The Dynamics of Non-Structural Carbohydrates in Different Types of Bamboo in Response to Their Phenological Variations: Implications for Managing Bamboo Plantations

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Abstract: Non-structural carbohydrate (NSC) dynamics in different bamboo types underlie the different management practices of bamboo plantations. The changes in moisture, soluble sugars, and starch content in response to phenological variations in different types of bamboo were measured to reveal the NSC dynamics in different bamboo forests for high yield. It was reported for the first time that new leaves of monopodial bamboo started sprouting after the completion of branching, while sympodial bamboo extended new leaves and branches simultaneously. NSC accumulation for shooting lasted about eight months for monopodial bamboo but only a short interval of two to three months for sympodial bamboo. The moisture content in different types of bamboo showed a similar trend of increasing from March to July and then decreasing until the coming January. From March to May, particularly in March, irrigation was required for the shooting of monopodial bamboo, whereas the shoot buds in sympodial bamboo showed sprouting, branching, and leafing. Total NSCs in different bamboo types remained relatively high in March, gradually decreased to the lowest in July, and then increased continuously until the coming January. Average soluble sugar and starch contents showed the same trend as the total NSCs in different types of bamboo. During the period from March to July, large amount of photoassimilates were required for shooting and height growth and then for branching and leafing in monopodial bamboo. Abundant photoassimilates were also required for branching, leafing, and shoot bud differentiation, as well as for subsequent shoot germination in sympodial bamboo. Thus, fertilizer application to both monopodial and sympodial bamboo plantations should be scheduled in early May and late July. Given the moisture, soluble sugar, starch, and total NSC content, January was the best season for harvesting monopodial bamboo for high production and future growth, whereas for sympodial bamboo, November was the best season.

Keywords: phenological variation; non-structural carbohydrates; sympodial bamboo; monopodial bamboo; management practices

1. Introduction

Bamboo is one of the most important and promising forest types in China and many of the tropical and subtropical economies due to easy propagation and fast growth. Bamboo forests are highly productive with irrigation and fertilizer. Bamboo consists of three broad types, viz. sympodial rhizomes with caespitose culms, sympodial rhizomes with scattered culms, and monopodial rhizomes with scattered culms [1]. Sympodial bamboo (Figure 1a,b)
generally produces shoots in autumn and reaches its maximal height in the same year but finishes branching and leafing in the coming spring. Monopodial bamboo with scattered culms (Figure 1c) generates shoots in spring, and then branching and leafing occur in the following months. Sympodial and monopodial bamboo differ significantly in phenology, implying that different cultivation and management practices are needed. Considerable efforts have previously focused on monopodial bamboo cultivation and management [2–4]. On the other hand, there has been little work with sympodial bamboo, and as a result, its management practices have been largely based on those for monopodial bamboo, which is inadequate.

Figure 1. Three typical bamboo types [1]. (a) Sympodial rhizomes with caespitose culms. (b) Sympodial rhizomes with scattered culms. (c) Monopodial rhizomes with scattered culms.

As the main product of photosynthesis, the accumulation of non-structural carbohydrates (NSCs) plays a critical role in the cultivation and management of bamboo plantation. Enhancing NSC accumulation is one of the effective measures for bamboo shooting and hence necessitates more research. NSCs are stored in the vegetative tissues of plants in the form of soluble sugars and starch, which essentially reflect the vitality and photosynthetic capacity of plants [5,6]. They serve as building blocks for growth, fuel for respiration, and solutes for osmoregulation and osmoprotection [7–9]. Conventionally, NSCs are expected to increase over the growing season when photosynthesis is high, while NSC reserves accumulate as growth slows down and decrease over the dormant season when photosynthesis is absent and they are drawn upon for respiration [10]. Bamboo relies on and replenishes stored NSCs throughout the year, and the seasonal patterns in storage are driven by the balance between sources and sinks. Theoretically, high NSC accumulation generates more bamboo shoots, improves growth, and benefits the overall development of the bamboo plantation. As the primary NSCs accumulate in bamboo culms, soluble sugar and starch concentrations and their seasonal patterns are closely related to the bamboo phenology; they reflect the physiological performance of bamboo, which to some extent underlies a range of bamboo conservation and management practices [11]. Previous studies estimated the seasonal fluctuation of starch and sugar contents in *Fargesia yunnanensis* [11], *Phyllostachys pubescens* [2], *Dendrocalamus giganteus*, and *Ph. edulis* [3], and the best season for harvesting bamboo culms was autumn when the sugar and starch contents were low [2,11,12], which decreased the damage from insects and microorganisms and improved their properties [2,3]. Moreover, for better bamboo forest management, researchers suggested fertilization and irrigation before and after branching, leafing, and shooting stage in *F. yunnanensis* [11]. However, knowledge on the seasonal patterns of NSC reserves in different types of bamboo remains limited. Specifically, an in-depth understanding of the phenological characteristics and NSC storage fluctuations in different bamboo types is critical for improving shoot and culm yield in bamboo plantation management.
Yunnan Province in southwest China harbors 39 genera and 282 native bamboo species, about half of the recorded bamboo species in China [1]. *Phyllostachys mannii* (monopodial bamboo with scattered culms), *Neosinocalamus affinis* (sympodial bamboo with caespitose culms), and *Fargesia yuanjiangensis* (sympodial bamboo with scattered culms) are the three most extensively distributed and commercially cultivated bamboo resources locally. They are primarily used for construction material, edible bamboo shoots, pulp and paper, biomass energy, and highly elastic bamboo strips for weaving farm tools, etc. These three species well represent the three broad types of bamboo. The current management practices for different types of bamboo forest, such as irrigation, fertilization, and proper harvest seasons, are inadequate and yet to be improved with proper justifications.

This study aimed to provide more detailed information on the variations in NSC levels in response to the phenological characteristics of the three types of bamboo. The findings shall furnish the theoretical basis for improving the management and production of different types of bamboo forests.

