 ***	0.42 *
SS								0.73 ***

3.2. Variations in Total Carbon and NSC in Needles Considering Light and Age

On DOY 174 and 210, the 1-year needles exhibited significantly ($p < 0.05$) higher concentrations of total carbon, glucose, NSC, and SS than the current-year needles under each light condition (Figures S1 and S2). DOY 249 revealed significant differences in NSC, glucose, and SS concentrations among the light conditions and needle ages (except for glucose between the two needle ages under low-light conditions) (Figure S3). On DOY 298 and 335, full-light 1-year needles demonstrated significantly lower total carbon, glucose, fructose, and SS concentrations than their low-light 1-year counterparts, while low-light current-year needles displayed higher glucose, starch, SS, and NSC concentrations (Figures S4 and S5).

The total carbon concentration was positively and significantly ($p < 0.05$) related to glucose, fructose, SS concentrations, and SS/NSC. Starch concentrations were negatively and significantly ($p < 0.05$) correlated with glucose, SS concentrations, and SS/NSC (Table 2).

3.3. Variations in Total Carbon and NSC in Twigs

On DOY 298, in a comparison between same-aged twigs under different light conditions, the full-light ones had significantly ($p < 0.05$) lower total carbon, glucose, sucrose, fructose, NSC, SS, and SS/NSC concentrations (except for fructose in low-light needles; Figure 3c). Generally, current-year twigs possessed higher carbon and NSC concentrations than 1-year twigs under the same light conditions in October, and more significant differences ($p < 0.05$) were found in October than in December. On DOY 335, the 1-year twigs of Korean pines had significantly ($p < 0.05$) higher total carbon concentrations than the current-year ones under same light conditions (Figure 4a). Between the two light conditions, full-light twigs had significantly ($p < 0.05$) lower total carbon and glucose concentrations than low-light ones; moreover, low-light 1-year twigs had significantly ($p < 0.05$) higher NSC and SS concentrations than full-light 1-year ones (Figure 4f,g).

