Plant Physiology and Biochemistry 124 (2018) 146–154

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Leaf non-structural carbohydrate allocation and C:N:P stoichiometry in response to light acclimation in seedlings of two subtropical shade-tolerant tree species

Hongtao Xie[1], Mukui Yu, Xiangrong Cheng*

National Research Station of Eastern China Coastal Forest Ecosystem, Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, 311400, China

ARTICLE INFO

Keywords:
Shade-tolerant species
Photosynthesis
Leaf C, N and P
Leaf non-structural carbohydrates
RDA
Light acclimation

ABSTRACT

Light availability greatly affects plant growth and development. In shaded environments, plants must respond to reduced light intensity to ensure a regular rate of photosynthesis to maintain the dynamic balance of nutrients, such as leaf non-structural carbohydrates (NSCs), carbon (C), nitrogen (N) and phosphorus (P). To improve our understanding of the nutrient utilization strategies of understory shade-tolerant plants, we compared the variations in leaf NSCs, C, N and P in response to heterogeneous controlled light conditions between two subtropical evergreen broadleaf shade-tolerant species, *Elaeocarpus sylvestris* (*E. sylvestris*) and *Illicium henryi* (*I. henryi*). Light intensity treatments were applied at five levels (100%, 52%, 33%, 15% and 6% full sunlight) for 30 weeks to identify the effects of reduced light intensity on leaf NSC allocation patterns and leaf C:N:P stoichiometry characteristics. We found that leaf soluble sugar, starch and NSC concentrations in *E. sylvestris* showed decreasing trends with reduced light intensity, whereas *I. henryi* presented slightly increasing trends from 100% to 15% full sunlight and then significant decreases at extremely low light intensity (6% full sunlight). The soluble sugar/starch ratio of *E. sylvestris* decreased with decreasing light intensity, whereas that of *I. henryi* remained stable. Moreover, both species exhibited increasing trends in leaf N and P concentrations but limited leaf N:P and C:P ratio fluctuations with decreasing light intensity, revealing their adaptive strategies for poor light environments and their growth strategies under ideal light environments. There were highly significant correlations between leaf NSC variables and C:N:P stoichiometric variables in both species, revealing a trade-off in photosynthesis production between leaf NSC and carbon allocation. Thus, shade-tolerant plants readjusted their allocation of leaf NSCs, C, N and P in response to light acclimation. Redundancy analysis showed that leaf morphological features of both *E. sylvestris* and *I. henryi* affected their corresponding leaf nutrient traits. These results improve our understanding of the dynamic balance between leaf NSCs and leaf C, N and P components in the nutritional metabolism of shade-tolerant plants.

Key message: Two species of understory shade-tolerant plants responded differently to varying light intensities in terms of leaf non-structural carbohydrate allocation and the utilization of carbon, nitrogen and phosphorus to balance nutritional metabolism and adapt to environmental stress.

1. Introduction

The availabilities of non-structural carbohydrates (NSCs), carbon (C), nitrogen (N), and phosphorus (P) in leaf reflect the nutrient levels that can be used by plants and have marked impacts on plant growth and development (Andersen et al., 2004; Li et al., 2008). Leaf NSCs (including soluble sugars and starch) represent the products of plant photosynthesis and are mainly involved in life processes, and the NSC concentration in tissue is a measure of the relationship between plant carbon uptake (photosynthesis) and carbon consumption (growth and respiration). NSCs also reflect the amount of plant carbohydrates that can be used to resist external adverse environments (Richardson et al., 2015; Hartmann and Trumbore, 2016). The C:N:P stoichiometry of plants is associated with important ecological processes, such as an organism's ability to adapt to environmental stresses (Woods et al., 2003). N and P are the main limiting resources for plant growth in

* Corresponding author. National Research Station of Eastern China Coastal Forest Ecosystem, Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, 311400, China.

E-mail addresses: xiehongtaode@126.com (H. Xie), chxr@caf.ac.cn (X. Cheng).

[1] Present address: National Research Station of Eastern China Coastal Forest Ecosystem, Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, 311400, China.

https://doi.org/10.1016/j.plaphy.2018.01.013
Received 11 May 2017; Received in revised form 15 January 2018; Accepted 16 January 2018

terrestrial ecosystems and are functionally connected in plants (Niklas and Cobb, 2006). Leaf N mainly consists of the soluble protein N and thylakoid protein N. Soluble protein N is principally Rubisco, whereas thylakoid protein N is the basis of chlorophyll formation (Stitt and Schulze, 1994). Approximately 75% of inorganic N is stored in the chloroplast for direct use in photosynthesis (Warren et al., 2000). Plants respond strongly to P availability because P is indispensable for the transportation of photosynthetic products. Thus, the C:N:P variations in leaves are directly affected by the rate of photosynthesis (Azcón et al., 1992). Leaf N concentration has been found to be positively correlated with NSC fixation ability, and P has been identified as the key element of plant metabolism (McGroddy et al., 2004; Reich and Oleksyn, 2004). Accordingly, leaf photosynthetic capacity and NSC synthesis are not only affected by leaf N concentration but also closely related to P concentration. Further, the individual and interactive effects of leaf NSCs, C, N and P affect plant survival and growth strategies. Consequently, it is of great importance to study the relationships between leaf NSCs and C:N:P stoichiometry to improve our understanding of plant life strategies, ecosystem carbon sink potential and the responses to increasing climate change (Hedin, 2004; He et al., 2006).