2. Materials and Methods

2.1. Plant Material

Culms of *N. affinis*, *F. yuanjiangensis*, and *Ph. mannii* at different phenological stages were sampled from the cultivated bamboo garden of Southwest Forestry University in Yunnan Province, China. The differences in the phenology, moisture, and NSC storage as a result of different environmental factors were minimal as the three bamboo species were collected from the same site. The phenological phenomena of each age class (1, 2, ≥3 years old) of the three species from three bamboo clumps were observed and recorded at the bamboo garden from March 2016 to January 2017.

The local rainy season lasts from June to October, while the months thereafter fall within the dry season. Five culms of each age class were harvested on 25 March, 25 May, 25 July, and 25 November 2016 as well as on 25 January 2017. The temperature and precipitation recordings were 14 °C and 11.4 mm in March, 19 °C and 89.6 mm in May, 20 °C and 179.3 mm in July, 12 °C and 71.5 mm in November, and 9 °C and 20.9 mm in January [13]. According to the culm heights and internode lengths, the culms were divided into three portions from the bottom to the top, i.e., 1st for bottom, 8th for middle and 15th for top.

The samples for measuring the endogenous soluble sugar and starch contents were obtained by cutting each culm with a sharp razor into small strips and freezing these strips immediately in liquid nitrogen. Samples for starch granule localization were fixed in FAA fixative (1.85% formaldehyde, 45% alcohol, and 0.25% acetic acid). All samples were measured for fresh weight in the bamboo garden. The moisture and endogenous soluble sugar and starch contents were expressed as percentages (%) based on the fresh weight.

2.2. Methods

2.2.1. Moisture Content Determination

Moisture content was calculated using the oven-dry test [14] with modification as the differences between the initial fresh weight and the oven-dry weight of samples. The samples were dried in an oven at 103 °C for 15 m and then dried at 85 °C to be constant for the oven-dry weight determination.

2.2.2. Soluble Sugar, Starch, and NSC Content Determination

The soluble sugar and starch contents were determined using phenol–sulfuric acid [15]. Samples (0.2 g) were powdered in a mortar and pestle with liquid nitrogen and then extracted with distilled water. The supernatants, centrifugally collected at 6000 rpm for 15 min, were mixed with 5% phenol and 98% sulfuric acid for 1 h. A spectrophotometer (752 N Hengping) was used to determine the absorbance at 485 nm. The sediments were collected and used to determine the starch contents. Each test was triplicated.
NSCs levels are defined as the sum of the starch and soluble sugar contents at each phenological stage in the culms of different age groups.

2.2.3. Starch Granule Localization

After fixation in FAA, the culm samples were dehydrated in a graded series of alcohol, then cut into sections using a sharp razor and observed with a scanning electron microscope (Hitachi TM3000, Hitachi High-Tech, Tokyo, Japan).

2.2.4. Statistical Analysis

The means derived from the experiments were statistically analyzed using multiple comparisons using the least significant difference method (LSD) to determine the level of significance at $p \leq 0.05$. These analyses were conducted using SPSS (Statistical Package for the Social Sciences) 20.0 for Windows software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Phenology of Three Types of Bamboo

Observing and recording the phenological phenomena in the three age classes of the different bamboo species in the bamboo garden showed significant difference (Table 1).

Table 1. Phenological characteristics of three bamboo types.

| Sample Timing       | Phyllostachys mannii (Monopodial Bamboo with Scattered Culms) | Neosinocalamus affinis (Sympodial Bamboo with Caespitose Culms) | Fargesia yuanjiangensis (Sympodial Bamboo with Scattered Culms) |
|---------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|
| March, 2016         | Shooting started.                                                | Branching and leafing started and few nodal buds on 1-year culms sprouted. | Branching and leafing started and a few nodal buds on 1-year culms sprouted. |
| Spring/dry season   | Most shoots completed height growth and started to extend branches and then sprouted new leaves. | Nodal buds of 1-year culms started sprouting. Branch and leaf extended simultaneously, and shoot buds started sprouting at the bases of 1- and 2-year culms. | Shoot buds at the base of 1 and 2-year culms started differentiating and formed pseudorhizomes. |
| May, 2016           | Most shoots completed height growth and started to extend branches and then sprouted new leaves. | Nodal buds of 1-year culms started sprouting. Branch and leaf extended simultaneously, and shoot buds started sprouting at the bases of 1- and 2-year culms. | Nodal buds of 1-year culms started sprouting. Branch and leaf extended simultaneously, and shoot buds started sprouting at the bases of 1- and 2-year culms. |
| Spring/dry season   | Culm sheaths dried out and eventually fell off. Branching, leafing, and shooting completed. New shoot buds started differentiating at the rhizome node underground. | Shoot buds continued forming and developing. Shoots generated out of the ground constantly. | Shoots started to sprout out of the ground, and August was the peak shooting season. |
| July, 2016          | No obvious change in bamboo culm.                                | Shooting completed, and most finished height growth.            | Shooting completed and most finished height growth. |
| Summer/rainy season | New culms with branches and leaves were dormant.                 | New culms enclosed in sheaths without branches and leaves were dormant. | New culms enclosed in sheaths without branches and leaves were dormant. |
| November, 2016      | New culms with branches and leaves were dormant.                 | New culms enclosed in sheaths without branches and leaves were dormant. | New culms enclosed in sheaths without branches and leaves were dormant. |
| Autumn/dry season   |                                                                  |                                                                 |                                                                 |
| January, 2017       |                                                                  |                                                                 |                                                                 |
| Winter/dry season   |                                                                  |                                                                 |                                                                 |

As a monopodial bamboo, Ph. mannii started shooting in March and completed shoot germination in late May, attaining their final height growth in the subsequent two months. The nodal buds of new culms started sprouting in early April, but culm sheaths did not fall off, and their internodes did not complete the elongation growth (Figure 2a). When attaining full height, most shoots have started their new branch extension (Figure 2b), and then most new leaves started spouting as the elongation of new branches completes (Figure 2c).
New leaves started sprouting after the completion of branch extension. The branching and leafing stage and the subsequent shooting stage lasted for about two months and completed by the end of July; then, the culm sheaths completely dried out and eventually fell off. During this period, new shoot buds also started differentiating at the rhizome node underground (Figure 2d). In November, new culms with branches and leaves were dormant.

