ORIGINAL PAPER

# Allocation patterns of nonstructural carbohydrates in response to $CO_2$ elevation and nitrogen deposition in *Cunninghamia lanceolata* saplings

Wenhui Zheng[1,2] · Renshan Li[4] · Qingpeng Yang[2,3] · Weidong Zhang[2,3] · Ke Huang[2,3] · Xin Guan[2,3] · Longchi Chen[2,3] · Xin Yu[5] · Qingkui Wang[2,3] · Silong Wang[2,3]

**Abstract** Stored nonstructural carbohydrates (NSC) indicate a balance between photosynthetic carbon (C) assimilation and growth investment or loss through respiration and root exudation. They play an important role in plant function and whole-plant level C cycling. $CO_2$ elevation and nitrogen (N) deposition, which are two major environmental issues worldwide, affect plant photosynthetic C assimilation and C release in forest ecosystems. However, information regarding the effect of $CO_2$ elevation and N deposition on NSC storage in different organs remains limited, especially regarding the trade-off between growth and NSC reserves. Therefore, here we analyzed the variations in the NSC storage in different organs of Chinese fir (*Cunninghamia lanceolata*) under $CO_2$ elevation and N addition and found that NSC concentrations and contents in all organs of Chinese fir saplings increased remarkably under $CO_2$ elevation. However, N addition induced differential accumulation of NSC among various organs. Specifically, N addition decreased the NSC concentrations of needles, branches, stems, and fine roots, but increased the NSC contents of branches and coarse roots. The increase in the NSC contents of roots was more pronounced than that in the NSC content of aboveground organs under $CO_2$ elevation. The role of N addition in the increase in the structural biomass of aboveground organs was greater than that in the increase in the structural biomass of roots. This result indicated that a different trade-off between growth and NSC storage occurred to alleviate resource limitations under $CO_2$ elevation and N addition and highlights the importance of separating biomass into structural biomass and NSC reserves when investigating the effects of environmental change on biomass allocation.

**Keywords** Biomass partition · $CO_2$ elevation · N deposition · Nonstructural carbohydrates · Structural biomass

✉ Qingpeng Yang
   yqp226@iae.ac.cn

✉ Qingkui Wang
   wqkui@163.com

✉ Silong Wang
   slwang@iae.ac.cn

1   Fujian Provincial Key Laboratory of Resources and Environmental Monitoring and Sustainable Management and Utilization, Sanming University, Sanming 365004, People's Republic of China

🄪 Springer

## Introduction

A fluctuation of nonstructural carbohydrates (NSC) in plant organs represents an asynchrony between carbon (C) supply and C demand (Kobe et al. 2010; Sala et al. 2012; Hartmann and Trumbore 2016). NSC are primarily derived from foliage photosynthates (Kozlowski 1992; Carbone et al. 2013) and translocate to other organs for energy metabolism, osmoregulation, and defense compound synthesis (Hartmann and Trumbore 2016). Given that NSC constitute 8%–17% of a tree's biomass (Wurth et al. 2005; Mei et al. 2015; Martínez-Vilalta et al. 2016), they are a vital component in terrestrial C cycling. Furthermore, NSC dynamics across plant organs reflect C source–sink status and organ function in adapting to environmental changes (Hartmann and Trumbore 2016). Despite its vital role in plant function and in stand-level terrestrial C cycling, NSC allocation of plant organs to environment stresses has been the focus of very limited research. With the current and expected changes to the global climate, however, a comprehensive understanding of NSC allocation in response to environmental variations is essential (Li et al. 2013; McDowell et al. 2008; Yang et al. 2016).

Because atmospheric $CO_2$ concentration has increased from 280 μmol mol$^{-1}$ before the Industrial Revolution to 414 μmol mol$^{-1}$ in 2020 (Dong et al. 2021), the impacts of $CO_2$ elevation on C cycling of forest ecosystems have received considerable attention (Oren et al. 2001; Hamilton et al. 2002; Norby et al. 2010). For instance, in a sweetgum forest, Norby et al. (2002) found that $CO_2$ elevation leads to differential increases in the biomass of various organs, but NSC allocation to different organs in response to $CO_2$ elevation has been rarely investigated (Li et al. 2018b). When $CO_2$ is elevated, C assimilation increases as confirmed by an upregulation of leaf photosynthetic capacity (Arp and Drake 1991; Long et al. 2004). Thus, under $CO_2$ elevation, it is reasonable to infer that much more NSC are stored in leaves. The translocation of carbohydrates to other organs to support organ growth also increases as the photosynthetic rate increases (Li et al. 2018a). Nevertheless, additional tree growth is likely limited by nutrient supply, particularly nitrogen or phosphorus, when $CO_2$ is elevated

(Oren et al. 2001; Norby et al. 2010). In response to nutrient limitation, plants may increase fine root production and mycorrhizal infection to intensify soil exploration (Zak et al. 2000; Luo et al. 2004). To support root and mycorrhizal growth, additional photosynthetic C might be partitioned to underground organs. Previous studies have mostly focused on NSC concentration responses to $CO_2$ elevation at the leaf or whole-plant level (Smart et al. 1994; Cheng et al. 2004; Mašková et al. 2017). Therefore, whether the NSC contents and concentrations in various organs vary in response to $CO_2$ elevation should be clarified.

