Non-structural carbohydrate levels of three co-occurring understory plants and their responses to forest thinning by gap creation in a dense pine plantation

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Abstract We investigated non-structural carbohydrates (NSC) levels and components (starch, glucose, fructose and sucrose) in the leaves of three typical co-occurring forest-floor plants, moss Eurhynchium savatieri (ES), fern Parathelypteris nipponica (PN) and forb Aruncus sylvester (AS) in a 30-year-old Chinese pine (Pinus tabulaeformis) plantation forest on the eastern Tibetan Plateau. We also explored their responses to three gap creation treatments (control and two gap creations of 80 and 110 m2) based on NSC levels. PN had the highest leaf NSC level of the three plants, with AS second and ES lowest. Starch was the predominant component of NSC and the contents of glucose were higher than those of fructose or sucrose for all three species. The NSC level of ES in intermediate gaps was significantly higher than at control sites. PN also had higher NSC levels in both small and intermediate gaps than in control sites. But the differences between treatments were not obvious for AS. Our results suggest that ES and PN benefit from gap formation while the two species have different NSC response sensitivities to gap size, but the leaf NSC level of AS is less sensitive to the disturbance.

Keywords Forest-floor plant · Gap thinning · Light radiation · Moss · Non-structural carbohydrates

Introduction

Non-structural carbohydrate (NSC) level is an important indicator to elaborate the carbon source and sink capacities of plants, and to a certain extent, can reflect their eco-physiological and growth status (Körner 2003; Wurth et al. 2005). Many researchers focused on trees and shrubs in forest in recent decades (Körner 2003; Wurth et al. 2005; Palacio et al. 2007; Li et al. 2008; Palacio et al. 2008). Nevertheless, we know little about the NSC levels of forest-floor herbaceous plants. Most previous studies only concerned species from single groups and comparative studies of plants with different growth forms have been few. Therefore, the first objective of the current research was to quantify and compare the NSC levels of understory plants in different growth form groups.

Forest thinning can improve environmental conditions of habitats such as the diurnal patterns, air temperature, vapor pressure deficit, and illumination, and can have great impacts on understory plants (Rambo and North 2009). However, most previous studies only focused on climatic fluctuation and vegetation renewal in the gap sites (Kariuki et al. 2006; Milakovsky et al. 2011). Thus we have limited
insight into the effect of gap formation on the physiological status of forest-floor herbaceous plants such as mosses, ferns and forbs. Therefore, our second objective was to study the responses to gap thinning of forest-floor herbaceous plants according to their NSC levels.

We addressed the following two questions: What are the differences in NSC levels and NSC components [Soluble sugars (SS) and starch] between different growth forms (moss, fern and forb); and what are the different responses of these plants to forest thinning according to their levels of NSC and its components. We hypothesized that in comparison to ferns and forbs, the NSC level of the shade-tolerant mosses would be lower because its photosynthetic capacity and NSC accumulation ability are relatively weak (Glime 2007; Waite and Sack 2010). Furthermore, since light is most likely to be the limiting factor in dense plantations, we presumed that NSC levels of these plants should be promoted by slight thinning because the resulting higher light intensity and longer irradiation duration could increase the NSC concentration in plant leaves (Montgomery and Chazdon 2002; Kuptz et al. 2010; Zhang et al. 2013). We also hypothesized that NSC levels of mosses would be lower in intermediate-sized gaps compared than in small gaps because the poikilohydric mosses might be more sensitive to the increased evaporation rate and light intensity caused by larger gap size (Marschall and Proctor 2004; Glime 2007).

Materials and methods

Focal species

We planned to select at least 12 understory plants (including mosses, ferns, forbs and sedges) growing in the experimental forest. However, due to the dense tree canopy cover, only a few understory species grew. Finally only three co-occurring forest-floor plants, *Eurhynchium savatieri* (ES), *Parathelypteris nipponica* (PN) and *Aruncus sylvester* (AS) were chosen. ES is a slender moss forming loose or dense mats on the soil, tree bases and rocks in moist habitats. PN is a fern with long creeping rhizomes, 40–60 cm in height and with leaves about 30–40 cm in length and 7–10 cm in width. This plant mainly grows under mature forest in hilly regions at elevations between 400 and 2500 m. And AS is a perennial forb, about 50–70 cm in height with leaves 5–13 cm in length and 2–8 cm in width. AS usually lives in open habitats under mixed forests on montane slopes and in valleys. Our nomenclature follows *Flore of China* and *Flora Bryophytorum Sinicorum*. Specimens were deposited in the herbarium at the Chengdu Institute of Biology, CAS.

Experimental layout and sample collection

We undertook field work at the thinning experimental sites of a 30-years-old Chinese pine (*Pinus tabulaeformis*) plantation forest near the Maoxian Mountain Ecosystem Research Station of Chinese Academy of Sciences at Fengyi Township of Maoxian County, Sichuan, China (103°54'E, 31°42'N, 1826 m a.s.l.). The region has a typical temperate climate with annual sunshine time of 1373.8 h, annual mean temperature of 9.3 °C, annual precipitation of 825.2 mm and annual evaporation of 968.7 mm. The thinning experiment had three treatments (control, small gap and intermediate gap with gap areas of 0, 80 and 110 m², respectively) and three replicates (totally six gaps and three controls) on similar blocks (Jiang et al. 2011).