![Figure 2](https://via.placeholder.com/150)

**Figure 2.** Different phenological phenomena of the three bamboo types. (a) Nodal buds of new Ph. mannii culms started sprouting in early April, while culm sheaths did not fall off and their internodes did not complete the elongation growth. Bar = 2 cm. (b) Branch extension was observed new Ph. mannii culms in late April. Bar = 8 cm. (c) New leaves appeared on the new branches of Ph. mannii in May. Bar = 8 cm. (d) New shoot buds started differentiating at the rhizome node underground in July. Bar = 4 cm. (e) Nodal buds of 1-year *N. affinis* culms started sprouting in March. Bar = 5 cm. (f) New leaves extended concurrently with branch extension in *N. affinis* in May. Bar = 8 cm. (g) Shoot buds appeared, differentiating and developing at the bases of 1- and 2-year *N. affinis* culms in May. Bar = 5 cm. (h) Nodal buds of 1-year *F. yuanjiangensis* culm started sprouting in March. Bar = 3 cm. (i) New *F. yuanjiangensis* culms extended branches and leaves simultaneously in March. Bar = 3 cm. (j) Shoot buds of *F. yuanjiangensis* culms started differentiating to form a pseudorhizome in March. Bar = 2 cm.

As a kind of sympodial bamboo with caespitose culms, the nodal buds of 1-year *N. affinis* culms (sprouted the previous July) started sprouting in March (Figure 2e), and their new leaves generally extended concurrently with branch extension (Figure 2f). This was significantly different from the monopodial bamboo. The optimum branching and leafing period generally commenced in mid-May and ceased in late June, prior to the onset of the rainy season. During this period, many new flourishing branches and leaves were observed on the 1-year culms, and shoot buds appeared, differentiated, and developed at the bases of 1- and 2-year culms (Figure 2g). *N. affinis* generated new shoots constantly from July to November. Shoots grew and completed their height growth in about three months. In November, most of the new culms finished their height growth. They were enclosed in culm sheaths and remained dormant without branches and leaves until late March. In November, most new culms finished their height growth, were enclosed in culm...
sheaths, and were dormant until late March without branches and leaves. The dormancy period of *N. affinis* lasted for nearly half a year.

For the sympodial bamboo with scattered culms, nodal buds of new culms of *F. yuanjiangensis* started sprouting in early March, which was a little bit earlier than in *N. affinis* (Figure 2h). Similarly, new culms extended their branches and leaves simultaneously (Figure 2i). Meanwhile, shoot buds at the bases of 1- and 2-year culms started differentiating and formed a pseudorhizome with a long neck (Figure 2j). In May, new culms completed branching and leafing. In July, shoots germinated from the bamboo clump, grew out of the ground, and then completed their height growth during the subsequent two months. August was the peak shooting season, a bit later than that of the *N. affinis*. *F. yuanjiangensis*, culms were dormant from November until the coming spring. New branches and leaves would not extend until the spring, which was more or less the same as that of *N. affinis*.

### 3.2. Phenological Patterns of Moisture Content in Three Types of Bamboo

Locally, the dry season lasts from November to the coming May. As one monopodial bamboo species, the average moisture content of *Ph. mannii* was low in March (32.72%) and May (32.15%), but it increased to 40.72% in July since July falls within the local rainy season. After July, it demonstrated a downward trend until the coming January (38.47%) (Figure 3). A similar trend was shown in the culms of all age groups (Figure 3, Table S1). It was also noticed that in March and May, the moisture content of *Ph. mannii* decreased with culm age, with higher moisture content in 1-year culms than in 3-year ones (Figure 3, Table S1).

![Figure 3](image-url). Dynamic changes in the moisture contents of three bamboo types at different phenological stages. Bars are mean values of the parameters from three replicates. Different letters above bars denote the statistical difference among the different phenological stages in each bamboo species at \( p < 0.05 \) according to LSD. Error bars represent 95% confidence intervals (\( n = 5 \)) (the same hereinafter).

For the sympodial bamboo with caespitose culms, it was observed that *N. affinis* had significantly higher moisture content in July compared with other months (Table S1, Figure 3). The moisture content in 1-year culms decreased from March (49.66%) to May (43.97%), but then increased to 48.37% in July. After July, it tended to decrease constantly until coming January. The moisture content in 2-year culms gradually increased from March and reached the greatest average in July (44.37%) but then demonstrated a downward trend until January (32.87%). Three-year culms showed a similar trend to that of two-year culms (Table S1, Figure 3). The moisture content of *N. affinis* also decreased with increasing culm age in March, May, and July, and 1-year culms had significantly higher moisture content than 3-year culms (Figure 3, Table S1).

For the sympodial bamboo with scattered culms, the average moisture contents of *F. yuanjiangensis* first generally decreased from March (43.14%) to May (39.92%) and then increased to the highest value of 48.88% in July, but demonstrated a downward trend later, similar to that in *N. affinis* (Figure 3, Table S1). A similar trend was observed across all ages.
of bamboo culms (Table S1). It was also noticed that young culms had considerably higher moisture content than mature ones (Figure 3, Table S1).