Nitrogen (N) deposition is another serious global problem that is intensifying in China. N deposition rate in China increased by 0.41 kg ha$^{-1}$ a$^{-1}$ between 1980 and 2010, reaching 21.1 kg ha$^{-1}$ a$^{-1}$ in 2010 (Liu et al. 2013). N deposition leads to an increase in NSC accumulation (Liu et al. 2016) by promoting photosynthetic C assimilation (Evans and Terashima 1988; Nakaji et al. 2001; Cechin and Fumis 2004; Granath et al. 2009). It may also intensify NSC consumption through several mechanisms. First, N deposition may amplify N assimilation and consequently elevate NSC consumption by increasing the amount of energy and C skeletons consumed in N assimilation (Invers et al. 2004; Monson et al. 2006). Second, during the stimulation of plant growth after N deposition, more carbohydrates may be allocated to structural biomass (Liu et al. 2016). Although previous studies have reported that N assimilation rates of leaves and roots show different responses to N addition (Invers et al. 2004), the changes in NSC consumption and allocation in various organs under N addition remain unclear. Thus, clarifying NSC dynamics in different organs under increased N deposition is urgently needed.

The allocation of biomass, which comprises structural biomass and NSC reserves, is a vital strategy used by plants to adapt to environmental conditions (Guo et al. 2016). Separating biomass into structural biomass and NSC storage is critical because the allocation of structural biomass and NSC reserves is the result of the trade-off between growth and carbohydrate storage (Canham et al. 1999; Kobe et al. 2010). Although the allocation of biomass under elevated $CO_2$ and N has received attention (Hättenschwiler and Körner 1997; Norby et al. 2002), few works have investigated the allocation of the separate biomass components at the whole-plant level under $CO_2$ elevation and N deposition. In addition, whether structural biomass or NSC reserves is the major contributor to alleviate resource limitations remains unclear. Optimal partitioning theory states that plants can increase biomass allocation to organs to acquire the resource(s) that most limits their growth (Bloom et al. 1985). Kobe et al. (2010), however, reported that incremental increases in NSC content rather than in structural biomass, are mainly responsible for the increase in biomass allocation to roots under nutrient limitation. Therefore, the responses of allocation of

[2]   CAS Key Laboratory of Forest Ecology and Management, Institute of Applied Ecology, Huitong Experimental Station of Forest Ecology, Shenyang 110016, People's Republic of China

[3]   Huitong National Research Station of Forest Ecosystem, Huaihua, Huitong 418307, People's Republic of China

[4]   Life Science Department, Luoyang Normal University, Luoyang 471934, People's Republic of China

[5]   Fujian Provincial Key Laboratory of the Development and Utilization of Bamboo Resources, Sanming University, Sanming 365004, People's Republic of China

structural biomass and NSC content to $CO_2$ elevation and N deposition require clarification.

Chinese fir [*Cunninghamia lanceolata* (Lamb.) Hook.] is widely planted in southern China and plays a vital role in timber production and C sequestration (Wang et al. 2017). Thus, further insight into the responses of NSC allocation in Chinese fir to $CO_2$ elevation and N deposition are needed to design strategies to address the effects of global climate change. Here we (1) investigated the responses of NSC concentrations and contents of various organs to $CO_2$ elevation or N deposition, and (2) compared the resultant changes in structural biomass and NSC storage among aboveground organs and roots.

## Materials and methods

### Study site and experimental design

The experiment was conducted at Huitong National Research Station of Forest Ecosystem (26°50′ N, 109°36′ E) in Hunan Province, China. This region has a typical humid mid-subtropical monsoon climate that is characterized by a warm and humid summer and a relatively dry and cold winter. The annual average air temperature is 16.5 °C, and the monthly mean maximum and minimum air temperatures of 29.0 °C and 1.9 °C occur in July and January, respectively. Annual precipitation ranges from 1200 to 1400 mm, mainly during April to July.

In February 2016, 50 one-year-old Chinese fir saplings were planted in plastic pots (25 cm diameter, 50 cm height) filled with surface soil (0–30 cm) that had been homogenized after its removal from a Chinese fir plantation. All pots were periodically watered to 60% field capacity to replace water lost via evapotranspiration.

In August 2016, 36 Chinese fir saplings with similar basal diameters and heights were selected and separated randomly into six equal groups. Each group was transferred to a randomly assigned closed climate-controlled chambers. From 06:00 to 18:00 every day, three chambers received ambient air $CO_2$ concentration (approximately 400 µmol mol$^{-1}$) and the other three elevated $CO_2$ concentration ($600 \pm 50$ µmol mol$^{-1}$) that was controlled using a $CO_2$ detection system (Shsen-QZD, Institute of Shengsen Numerical Control Technology, Qingdao, China). Three Chinese fir saplings in each chamber were randomly chosen as parallel samples to receive N fertilizer in the form of $NH_4NO_3$ at the rate of 10 g N m$^{-2}$ a$^{-1}$ via irrigation once a month. The three other saplings received an equal amount of deionized water. Soil moisture was maintained at approximately 60% of field capacity during the experiment. Thus, four treatments were imposed: (1) ambient air $CO_2$ concentration and no N (EC0N0), (2) ambient air $CO_2$ concentration and N addition (EC0N1), (3) elevated $CO_2$ concentration and no N (EC1N0), and (4) elevated $CO_2$ concentration and N addition (EC1N1).