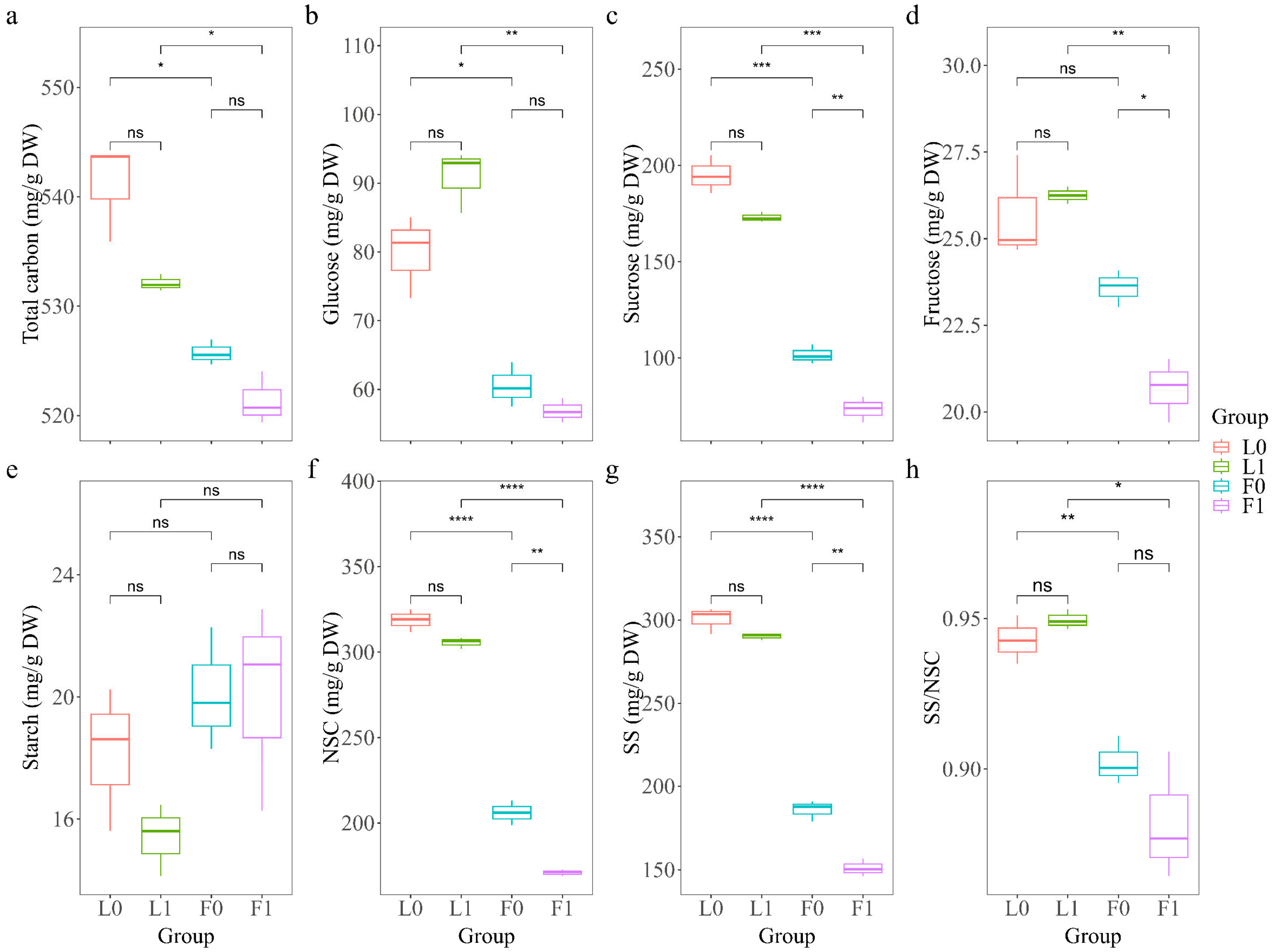

Figure 3. Comparisons of total carbon (**a**), glucose (**b**), sucrose (**c**), fructose (**d**), starch (**e**), NSC ((**f**), calculated by adding sucrose, fructose, glucose and starch), SS ((**g**), soluble sugar, calculated by adding sucrose, glucose and frucrose) and SS/NSC (**h**) among twigs on DOY 298 (25 October 2021). SS, soluble sugar; 0, current-year twigs; 1, 1-year twigs; L, low light; F, full light. ns, not significant, **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