3.3. Phenological Patterns of NSCs in Three Types of Bamboo

NSCs are the sum of soluble sugars and starch, and the soluble sugar content in three different types of bamboo varied with seasons (Figure 4 and Table S2). As a monopodial bamboo species, *Ph. mannii* started shooting in March, attained its final height growth in May, and then finished branching and leafing in July. During this period, the average soluble sugar content in *Ph. mannii* sharply decreased from March (3.94%) to July (1.14%). In November, it demonstrated an increasing trend until January (2.46%), implying that normal photosynthesis was carried out by the new branches and leaves. Culms of different age groups showed a similar trend of having the lowest soluble sugar contents in July, with 2-year culms being particularly low (0.95%) (Figure 4 and Table S2). The top portions in each age group had greater soluble sugar contents than the middle and bottom portions (Table S2).

![Figure 4. Dynamic changes in the soluble sugar contents of three bamboo types at different phenological stages.](image)

As a sympodial bamboo with caespitose culms, nodal buds of *N. affinis* culms started sprouting in March, and the optimum branching and leafing was in May. Thus, it was observed that the average soluble sugar content of *N. affinis* culms in March (2.81%) decreased to 1.65% in May, which implied that the node bud germination and branch and leaf extension caused a significant decrease in soluble sugar content in the culms. The lowest average soluble sugar content was observed in July (1.09%), since *N. affinis* generated new shoots in July, consuming lots of sugar. After July, it increased constantly until the coming January (2.39%) (Figure 4 and Table S2). A same variation pattern was found in the culms of all age groups. Additionally, in March, May and July, the soluble sugar content of *N. affinis* increased with culm age and reached the greatest value in the 3-year culms, while in November and January, lower soluble sugar content was observed in 3-year culms. Additionally, the soluble sugar content showed an increasing trend with culm height: The top and middle portions had higher soluble sugar contents than the bottom ones (Table S2).

The variations in soluble sugar content in *F. yuanjiangensis* were roughly similar to those in of *N. affinis*, which decreased from March to July and then increased in January (Figure 4 and Table S2). The 1-year culms also showed a decreasing trend from March (0.65%) to July (0.54%) but then continuously increased to 1.28% in January. The time when the soluble sugar content began decreasing was in accordance with the branching and leafing of *F. yuanjiangensis*, which implied that this drop was mainly caused by the consumption of sugars for the new branch and leaf extension. The 2-year culms in March had the greatest soluble sugar content (1.62%), but it tended to decrease and reached the lowest average in November (0.86%) before increasing again to 1.39% in January. This same trend was found for 3-year culms (Figure 4 and Table S2). The increasing soluble
sugar content in *F. yuanjiangensis* in January implied that photosynthesis remained active in winter. Additionally, the soluble sugar content of *F. yuanjiangensis* did not increase significantly with culm age but did increase significantly with culm height. The top portion of the culms had higher soluble sugar contents than the middle and bottom ones in each age group in different months (Table S2).

The starch content also varied with seasons in three different types of bamboo species (Figure 5 and Table S3). The highest starch content average in *Ph. mannii* was observed in March (3.99%). *Ph. mannii* started shooting in March and then extended branches and leaves. As a result, the starch content average demonstrated a significant decreasing trend after March, reaching its lowest value in July (1.09%). This was particularly observed for the 1- and 2-year culms, which showed a sharp drop. However, the starch content in the 3-year culms slightly increased from March (3.13%) and reached its highest average of 4.24% in May. This indicated that stored carbohydrate consumption for shooting, as well as branching and leafing, mainly occurred in the 1- and 2-year culms, while the contribution of 3-year-culms to the bamboo cluster was relatively minor. In July, the branching and leafing of *Ph. mannii* completed, and at that time, the starch content in all age groups of culms decreased to the lowest. However, after July, it continuously increased, reaching 2.21% in January; in particular, content in the 1-year culms significantly increased up to 2.91% (Figure 5 and Table S3). The starch content in the 3-year culms in January was significantly lower than that of 1 and 2-year culms, indicating that their contribution to the bamboo cluster declined. Scanning electron microscope (SEM) results indicated larger quantities of starch granules in March, while very few granules were detected in July (Figure 6). Additionally, the starch content increased with culm height, and higher starch content was observed at the top portions in each age group at different months (Table S3), which was also supported by observations under SEM (Figure 6).

![Figure 5. Dynamic changes in the starch contents of three bamboo types at different phenological stages.](image)

As the nodal buds of *N. affinis* started sprouting and their branches and leaves extended simultaneously in March, the average starch contents also demonstrated a decreasing trend from March (2.60%) to May (1.53%) (Figure 5 and Table S3). *N. affinis* started shooting in July, and thus, the average starch contents showed a continual drop to 1.30%. It could be also noted that the starch content in the 1- and 2-year culms showed a sharp decrease, particularly in the 2-year culms, whereas the starch content in the 3-year culms did not decline but increased slightly. This indicated that the starch reserves in the 1- and 2-year culms was the main source for shooting (Figure 5 and Table S3). This was consistent with the phenomenon we observed in the field that new shoots mainly generated from 1- and 2-year bamboo, particularly in the 2-year culms, which consumed the most starch. Under SEM, fewer starch granules were detected in the parenchyma cells of the culms in July, especially for the younger culms, which was consistent with the experimental data (Table S3 and Figure 7). After July, the average starch contents of *N. affinis* increased continuously and reached 2.97% in January. In November and January, the starch content
demonstrated a decreasing trend with culm age, and 3-year culms maintained the lowest average in November and January compared with the 1 and 2-year culms (Figure 5, Table S3). In each age group, the starch content increased with culm height, and the middle and top portions of the culms had higher starch contents than the bottom ones (Table S3). Under SEM, starch granules were easier to observe in the parenchyma cells of the culms in January and March, while in May, July, and November, few starch granules could be detected in the 1- and 2-year culms (Figure 7). In addition, more starch granules were also observed in the middle and top portions in all age groups of the culms (Figure 7). The SEM results corroborated well with the experimental data.