### Sample collection and NSC measurement

In September 2017, all saplings in the chambers were harvested and separated into different organ types (needles, branches, stems, and roots). Needles and branches were each separated into current and previous year groups. Roots were separated into coarse (diameter ≥ 2 mm) and fine (diameter < 2 mm) roots. To reduce variability, we prepared mixed samples by combining the parallel samples from each chamber on the basis of organ types. All samples from different organs were oven-dried at 80 °C to a constant mass then weight to estimate biomass. The samples were then milled to a fine powder to measure NSC using the method described by Li et al. (2018a) and Yang et al. (2016): Soluble sugars (i.e., glucose, sucrose, and fructose) and starch concentration were measured and added to estimate total NSC concentration.

The NSC content for each organ was calculated as Organ-specific concentration × Organ-specific biomass. Structural biomass was then calculated as Biomass minus NSC content similar to the methods of Canham et al. (1999) and Kobe et al. (2010).

### Statistical analyses

The effects of $CO_2$ elevation, N addition, and their interaction on concentrations and contents of NSC, concentrations and contents of starch and of soluble sugars in stems, coarse roots, and fine roots were all evaluated using a two-way ANOVA implemented in R version 4.1.3 (R Core Team 2022). The mixed effects model was applied to evaluate the effect of $CO_2$ elevation, N addition, needle age or branch age, and their interactions on NSC concentrations and NSC contents in needles and branches. This model was run using the lmer function in R by loading the lme 4 and lmer Test packages. The ANOVA function in R was used for parametric tests. In this model, $CO_2$ elevation, N addition, and needle age or branch age were used as fixed effects; the chamber was used as a random effect. The significance level was set at $P < 0.05$ for all analyses unless otherwise stated.

## Results

### Concentrations of NSC and its two fractions in different organs

Needle NSC (Fig. 1a, $P = 0.006$), soluble sugars (Fig. 1c, $P = 0.028$), and starch (Fig. 1e, $P = 0.002$) concentrations greatly decreased with needle age. $CO_2$ elevation remarkably increased needle NSC concentrations (Fig. 1a, $P = 0.001$) and its two fractions (soluble sugars (Fig. 1c, P < 0.001) and
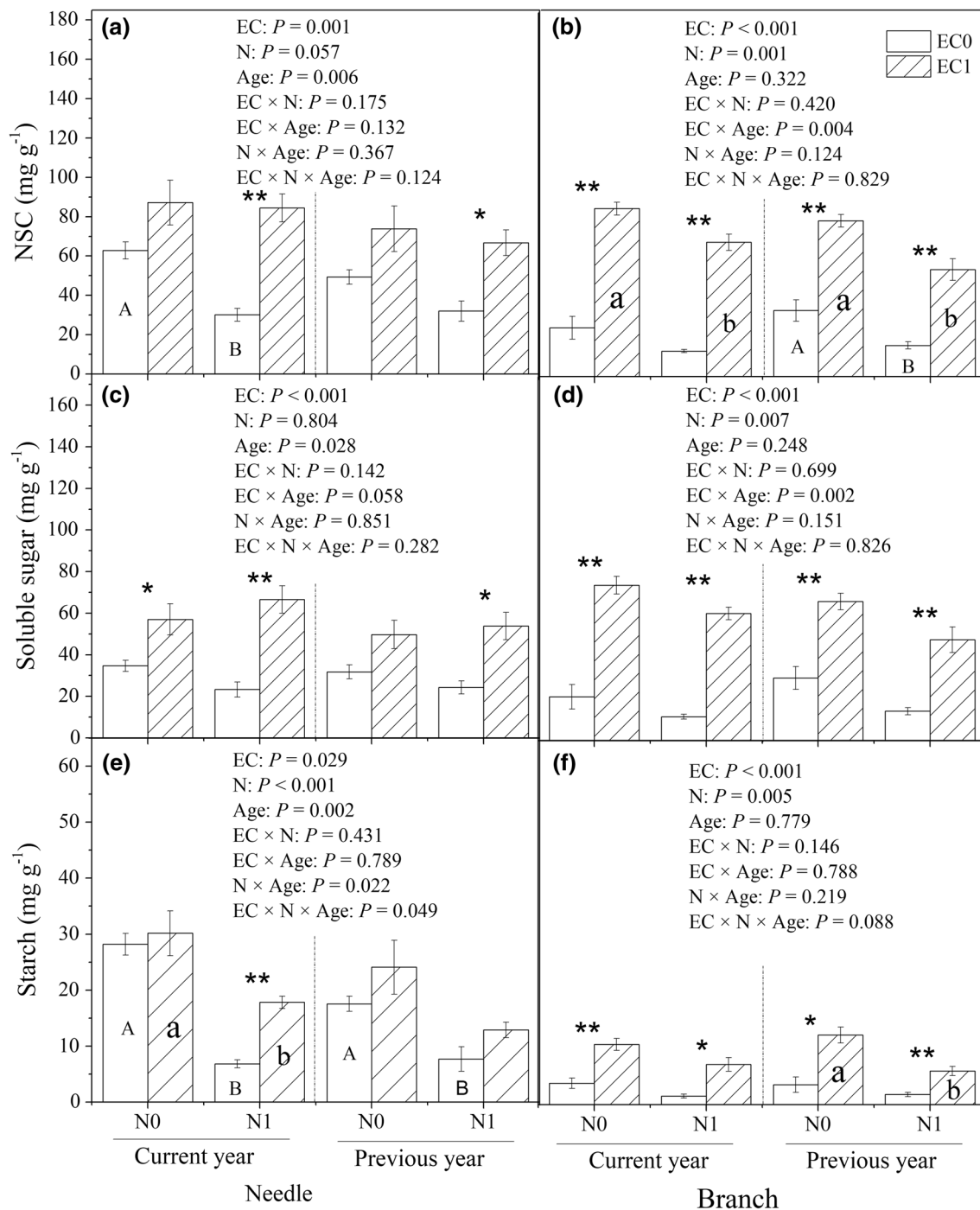
**Fig. 1** NSC (**a**, **b**), soluble sugars (**c**, **d**), and starch (**e**, **f**) concentrations in needles (**a**, **c**, **e**) and branches (**b**, **d**, **f**) from Chinese fir saplings under $CO_2$ elevation and N deposition. EC0: ambient air $CO_2$ concentration; EC1: elevated $CO_2$ concentration; N0: no N; and N1: N addition. Different uppercase letters denote significant differences between EC0N0 and EC0N1 ($P < 0.05$). Different lowercase letters denote significant differences between EC1N0 and EC1N1 ($P < 0.05$). Significant differences between EC0N0 and EC1N0 or between EC0N1 and EC1N1 are denoted by asterisks: *$P < 0.05$, **$P < 0.01$. Values are expressed as means ± SE ($n = 3$)