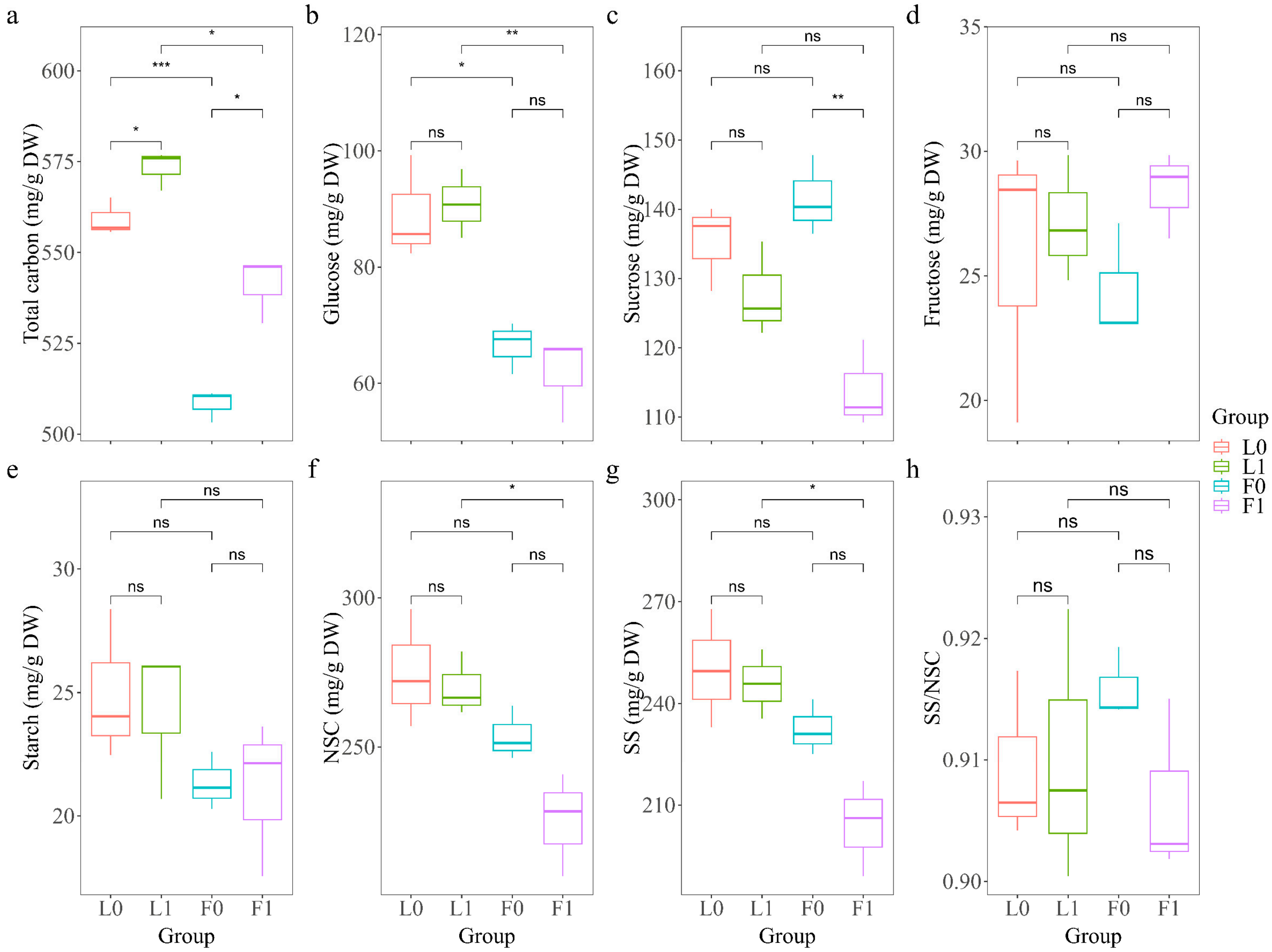

Figure 4. Comparisons of total carbon (**a**), glucose (**b**), sucrose (**c**), fructose (**d**), starch (**e**), NSC ((**f**), calculated by adding sucrose, fructose, glucose and starch), SS ((**g**), soluble sugar, calculated by adding sucrose, glucose and frucrose) and SS/NSC (**h**) among twigs on DOY 335 (1 December 2021). SS, soluble sugar; 0, current-year twigs; 1, 1-year twigs; L, low light; F, full light. ns, not significant, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

4. Discussion

4.1. Light Affects Variations in Total Carbon and NSC in Needles Less Than Season Does

Seasonal changes in total carbon and NSC in the low- and full-light needles all followed similar patterns, which indicated that new needles adapted to the environments as quickly as last year's needles. At the first and second sampling times, the current-year needles tended to have lower NSC concentrations than 1-year ones, which may be a result of the needles' development, since the needles were not fully grown. SS and NSC first decreased and then increased, and starch decreased all the time, in accordance with what Zhou et al. [27] found in current and 1-year-old needles of understory Korean pine saplings. However, Zhu et al. [28] found that sucrose increased continuously throughout the leaf development of *Hevea brasiliensis*, which was the opposite to our results. This conflict may be attributed to diurnal changes in soluble sugar because of its energetic role in metabolism [29]. Once new leaves had formed, they relied more on themselves for photosynthesis rather than depending on older leaves [30].

Light is vital for photosynthesis and plants' life [31], and its availability strongly affects the products of photosynthesis, such as leaf NSC [32]. Research by Zhang et al. [33] revealed that NSC concentrations did not vary significantly ($p < 0.05$) under four different light levels in the needles of Korean pines, but not in another deciduous species, *Quercus mongolica*. In our study, we found significant ($p < 0.05$) differences in NSC and SS concentrations between full-light and understory same-aged needles on DOY 249 (Figure S3f,g), 298 (Figure S4g), and 335 (Figure S5f,g), all of which occurred after the needles' maturation. Xie et al. [34] also found that leaf SS, starch, and NSC concentrations differed significantly between 100% light and 6% light.