Figure 6. Starch granule distributions in *Phyllostachys mannii* culms at different phenological stages. 1Y denotes the 1-year culm; 2Y denotes the 2-year culm; 3Y denotes ≥3-year culm; T denotes the top portion of the culm. M denotes the middle portion of the culm; B denotes the bottom portion of the culm. Bar = 20 μm (the same hereinafter).
The starch content in *F. yuanjiangensis* exhibited roughly the same fluctuating pattern as that for *N. affinis* (Figure 5, Table S3). The average starch content increased slightly from March (1.22%) to May (1.33%). During this period, new culms of *F. yuanjiangensis* extended their branches and leaves simultaneously, and shoot buds at the base of 1- and 2-year culms started differentiated, which consumed a great deal of carbohydrates. Thus, a decreasing trend in starch was observed in the 1- and 2-year culms. However, a slightly increasing trend from March (1.16%) to May (1.71%) was found in the 3-year culms. In July, the average starch content decreased slightly from May (1.33%) to July (1.05%) due to shoot sprouting. Starch content in the 2- and 3-year culms decreased, particularly for the 3-year culms, which showed a drop from 1.71% (May) to 0.96% (July). This implied that the 2- and 3-year culms provided ample starch reserves for the bamboo clumps during the period of shooting. The completion of branching and leafing in the 1-year culms in July made photosynthesis easier, and thus, the starch content in the 1-year culms increased to 0.93%. In November, the new culms completed their height growth, which resulted in a significant decrease in starch content for the 2-year culms. This indicated that 2-year culms provided much more energy to the clump for the height growth of new culms. In January, the starch
content in 3-year culms slightly increased. It was also observed that the starch content in March, May, and July increased with culm age, whereas in November and January, it demonstrated a slightly decreasing trend with culm age. Additionally, the starch content increased with culm height, and the starch content was higher in the top than in the middle and bottom portions (Table S3), which was consistent with the SEM observation (Figure 8).

The total NSC reserves in the three bamboo species also varied with seasons. In March, more NSCs were observed in all three bamboo types (Figure 9). In May, NSCs decreased slightly *Ph. mannii*, and a decreasing trend was also found for the sympodial bamboo (*N. affinis* and *F. yuanjiangensis*). In July, the NSCs in the three types of bamboo species decreased and reached the lowest average. After July, NSC content followed a continuous increasing trend until January (Figure 9).

**Figure 8.** Starch granule distributions in *Fargesia yuanjiangensis* culms at different phenological stages.

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The total NSC reserves in the three bamboo species also varied with seasons. In March, more NSCs were observed in all three bamboo types (Figure 9). In May, NSCs decreased slightly *Ph. mannii*, and a decreasing trend was also found for the sympodial bamboo (*N. affinis* and *F. yuanjiangensis*). In July, the NSCs in the three types of bamboo species decreased and reached the lowest average. After July, NSC content followed a continuous increasing trend until January (Figure 9).
Figure 9. Non-structural carbohydrates (NSCs) in the three bamboo types at different phenological stages.

4. Discussion

4.1. Phenology of Different Types of Bamboo

Vegetative phenology reflects the adaptation of graminoid processes to the giant, arborescent form and the need to complete the elongation of growth axes in a single growth period [16]. In the present study, three different bamboo species had different phenological presentations. *N. affinis* and *F. yunnanensis* are sympodial bamboo, generating shoots in July, while *Ph. mannii* is a monopodial bamboo that started shooting in March. The sympodial bamboo species (*N. affinis* and *F. yunnanensis*) generally extended their branches and leaves simultaneously on the 1-year culms from May to July. New shoots of the monopodial bamboo species (*Ph. Mannii*) sprouted and started their buds and branch extension even before height growth completion. They generally started branching in May first and sprouted new leaves later. The branching and leafing stage of the monopodial bamboo did not overlap with that of the sympodial bamboo. This finding is the first of its kind and has never been noticed and reported before. In the field, a similar phenomenon could also be observed in the new culms of *P. edulis* and other monopodial bamboo. This was a critical difference between monopodial and sympodial bamboo.

For the monopodial bamboo (*Ph. mannii*), shoot buds differentiated in autumn, and shooting started in the coming March and lasted about eight months. This was similar to the phenology of *Ph. edulis* [17]. This period was sufficient for stored carbohydrates to be translocated from shoot buds to the sprouting shoots. For the sympodial bamboo (*N. affinis* and *F. yunnanensis*), new branch and leaf extension occurred in May, while their shoot buds differentiated at the bases of the 1- and 2-year culms. It took only two to three months from branching and full leaf extension to shoot germination, which caused the short interval for the carbohydrate storage for shooting. This was in accordance with the report from Wang et al. on *F. yunnanensis* [11]. The short time from branching to shoot germination might be the critical reason for the simultaneous branching and leafing in sympodial bamboo since it might be beneficial to the photosynthetic product accumulation for the subsequent shooting stage. In addition, the branching and leafing stage in sympodial bamboo (*N. affinis* and *F. yunnanensis*) occurred at the end of the dry season and preceded the onset of the rainy season, which was significantly different from the pattern in *Bambusa arnhemica* [16]. The adaptive advantages of leaf flush prior to the onset of the wet season are unclear but may involve the use of optimal conditions for photosynthesis and the avoidance of herbivory [11,18].

In winter, photosynthesis was absent or low, and NSC reserves were insufficient for the branching and leafing of sympodial bamboo. Most new culms finished their height growth enclosed in culm sheaths but without branches and leaves; they were dormant...
from November until late March, nearly half a year. Sympodial bamboo had a long phase of storing photoassimilates for the new branching and leafing in the coming year. The unique biological and phenological characteristics of monopodial and sympodial bamboo should be taken into consideration when conducting relevant cultivation and management research in the future.