starch (Fig. 1e, $P = 0.029$). However, a marginally significant decrease in needle NSC concentration (Fig. 1a, $P = 0.057$) and a significant decrease in needle starch concentration (Fig. 1e, P < 0.001) were found under N addition. The soluble

sugars concentration in needles did not change in response to N addition (Fig. 1c, $P = 0.804$). N addition and needle age exerted an interactive effect on starch concentration (Fig. 1e, $P = 0.022$). This effect indicates that the decrease in the starch

concentration of current year needles after N addition was greater than the decrease in the previous year needles (Fig. 1e).

Unlike the NSC concentration in needles, the concentration of NSC and its two fractions in branches did not differ based on branch age (Fig. 1b, d, f, $P > 0.05$). $CO_2$ elevation significantly increased branch NSC (Fig. 1b, $P < 0.001$), soluble sugars (Fig. 1d, $P < 0.001$), and starch (Fig. 1f, $P < 0.001$) concentrations. Conversely, N addition significantly decreased branch NSC (Fig. 1b, $P = 0.001$), soluble sugars (Fig. 1d, $P = 0.007$), and starch (Fig. 1f, $P = 0.005$) concentrations. $CO_2$ elevation and branch age had an interactive effect on branch NSC (Fig. 1b, $P = 0.004$) and soluble sugars concentrations (Fig. 1d, $P = 0.002$). These results demonstrate that the increase in NSC and soluble sugars concentrations in current year branches was more prominent than in previous year branches under $CO_2$ elevation.

$CO_2$ elevation significantly increased the concentrations of NSC (Fig. 2a, $P < 0.001$), soluble sugars (Fig. 2b, $P < 0.001$), and starch (Fig. 2c, $P = 0.004$) in stems, whereas N addition significantly decreased the concentrations of NSC (Fig. 2a, $P = 0.009$) and soluble sugars (Fig. 2b, $P = 0.018$). $CO_2$ elevation and N addition exerted a significant interactive effect on NSC (Fig. 2a, $P = 0.025$) and soluble sugars concentrations (Fig. 2b, $P = 0.005$) in stems. This effect showed that the decrease in stem NSC and soluble sugars concentrations with N addition only happened in ambient $CO_2$, not in elevated $CO_2$.

$CO_2$ elevation significantly increased the starch (Fig. 3e, $P < 0.001$) and NSC concentrations (Fig. 3a, $P < 0.001$) in coarse roots and starch (Fig. 3f, $P < 0.001$) and NSC (Fig. 3b, $P < 0.001$) concentrations in fine roots. However, the NSC, soluble sugars, and starch concentrations in coarse roots did not change in response to N addition (Figs. 3 a, c, and e, $P > 0.05$). N addition significantly decreased the NSC (Fig. 3b, $P = 0.004$) and soluble sugars concentrations (Fig. 3d, $P = 0.001$) in fine roots. A significant interactive effect between $CO_2$ elevation and N addition on the NSC concentration of coarse roots was found (Fig. 3a, $P = 0.041$).