Seasonal fluctuations are more important to SS than the effect of growth conditions, as Terziev et al. [35] found in *Pinus sylvestris* L. sapwood. In our study, carbon and NSC (except sucrose) were all significantly ($p < 0.05$) related to the sampling time (DOY). The observed seasonal variations in NSC concentrations reflected the joint effects of soluble sugars and starch, with the former increasing and the latter decreasing. Conifers' leaves were also characterized by opposite temporal dynamics for starch and SS in leaves, with a peak in starch in late spring–early summer coinciding with the seasonal minimum of SS, which was distinctly different from the other studied functional groups [1]. The SS/NSC ratio reflects the allocation pattern of NSC within leaves [34]. SS/NSC in Korean pine needles increased over time, which demonstrated that the increase in NSC was mainly caused by the marked increase in soluble sugars, while the starch contributed less. Korean pine needles grown under the canopy showed more variations in glucose, SS, and NSC (Figure 1). In needles, NSC and SS concentrations almost doubled, and glucose concentrations nearly quadrupled, if we compare the first sampling time with the last (Figure 2b,f,g). This demonstrates the powerful effect of season/DOY on their variation, especially for those grown under low-light conditions. No significant relationships ($p < 0.05$) were discovered (Figure S6) between light and other parameters. This phenomenon may be attributed to the classifications of season and light, since there were three seasons in the study but only two light conditions.

4.2. Variations in Total Carbon and NSC Respond to Cold and Future Growth

As plants are fixed in place, they have to withstand environmental stresses to ensure their sustainability [36]. Repeated challenges such as the onset of winter and the lack of light during the whole growing season are both environmental stresses that plants have to tolerate. Cold is acknowledged to be an important factor limiting the distribution of plants [37]. SS reached its maximum in winter, as found in *Pinus koraiensis* [27], *Pinus sylvestris* [38], and other species [39]. In this study, we could only see that NSC reached its maximum on DOY 335, the coldest sampling time, but the lack of comparisons among different species, sites, and measurements during the following months made it impossible to draw further conclusions.

Plants are mostly dormant below 0 °C, and at this temperature, the living cells evergreens' foliage face extracellular freezing of cellular water and the associated desiccation [40]. SS increased with colder conditions [41], which would be helpful for increasing a plant's tolerance of cold by serving as a cryoprotectant [42]. Korean pine is one of the native species in northeast China, where the lowest temperature hits −40 °C in winter; knowing more about its metabolism in winter is helpful for exploring cold tolerance mechanisms in plants.

As SS reaches the maximum and starch reaches the minimum, they may play an important role in the cold tolerance of a tree in winter, maintaining basal respiration [43], as well as providing energy for early growth in spring [39,44]. Palacio et al. [45] also found that overwintering and spring leaves could serve as N and NSC sources for shoot growth in Mediterranean sub-shrub evergreen species.

There is also a dual view about how NSC function: starch acts mostly as a reservoir for future use, while soluble sugars perform immediate functions (e.g., osmoregulation) and are kept above some critical threshold [1]. In needles, we found that sucrose and fructose concentrations were mainly stable, and starch decreased all the time, while glucose increased greatly when winter came, which contributed extensively to the increase in NSC, SS, and SS/NSC. Glucose is arguably one of the most critical elements for life; it is the primary fuel for glycolysis and respiration, and is responsible for generating much of the energy required for future growth [46]. The sucrose concentration maintained a stable level and did not decompose itself entirely into glucose and fructose, which suggests that it may also work as an energy reserve. This implies that plants are preparing all the time, whether for more cold weather or spring growth, and are ready to condense glucose to form starch again, similar what happens during the night–day cycle [10]. In twigs, things were a little different. Sucrose was more abundant than glucose. Sucrose is one of the dominant NSC exported from the leaves into the phloem [10]. From these results, we inferred that twigs are less active than needles at the same time, since needles are the primary sources of NSC and the twigs are sinks. Chang et al. [47] also found that Eastern white pine is most sensitive to temperature changes during October and November. With the weather becoming more difficult to forecast, warmer and shorter winters occurring, and the risk of late spring and early autumn frosts increasing, this preparation may help plants greatly during the coming years. However, distinguishing NSC between immediate vs. future needs is difficult [1]. Basically, we only measured NSC concentrations in different organs within plants and still found it hard to separate their different functions with their complicated metabolism.