4.2. Moisture Content Variation in Different Types of Bamboo

The moisture content of bamboo was affected by different phenological stages since the temperature and rainfall in different phenological periods were inconsistent, which affected the transpiration and metabolic rate of bamboo. Knowledge of the water status in plant tissue may give an indication of the water requirements and the timing of irrigation [11,19]. Overall, similar seasonal patterns in moisture content were observed in three different bamboo species. It increased from March to July and then demonstrated a decreasing trend until the coming January, which is similar to the result from Wang et al. on *F. yunnanensis* [11]. On the one hand, this was relevant to the local climate and precipitation since March and May were the local dry seasons, resulting in relatively low moisture content in the bamboo culms. The greatest moisture content was observed in July due to the water supply from the adequate rainfall in local rainy season.

On the other hand, the significant decrease in moisture content in the dry season (March to May) in the 1-year-old culms of *N. affinis* and *F. yuanjiangensis* was in accordance with their new branch and leaf extension period, which implied that branching and leafing of new culms consumed an abundance of water. Hence, it was essential to supply sufficient water for the sympodial bamboo clumps during this period. This was also in accordance with the observation in the field, where sympodial bamboo was much more susceptible to drought stress than bamboo in other seasons. March to May was the shooting stage for the monopodial bamboo (*Ph. mannii*), but a lower moisture content in *Ph. mannii* culms was observed. This implied that adequate irrigation needs to be supplied between March and May to ensure active shooting and growth. In brief, both the sympodial and monopodial bamboo required adequate watering during the dry season (March to May), particularly in March (lowest moisture average). Irrigation was required for new culm branching and leafing for sympodial bamboo, whereas ample water was needed for shooting in the monopodial bamboo. Traditionally, monopodial bamboo species are mainly distributed in Jiangsu, Zhejiang, and Hunan Province of China. The shooting stage from March to May was the local plum rain period, while it was the dry season in Yunnan in southwest China. Therefore, monopodial bamboo in the southwest regions of China required adequate irrigation to meet the moisture needs from March to May, which was the particular point that needs to be considered. In July, moisture content in the 2- and 3-year culms was lower than that in the 1-year culms for the sympodial bamboo. Similar trend was also observed in *F. yunnanensis* [11]. As the mature culms were the main source for shooting in the sympodial bamboo clump, a large amount of water was consumed during the shooting stage [11]. After July, a decreasing trend in moisture was observed in the three different bamboo types until the coming January. This reflected that the bamboo’s moisture content decreased for dormancy and to avoid freeze injury. Furthermore, it was in the local dry season when moisture content decreased.

In addition, the moisture content in the three bamboo types decreased with culm age, which coincided with the observation from Wang et al. on *F. yunnanensis* [11]. Along with the aging of the culms, their cell walls thickened with smaller cell cavities [20], and the water storage ability was weakened [21]; therefore, the 3-year culms generally had lower moisture content than that of the 1- and 2-year culms.

4.3. NSCs Variations in Different Types of Bamboo

Soluble sugar and starch are the principal forms of carbohydrate stored in the vegetative tissues of plants [7]. Soluble sugars, including monosaccharides and oligosaccharides, are the main photosynthetic products and the substrate for metabolism [22,23] that can
be reciprocally transformed and reused by plants. Starch accumulation in stem tissues occurs when carbohydrate supply is in excess of the demand [7]. Soluble sugar was the substrate for starch synthesis and there was a close relationship between the soluble sugar content and starch synthesis in plants. The carbohydrate patterns of the three bamboo types varied, consistent with the observed phenological events and the physiological processes of bamboo.

A previous study has shown that free sugar contents were generally lower in autumn and winter than in spring and summer in *P. pubescens* [2], whereas in *F. yunnanensis*, the soluble sugar content was much lower in spring [11]. In this study, although a decline was observed in both soluble sugar and starch content from March to July in the different bamboo species, the reasons were different. For the monopodial bamboo, most photoassimilates consumed by shooting and height growth reduced the soluble sugar content from March to May, followed by continuous dropping from the branching and leafing in July. Starch accumulation was limited as the carbohydrate supply was short. Therefore, the starch content also showed a continual drop from March and remained at the lowest level in July. Very few starch granules could be detected under SEM in July.

By contrast, for the sympodial bamboo, branching and leafing decreased the soluble sugar from March to May, followed by decreases from the shoot buds sprouting underground and shoots germinating above ground in July. Almost all the starch was depleted and reutilized, so the starch content also declined. Therefore, fertilizer application to both the monopodial and sympodial bamboo plantations could be scheduled at the start of May and at the end of July. In winter, both the soluble sugar content and starch content continuously increased from November to the coming January for the sympodial bamboo, which might be related to the adaptation of bamboo clumps to the cold season. Stored carbohydrates are critically important for the maintenance of bamboo during the cold season and for vernal growth. High levels of starch stored following a stress event are indicative of trees with high vitality, while low starch levels after a similar stress event, reflect low tree vitality [6]. The monopodial bamboo, by November, had completed its height growth and crown construction. Its flourish branches and leaves started normal photosynthesis to synthesize a certain amount of sugar since it was warm; the local mean temperature in November was 12 °C, which caused the slight increase in the soluble sugar. Starch accumulated when sugar supply was in excess of the demand, and thus, the increasing trend that was observed for starch concentration and starch granules could be easily detected under SEM during this period for monopodial bamboo. This was in accordance with the report from Okahisa et al. that the starch content in *P. pubescens* culms was higher in winter [2].

In this study, the soluble sugar increased with culm age from March to May for the sympodial bamboo. Shoot buds usually sprouted at the bases of the 1- and 2-year culms, which consumed considerable sugar. Moreover, the 2- and 3-year culms of the sympodial bamboo had flourish branches and leaves producing abundant photoassimilates by photosynthesis, while the 1-year culms had few branches and leaves, and most of their carbohydrates came from their mother bamboo. Therefore, the 2- and 3-year culms had higher stored soluble sugar contents. For the monopodial bamboo, from May to July, more carbohydrates were consumed by shoot height growth, and their crown construction caused lower soluble sugar and starch content in the 1- and 2-year culms. In January, as the 3-year culms became 4-year, weak sugar storage capacity of the old culms decreased the sugar content.