## Contents of NSC and its two fractions in different organs

Similar to needle NSC concentration, the contents of needle NSC and its two fractions decreased with needle age (Figs. 4a, c, e, $P < 0.001$). Similarly, branch NSC (Fig. 4b, $P = 0.045$) and soluble sugars (Fig. 4d, $P = 0.047$) contents decreased with branch age. With the exception of soluble sugars content in fine root, NSC, soluble sugars and starch contents significantly increased in all organs under $CO_2$ elevation (Figs. 4, 5, and 6, $P < 0.05$). However, NSC content and its two fractions in various organs varied in their responses to N addition. In needles, N addition significantly increased soluble sugars content (Fig. 4c, $P = 0.005$) but
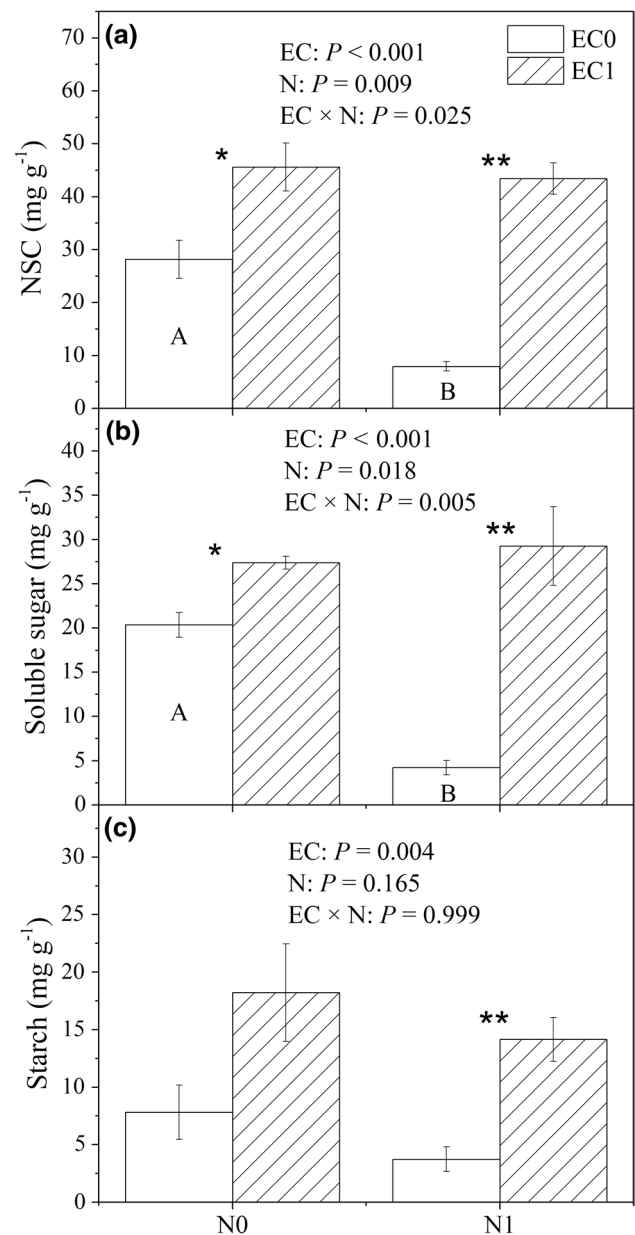


**Fig. 2** NSC (**a**), soluble sugars (**b**), and starch (**c**) concentration in stems from Chinese fir saplings under $CO_2$ elevation and N deposition. EC0: ambient air $CO_2$ concentration; EC1: elevated $CO_2$ concentration; N0: no N; and N1: N addition. Different uppercase letters denote significant differences between EC0N0 and EC0N1 ($P < 0.05$). Significant differences between EC0N0 and EC1N0 or between EC0N1 and EC1N1 are denoted by asterisks: *$P < 0.05$, **$P < 0.01$. Values are expressed as means $\pm$ SE ($n = 3$)

significantly decreased starch content (Fig. 4e, $P = 0.040$). In branches, NSC (Fig. 4b, $P = 0.007$) and soluble sugars (Fig. 4d, $P = 0.010$) contents significantly increased with N addition, as did starch content (Fig. 6e, $P = 0.003$) and NSC content (Fig. 6a, $P = 0.003$) in coarse roots. However, soluble sugar content in fine roots significantly decreased