Of all the environmental factors that affect plant performance, light is perhaps the most spatially and temporally heterogeneous (Nascimento et al., 2015). Under evergreen canopies, light is unpredictably interrupted by leaves and branches, which form shaded light environments for understory plants (Zhang et al., 2012). Light availability strongly affects the products of photosynthesis, such as leaf NSCs (Hartmann and Trumbore, 2016). Distinct light environments create different selective pressures that can drive structural carbohydrate and elemental changes in plants, for example, changes in leaf C, N and P (Enquist and Niklas, 2002). Although numerous studies have evaluated changes in NSCs, C, N and P in leaf chemistry in response to different growth conditions, such as different temperature, precipitation and nitrogen-deposition conditions (Villar et al., 2015; Henry et al., 2006), few have focused on the effects of varying light intensity on shade-tolerant species in terms of NSC allocation and C:N:P stoichiometry and their mutual relationships.

Trees growing under the forest canopy must cope with low light conditions, and their development depends on their abilities to utilize resources. Light reduction affects the rate of plant photosynthesis; thus, the availability of various nutrients in leaves can change to establish diverse growth strategies among different species in heterogeneous light environments. Previous studies have demonstrated that under high-light conditions, plants tend to allocate more biomass to underground parts to increase the absorption of water and nutrients, whereas in low-light environments, more biomass is allocated to the aboveground parts, characterized by an increase in leaf biomass (Wang et al., 2011). Leaf NSCs, C, N and P are all considered important factors that determine leaf biomass. However, it remains to be determined whether they show similar trends as that of biomass with increasing light in the environment and what relationships exist among them. Understory species are crucial to the sustainable development of forests and, in particular, forest plantations, where shaded species provide not only higher biodiversity and stronger ecosystem functionality but also higher economic returns. For example, *Schima superba*, *Michelia macclurei* and *Elaeocarpus sylvestris* (*E. sylvestris*) have been planted for years in the understory of a *Cunninghamia lanceolata* stand in South China for timber provision (Xiong, 2007). Consequently, underplanting is being adopted by an increasing number of forest managers, and several studies have explored how shade-tolerant plants respond to environmental stress. However, little information is available on leaf NSC diversification and leaf C:N:P stoichiometry in response to canopy shading or reduced light availability in contrasted, shade-tolerant plant species in the subtropical region of Eastern China.

In this context, we conducted a manipulative field experiment to evaluate the effects of different light intensity levels along a gradient on leaf NSC accumulation and allocation patterns as well as on the leaf C, N and P availability and stoichiometry in two large-scale distributed shade-tolerant tree species. We also investigated the relationships among leaf NSCs, C, N and P and their combined effects on plant survival strategy. Specifically, the study aims were 1) to determine how leaf soluble sugar, starch and total NSC concentrations respond to different levels of light availability along a gradient in two shade-tolerant species; 2) to determine how leaf C, N and P concentrations and the leaf C:N:P ratio respond to different controlled light intensities in two shade-tolerant species; 3) to identify the relationships among leaf NSCs and leaf C, N and P at different levels of light availability; and 4) to identify how leaf morphological features affect the leaf nutrient traits in two shade-tolerant species.

2. Materials and methods

2.1. Study site

The study site was located at the Qianjia Village Tree Nursery Station (30° 14′ N, 119° 86′ E, elevation 265 m a.s.l.) in Fuyang, Hangzhou, China. The area has a subtropical monsoon climate with an average annual temperature of 16.4 °C and average annual precipitation of 1814 mm. The frost-free period is 252 days, and the total annual sunshine is 1334.1 h. The experimental site was farmland before it was transformed into a tree nursing station in 2005. The site has red-yellow soil with a pH of 6.5.

2.2. Plant material and experimental design

Two subtropical evergreen broadleaf tree species, *E. sylvestris* and *Illicium henryi* (*I. henryi*), were selected as our research objects based on their wide distributions and multiple functions. For example, *E. sylvestris* has high timber and ecological value, and *I. henryi* has great pharmaceutical value. Both species are widely distributed along coastal areas of Eastern China. Based on our previous study of the growth and leaf morphological characteristics of these two species under varying controlled light conditions, we assume that *E. sylvestris* is more likely to be a mild shade-tolerant species, whereas *I. henryi* is more likely to be a moderate shade-tolerant species. Before the experiment, seeds of both species were collected near Hangzhou (30° 31′ N, 120° 16′ E). In April 2008, the seeds of both species were sown in the field at the Qianjia Village Tree Nursery Station. Similar tending management was conducted for the two species after seed germination.

The experiment was conducted in a flat, open area in Qianjia Village. For each species, 150 two-year-old seedlings, approximately uniform in size, were randomly divided into five groups, with 30 seedlings of each species per group. The two tree species of each group were randomly planted (with trees of the same species grown together) in 15 × 2 m plots on March 14, 2010.

One month later, a light treatment was randomly assigned to each plot. Seedlings planted in the same plot were thus subjected to the same controlled light condition. Five light treatments (100, 52, 33, 15 and 6% full sunlight) were created by building a shade house covered with an increasing number of layers of black nylon shade netting. The 17 × 2.5 m shade house was 2.5 m high. Relative irradiance in each shading house was estimated with a light meter (TES-1335, Taiwan) during clear days in the summer. A 20 cm slit was retained between the soil surface and the shade netting to allow ventilation in the shade house. In the experimental plot, weeds were periodically cleared, and the seedlings were watered as needed.

2.3. Plant sampling

Thirty weeks after the initiation of the light treatments, the final harvest was conducted in mid-November of 2010. Six seedlings of each species and light treatment were randomly selected and destructively sampled before the final harvest. A subsample of 50 mature leaves of