It was observed that the soluble sugar and starch were higher in the top and middle portions than the bottom at all age classes for both the sympodial and monopodial bamboo. This is because the top culm portions were close to leaves, and more carbohydrates were produced. This trend was similar to that in *F. yunnanensis* [7,24], while in *P. pubescens*, no special trend was observed [2].

Generally, the total NSCs showed a similar trend to that of starch in the different bamboo types. In March, the total NSCs in the three bamboo types remained at a relatively
high level, which was related to higher soluble sugar and starch concentrations. However, after March, the NSCs in all three bamboo types decreased to the lowest average in July, then increasing continuously until January. Richardson et al.’s standard conceptual model for temperate forest trees showed that NSCs are replenished over the course of the summer growing season (e.g., increasing from June to August), when photosynthesis exceeds metabolic demands for C and drawn down over the course of the dormant season (e.g., decreasing from November to March), when there is no photosynthesis and reserves are used to provide the energy required for maintaining respiration [25].

The decrease in total NSCs in sympodial bamboo in July might be mainly attributed to the depletion by shooting, while the decrease in the NSCs in monopodial bamboo was due to the branching and leafing. Therefore, fertilizer application to both the monopodial and sympodial bamboo plantations could be scheduled at the start of May and at the end of July. The increase in the total NSCs of the three bamboo we observed from November to January may reflect the remobilization of sugars from storage compartments in other organs to satisfy the demand associated with the vernal growth in winter. Moreover, the local mean temperature in November was 12 °C, and NSCs in the bamboo might still accumulate through photosynthesis.

5. Implications for Managing Bamboo Plantations

The local dry seasons (March and May) resulted in relatively low moisture content in both monopodial bamboo (Ph. mannii) and sympodial bamboo (N. affinis and F. yuanjiangensis). This corresponded with the shooting period for monopodial bamboo (Ph. mannii) and the branching and leafing stage for sympodial bamboo (N. affinis and F. yuanjiangensis). Insufficient water supply caused lower yield of bamboo shoots and smaller leaves during the leafing stage [26]. Therefore, ample irrigation was required for both the sympodial and monopodial bamboo plantations from March to May.

The soluble sugar, starch, and total NSCs of both the monopodial bamboo (Ph. mannii) and sympodial bamboo (N. affinis and F. yuanjiangensis) decreased from March to July. During this period, large amounts of photoassimilates were required for shooting, height growth, and then branching and leafing for the monopodial bamboo. Ample photoassimilates were also required for branching, leafing, and shoot bud differentiation, as well as for subsequent shoot germination in the sympodial bamboo. Thus, fertilizer application to both the monopodial and sympodial bamboo plantations should be scheduled in early May and late July.

In January, the soluble sugar content in the 3-year culms of monopodial bamboo (Ph. mannii) was moderate, and their starch content was significantly lower than that in the 1- and 2-year culms, indicating that their contribution to the bamboo cluster declined; culm harvest at this time suffered less damage from insects and microorganisms. Thus, January was the optimum harvest time for the 3-year monopodial bamboo culms. Though the starch content of 3-year culms of the sympodial bamboo (N. affinis and F. yuanjiangensis) showed relatively lower levels in July, their moisture content was high. Since it was the local rainy season and the time for the growth of the new culms, it was not a suitable time for harvesting bamboo culms. At the end of the rainy season in November, the moisture content in the 3-year culms of the sympodial bamboo (N. affinis and F. yuanjiangensis) decreased, while soluble sugar and starch content were moderate. Culms harvested at this time were most resistant to mildew growth and had minimal shrinkage rates; which favor high-quality processing and utilization [26]. Moreover, low soluble sugar and starch content implied less damage from insects and microorganisms [2,3]. Therefore, the best time to harvest sympodial bamboo was November.

6. Conclusions

Monopodial bamboo started shooting in March, while sympodial bamboo generated shoots in July. New leaves of monopodial bamboo started sprouting after the completion of branching, while sympodial bamboo generally extended new leaves and branches
Simultaneously. The shoot buds of monopodial bamboo differentiated in autumn, and shoot germination started in the coming March and lasted about eight months. This period was sufficient for the stored NSCs to be translocated from shoot buds to the shoots for shooting, while the shoot buds of sympodial bamboo differentiated in May and shooting started in July. Thus, these bamboo types had only a short interval of two to three months for NSC accumulation for storage for shooting.

Similar seasonal patterns in moisture content were observed in the three different types of bamboo, increasing from March to July and then demonstrating a decreasing trend until the coming January. From March to May, particularly in March, irrigation was required for the shooting of monopodial bamboo and for the new culm branching and leaching of sympodial bamboo. Total NSCs in the three bamboo species remained relatively high in March, decreased gradually to the lowest average in July, and after July, increased continuously until the coming January. Average soluble sugar and starch contents showed the same trend as the total NSCs in different types of bamboo. During the period from March to July, large amounts of photoassimilates were required for shooting and height growth as well as branching and leaching in monopodial bamboo. Ample photoassimilates were also required for branching, leaching, and shoot bud differentiation, as well as for subsequent shoot germination in sympodial bamboo. Thus, fertilizer application to both the monopodial and sympodial bamboo plantations should be scheduled in early May and late July. Given the moisture, soluble sugar, starch, and total NSC content, January was the best season for harvesting monopodial bamboo for high production and future growth, whereas for sympodial bamboo, November was the best season.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13081218/s1, Table S1: Moisture content (%) of three bamboo types at different phenological stages. Table S2: Soluble sugar content (%) of three bamboo types in different phenological stages. Table S3: Starch content (%) of three bamboo types in different phenological stages.

Author Contributions: H.Z. co-designed the experiment, ran the data analysis and wrote the paper; W.H., L.Y. and J.L. performed the experiments; M.L. revised the manuscript; C.W. and S.W. designed the experiment, supervised the research work and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was jointly funded by National Natural Science Foundation of China (No. 32160415); Natural Science Foundation of Yunnan Province (No. 202001AT070108; 202001AT070133); Yunnan Provincial Joint Special Project for Basic Research in Agriculture (202101BD070001-114).