**Fig. 3** NSC (**a**, **b**), soluble sugars (**c**, **d**), and starch (**e**, **f**) concentrations in coarse (**a**, **c**, **e**) and fine roots (**b**, **d**, **f**) from Chinese fir saplings under $CO_2$ elevation and N addition. EC0: ambient air $CO_2$; EC1: elevated $CO_2$; N0: no N; and N1: N addition. Different uppercase letters denote significant differences between EC0N0 and EC0N1 ($P < 0.05$). Significant differences between EC0N0 and EC1N0 or between EC0N1 and EC1N1 are denoted by asterisks: *$P < 0.05$, **$P < 0.01$. Values are expressed as means $\pm$ SE ($n = 3$)
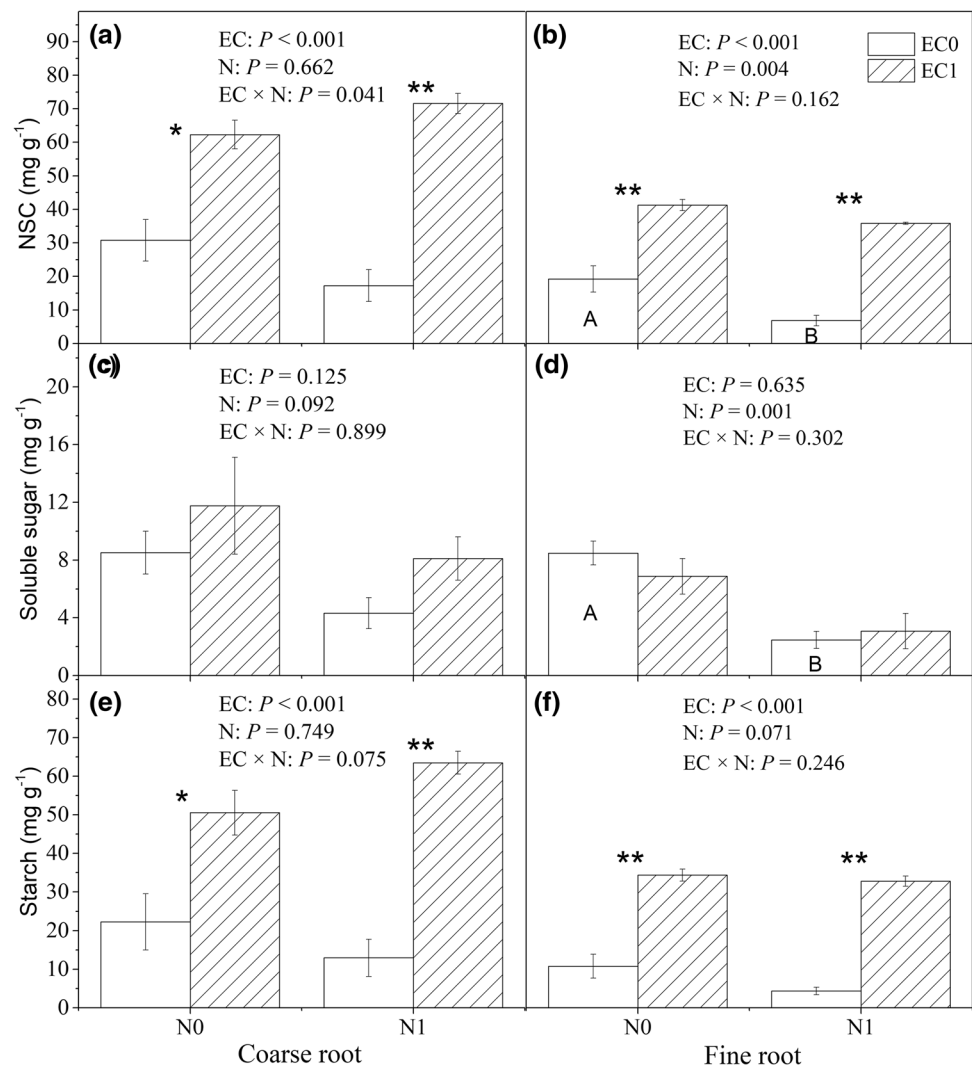


with N addition (Fig. 6d, $P = 0.036$). $CO_2$ elevation and N addition had significant interactive effects on needle NSC (Fig. 4a, $P = 0.004$) and soluble sugars (Fig. 4c, $P = 0.002$), branch NSC (Fig. 4b, $P = 0.003$) and soluble sugars (Fig. 4d, $P = 0.006$), stem NSC content (Fig. 5a, $P = 0.049$), and on coarse-root starch (Fig. 6e, $P = 0.003$) and NSC content (Fig. 6a, $P = 0.003$). $CO_2$ elevation and needle age had a significant interactive effect on needle NSC (Fig. 4a, $P < 0.001$), starch (Fig. 4e, $P < 0.001$) and soluble sugars content (Fig. 4c, $P < 0.001$).

## Comparison of allocation of structural biomass and NSC content to aboveground organs and roots

Under $CO_2$ elevation, NSC content was preferentially allocated to roots over aboveground organs (Fig. 7a, $P = 0.027$), but the proportion of the structural biomass of aboveground organs to the structural biomass of roots did not change in response to $CO_2$ elevation (Fig. 7b,

$P = 0.139$). By contrast, N addition promoted the preferential partitioning of structural biomass to aboveground organs instead of underground roots, as indicated by the significant increment in the structural biomass of aboveground organs relative to that of roots (Fig. 7b, $P = 0.008$). The proportion of the NSC content of aboveground organs to the NSC content of roots did not change in response to N addition (Fig. 7a, $P = 0.473$).