4.3. Light Conditions during the Growing Season Have Little Effect on Cold Tolerance

As sessile organisms, how plants tolerate different abiotic stresses is a key factor in their distribution patterns [36]. Traditional ecological knowledge tells us that physiochemical constraints largely determine plants' tolerance or polytolerance—the capacity to tolerate multiple stress factors simultaneously—to stress. However, the adaptation of woody plants to different abiotic stress is highly complex [48].

Light is important for plants as an energy source and as a developmental signal, which can also modulate their responses to stress. Learning how plants perceive light as a signal for adequate responses to abiotic stress is essential, which will support breeding strategies to enhance plants' stress resilience [49]. Early research found that shading decreased soluble carbohydrates in peach shoots and hardiness in cherry and peach shoots [50]; shading combined with a low-temperature treatment caused greater damage to soybeans [51]. The results indicated that light substantially influenced the process of acclimation to both low and high temperatures, and it may affect the level of injury from stress [52]. Our study found that even though the light environment was unfavorable to understory Korean pines during the growing season, cold tolerance in December was not inhibited; moreover, understory twigs may act in preparation for winter earlier than full-light ones. There were more significant ($p < 0.05$) differences found in the twigs on DOY 298 (when the temperature dropped obviously, Figure S7) than on DOY 335, which indicated that understory twigs might be sensitive to the mutual effect of understory light and temperature and responded earlier than full-light ones. This is similar to what Oberschelp et al. [5] found in *Eucalyptus benthamii*, where the earlier accumulation of sugar gave it a higher capacity to acclimate to cold temperatures. Short days or shady environments may increase cold acclimation [49]. However, after summarizing the combinations of stress tolerance, Puglielli et al. [53] found strong independence between shade tolerance and cold tolerance.

5. Conclusions

Plants have to endure many adverse conditions to ensure their survival and growth, among which light and low temperature are noteworthy abiotic stresses that affect plants' distribution around the world. Our research showed that during the growing season, understory Korean pines can handle low light levels as well as they can handle full light, where the needles' concentrations of total carbon, NSC, and their components changed in the same way. Compared with trees grown under full light, needles under the canopy exhibited greater variations in these components, while the season had a bigger impact on them than light conditions. Total carbon and NSC (except for sucrose) were all significantly ($p < 0.05$) related to the DOY (day of the year 2021), from late June to early December. Moreover, low light during the growing season did not affect how well understory Korean pine could handle cold, and twigs that grew in the shade may have prepared to handle stress sooner than those that grew in full light. Finally, plants' tolerance of interacting stresses is complicated, and as the future climate becomes more volatile, it is important to know the dynamics of carbon and carbohydrates, which help plants adapt to constantly changing conditions.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f14122296/s1. Figure S1. Comparisons of total carbon and NSCs between needles on DOY 174 (23 June 2021). SS, soluble sugar; 0, current-year needles; 1, 1-year needles; L, low light; F, full light. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Figure S2: Comparisons of total carbon and NSC between needles on DOY 210 (29 July 2021). SS, soluble sugar; 0, current-year needles; 1, 1-year needles; L, low light; F, full light. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Figure S3: Comparisons of total carbon and NSC between needles on DOY 249 (6 September 2021). SS, soluble sugar; 0, current-year needles; 1, 1-year needles; L, low light; F, full light. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Figure S4: Comparisons of total carbon and NSC between needles on DOY 298 (25 October 2021). SS, soluble sugar; 0, current-year needles; 1, 1-year needles; L, low light; F, full light. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Figure S5. Comparisons of total carbon and NSC between needles on DOY 335 (1 December 2021). SS, soluble sugar; 0, current-year needles; 1, 1-year needles; L, low light; F, full light. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Figure S6: Correlations of all parameters in needles. TC, total carbon concentration; DOY, day of the year; SS, soluble sugar. Non-significant correlations are omitted ($p < 0.05$), negative correlations are shown in green, and positive correlations are in red; the color gradient shows the strength of the correlation. There were three seasons included in the analysis: summer, autumn, and winter. Table S1: Comparison of the means of total carbon and NSC in Korean pine needles. Figure S7: Low, average (Ave), and high air temperatures in the sampling site from June to November. Data are from NCEI (National Centers for Environmental Information) and NOAA (National Oceanic and Atmospheric Administration). DOY, day of the year 2021. Expanded black dots represented the average temperature on each sampling day.

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Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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

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