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

References
1. Yang, Y.; Wang, K.; Wang, J. Bamboo Flora of Yunnan; Yunnan Press: Kunming, China, 2018; pp. 1–11.
2. Okahisa, Y.; Yoshimura, T.; Iinuma, Y. Seasonal and height-dependent fluctuation of starch and free glucose contents in moso bamboo (Phyllostachys pubescens) and its relation to attack by termites and decay fungi. J. Wood Sci. 2006, 52, 445–451. [CrossRef]
3. Fan, Y. Seasonal Variation of Starch Content in Dendrocalamus giganteus and Phyllostachys edulis and Its Relationship to Insect Damage. Master’s Thesis, Southwest Forestry University, Kunming, China, 2010; pp. 21–36.
4. Troya Mera, F.A.; Xu, C. Plantation management and resource economics of bamboo in China. Cienc. Tecnol. 2014, 7, 1–12. [CrossRef]
5. Kramer, P.J.; Kozlowski, T.T. Physiology of Woody Plants; Academic Press: New York, NY, USA, 1979.
6. Wong, B.L.; Baggett, K.L.; Rye, A.H. Seasonal patterns of reserve and soluble carbohydrates in mature sugar maple (Acer saccharum). Can. J. Bot. 2003, 81, 780–788. [CrossRef]
7. Scofield, G.N.; Ruuska, S.A.; Aoki, N.; Lewis, D.C.; Tabe, L.M.; Jenkins, C.L.D. Starch storage in the stems of wheat plants: Localization and temporal changes. Ann. Bot. 2009, 103, 859–868. [CrossRef] [PubMed]
8. Galvez, D.A.; Landhäusser, S.M.; Tyree, M.T. Low root reserve accumulation during drought may lead to winter mortality in poplar seedlings. New Phytol. 2013, 198, 139–148. [CrossRef]
9. Hartmann, H.; Trumbore, S. Understanding the roles of nonstructural carbohydrates in forest trees-from what we can measure to what we want to know. New Phytol. 2016, 211, 386–403. [CrossRef]
10. Kozlowski, T.T. Carbohydrate sources and sinks in woody plants. Bot. Rev. 1992, 58, 107–222. [CrossRef]
11. Wang, S.; Ding, Y.; Lin, S.; Ji, X.; Zhan, H. Seasonal changes of endogenous soluble sugar and starch in different developmental stages of *Fargesia yunnanensis*. *J. Wood Sci.* 2016, 62, 1–11. [CrossRef]

12. Hirano, Y.; Shida, S.; Arima, T. Effect of cutting season on biodeterioration in Madake (*Phyllostachys bambusoides*). *Mokuzai Gakkaishi* 2003, 49, 437–445. (In Japanese)

13. National Meteorological Science Data Center of China. Available online: http://data.cma.cn/analysis/yearbooks.html (accessed on 23 January 2019).

14. Reeb, J.; Milota, M. Moisture content by the oven-dry method for industry testing. In Proceedings of the 50th Annual Dubois Meeting of the Western Dry Kiln Association, Portland, OR, USA, May 1999; pp. 66–74. Available online: https://ir.library.oregonstate.edu/concern/conference_proceedings_or_journals/tq977v782 (accessed on 23 January 2019).

15. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 1956, 28, 350–356. [CrossRef]

16. Franklin, D.C. Vegetative phenology and growth of a facultatively deciduous bamboo in a monsoonal climate. *Biotropica* 2005, 37, 343–350. [CrossRef]

17. Zhou, F. *Silviculture of Bamboo*; China Forestry Publishing House: Beijing, China, 1998; pp. 93–101.

18. Bhat, D.M.; Murail, K.S. Phenology of understory species of tropical moist forest of Western Ghats region of Uttara Kannada district in South India. *Curr. Sci.* 2001, 81, 799–805.

19. Yaron, B.; Danfors, E.; Vaadia, Y. *Arid Zone Irrigation*; Springer: New York, NY, USA, 1973; p. 200.

20. Liese, W. *The Anatomy of Bamboo Culms*; INBAR Technical Report, No. 18; International Network for Bamboo and Rattan: Beijing, China, 1998; pp. 90–93.

21. Zheng, Y.; Hong, W. *Silviculture of Moso Plantation*; Xiameng University Press: Xiameng, China, 1999; pp. 34–58.

22. Luo, X.; Huang, Q. Relationships between Leaf and Stem Soluble Sugar Content and Tuberous Root Starch Accumulation in Cassava. *J. Agric. Sci.* 2011, 3, 64–72. [CrossRef]

23. Bodelón, O.G.; Blanch, M.; Sanchez-Ballesta, M.T.; Escirbano, M.I.; Merodio, C. The effects of high CO₂ levels on anthocyanin composition, antioxidant activity and soluble sugar content of strawberries stored at low non-freezing temperature. *Food Chem.* 2010, 122, 673–678. [CrossRef] [PubMed]

24. Aoki, T. *Studies on Major Bamboo Specie in Japan*; Ashishobo: Fukuoka, Japan, 1987; pp. 267–270. (In Japanese)

25. Richardson, A.D.; Carbone, M.S.; Keenan, T.F.; Czimczik, C.I.; Hollinger, D.Y.; Murakami, P.; Schaberg, P.G.; Xu, C. Seasonal dynamics and age of stemwood nonstructural carbohydrates in temperate forest trees. *New Phytol.* 2013, 197, 850–861. [CrossRef] [PubMed]

26. Chen, B.; Yang, Y.; Zhang, G.; Sun, M.; Shi, M. A Study on Cultivation and Integrated Utilization of Large-size Cluster Bamboo. *J. West China For. Sci.* 2007, 36, 1–9, (In Chinese with English Abstract).