## Discussion

### Effect of $CO_2$ elevation on NSC concentration and content of different organs

The positive effect of $CO_2$ elevation on NSC accumulation in needles could be due to the following reasons. First, NSC are mainly produced in leaves through photosynthetic C assimilation (Kozlowski 1992), the upregulation of leaf
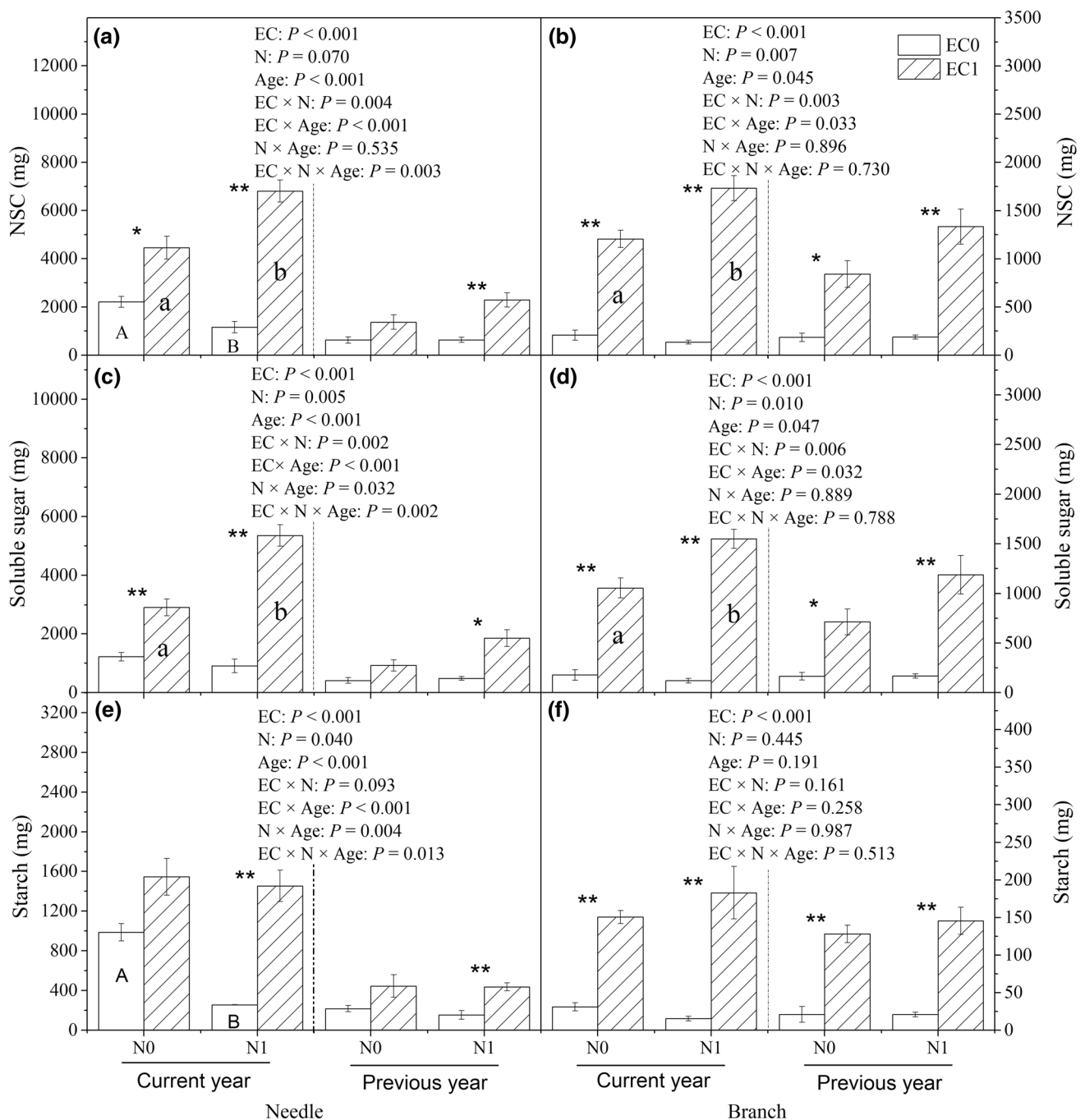
**Fig. 4** NSC (**a**, **b**), soluble sugars (**c**, **d**), and starch (**e**, **f**) contents in needles (**a**, **c**, **e**) and branches (**b**, **d**, **f**) from Chinese fir saplings under CO₂ elevation and N addition. EC0: ambient air CO₂ concentration; EC1: elevated CO₂ concentration; N0: no N; and N1: N addition. Different uppercase letters denote significant differences between EC0N0 and EC0N1 ($P < 0.05$). Different lowercase letters denote significant differences between EC1N0 and EC1N1 ($P < 0.05$). Significant differences between EC0N0 and EC1N0 or between EC0N1 and EC1N1 are denoted by asterisks: *$P < 0.05$, **$P < 0.01$. Values are expressed as means ± SE ($n = 3$)

photosynthetic capacity under CO₂ elevation increases C assimilation (Arp and Drake 1991; Long et al. 2004; Luo et al. 2006). Second, CO₂ elevation is likely to reduce needle respiration rates (Idso and Kimball 1992; Zha et al. 2002;