We measured fundamental environmental parameters at the sites before sample collection. Photosynthetically active radiation (PAR) was measured by Skye Instruments type SKR1800 dual channel radiometers (Skye Instruments, Llandrindod, Powys, UK) at several spots in the center of each site. Temperature and moisture at soil surface were also measured using a temperature/humidity data logger (DS1923 iButton, Maxim Integrated Products). All parameters were synchronously obtained. The data indicated that PAR, temperature and moisture at soil surface were significantly higher in intermediate and small gaps than in the controls (Table 1). All monitored parameters were similar at the two gap treatments.

We collected samples from three sampling points in the central zone of each site (about 2-g fresh weight for each sample) on 21 September 2010, a sunny day. We collected only mature leaves at the top layer of PN and AS, and ES tissues without cover from other plants or litter. The samples were stored in a refrigerator (about 4 °C) and brought to the station laboratory within 3 h. Only green tissues of ES, the 10–20th pinna from the leaf apexes of PN and the first pair of leaves behind the spiry leaf of AS were selected as the final samples. All samples were swiftly washed with deionized water and put into a pre-heated oven at 115 °C for 10 min to stop physiological activities. Samples were then oven-dried at 65 °C for 48 h. Dried samples were ground and stored at −4 °C.

Chemical analysis

The method of Baninasab and Rahemi (2006) was adopted for sample pre-treatment. HPLC with evaporative light-scattering detector was used to measure the concentrations of sugars: glucose, fructose and sucrose (Sun et al. 2004). Starch content was determined using an anthrone colorimetric protocol following Li et al. (2008). All results were recorded on a dry weight basis (mg g⁻¹).
Statistical analysis

The SS content of each sample was calculated as the sum of glucose, fructose and sucrose. And NSCs were counted by summing values for starch and SS. Data for all components (NSC, starch, SS, glucose, fructose and sucrose) were checked for normality and homoscedasticity prior to statistical analyses. Sucrose concentration values were transformed by ln (y+1) to meet the requirement of normal distribution. Data were analyzed by two-way ANOVA with species and thinning treatment as the main factors. LSD multiple comparison tests were used to compare differences between means and the Games-Howell post hoc test was used when the data did not meet the ANOVA assumption of homogeneity of variance. All statistical analyses were performed using SPSS (version 16.0) and Origin (version 8.6). All values were considered significant when \( p \leq 0.05 \).

Results

NSC level and proportion

Levels of NSC and its components varied significantly among the three understory plants (Table 2). PN had the highest NSC level, with AS second and ES lowest. PN and AS had similar starch levels and both had significantly higher starch concentrations than ES. ES and AS had similar SS levels, both significantly lower than that of PN.
Three species also had different levels of glucose and fructose, ranking as PN ≈ AS > ES and PN > AS ≈ ES, respectively. PN had higher sucrose levels than ES and sucrose concentrations in the leaves of AS were too low to be detected (Table 2). For all of these understory species, starch was the predominant component of NSC and the level of glucose was higher than the other two sugars (Fig. 1). Starch contents of ES and AS were higher than SS while the pattern was reversed for PN.

Response of NSC to thinning

The NSC level of ES was higher at intermediate gaps than at control sites but there were no significant differences between the NSC levels at small gaps and at the other two treatments. PN had higher NSC level at the thinning gaps than at the control sites. NSC levels for AS were similar at all treatments (Fig. 2a). There were no differences in starch levels among treatments for all three plants (Fig. 2b; Table 3). Much higher contents of glucose, fructose,
The varied NSC levels of the co-occurring understory plants reflect their different carbon supply status (Wurth et al. 2005). Here we firstly focus on moss and compare it with other herbaceous species under the weak light environment. Our results supported the initial hypothesis that the moss ES had the lowest levels of NSC (Table 2). Furthermore, the NSC levels of ES in intermediate gaps at both gap sizes (Fig. 2b–f) were all increased with increasing gap sizes (Fig. 2a–e). These results demonstrate that the metabolic activity of PN is stronger in gaps than in understory because the increase of NSC levels is due to SS, not starch (Palacio et al. 2007). AS did not differ in any of the NSC components among the three treatments, indicating that this species was less sensitive to the impact of moderate thinning. Moreover, for the three species, the similar NSC levels between the intermediate gaps and the small gaps (Fig. 2a) could be attributed to light saturation due to increasing gap size (Zhu et al. 2010) or perhaps due to the sink regulation of the photosynthetic process (Paul and Foyer 2001; Kasai 2008). Taken together, our results only support part of the initial prediction that leaf NSC levels of ES and PN would be promoted by slight thinning. However, there was no indication that the intermediate gap restricted the accumulation of NSC for the moss. Conversely, the gap creation at intermediate size may be a better scheme for ES and PN than creation of smaller gaps.

In conclusion, the moss ES had the lowest levels of NSC and its components while the fern PN had highest levels among the three species under the pine plantation forest (Table 2). Furthermore, the NSC levels of ES in intermediate gaps and PN at both gap sites were significantly higher than at control sites. There were no significant differences among treatments for the forb AS. Our results suggest that ES and PN can benefit from the lighter and warmer habitats created by gap formation in the understory of the pine plantation. In contrast, AS proved less sensitive to gap formation.
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