Long et al. 2004). Thus, the increase in C sources and the reduction in C sinks resulted in needle NSC accumulation under CO₂ elevation. In addition, the response of NSC accumulation to CO₂ elevation was dependent on needle age; the
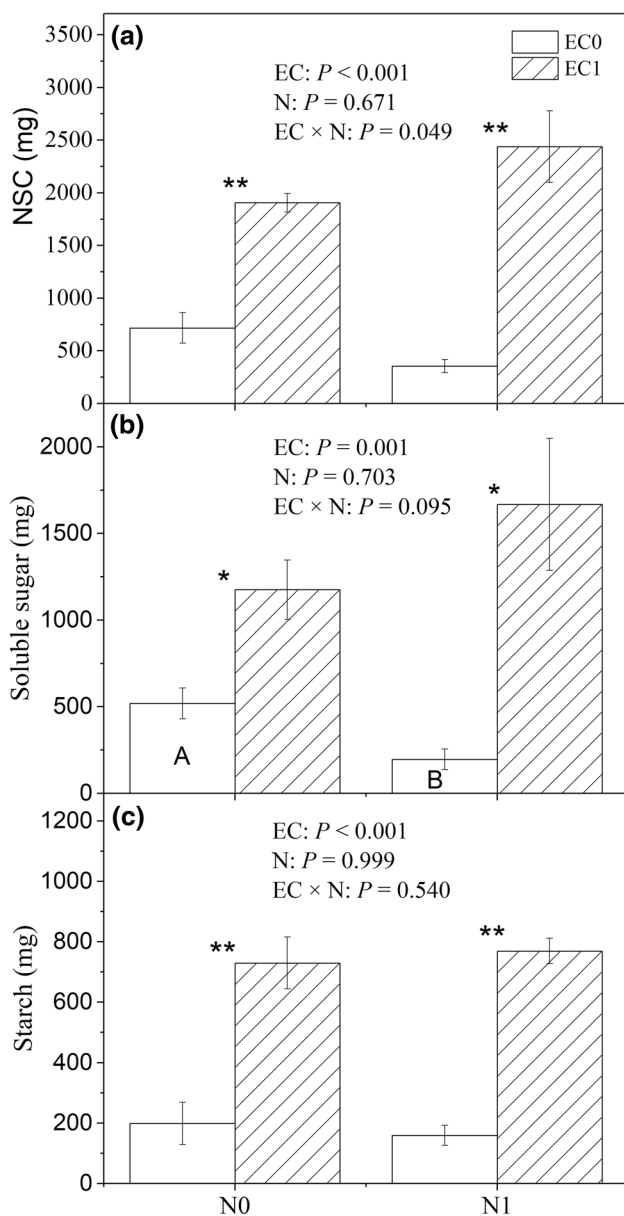
**Fig. 5** NSC (**a**), soluble sugars (**b**), and starch (**c**) contents in stems from Chinese fir saplings under $CO_2$ elevation and N addition. EC0: ambient air $CO_2$ concentration; EC1: elevated $CO_2$ concentration; N0: no N; and N1: N addition. Different uppercase letters denote significant differences between EC0N0 and EC0N1 ($P < 0.05$). Significant differences between EC0N0 and EC1N0 or between EC0N1 and EC1N1 are denoted by asterisks: *$P < 0.05$, **$P < 0.01$. Values are expressed as means ± SE ($n = 3$)

accumulation in current year needles was more pronounced than in previous year needles (Fig. 4a, c, e) because photosynthetic rate in current year needles is more sensitive than in previous year needles to $CO_2$ elevation (Tissue et al. 2001).

$CO_2$ elevation increased branch and stem NSC concentration and content by augmenting the translocation of

photosynthates from needles to branches and stems. We found that $CO_2$ elevation promoted sapling growth (Fig. S2) as reported previously (Curtis and Wang 1998; De Souza et al. 2008). Therefore, the excess photosynthate fixed by needles is translocated to branches and stems to promote their elongation and maximize space occupation and light harvest (Li et al. 2018a). Second, the additional growth of biomass under $CO_2$ elevation increased the amounts of soil nutrients required for organic matter production (Huang et al. 2007). Thus, large amounts of NSC must be translocated to branches and stems for nutrient transport given their vital roles in phloem transport (Dietze et al. 2014).

Similar to the accumulation in aboveground organs, NSC accumulation in coarse and fine roots increased under $CO_2$ elevation. This response may be related to the nutrient limitations experienced by plants under $CO_2$ elevation (Fig. S3). Plants could alleviate nutrient limitation by amplifying their exploration of soil for available nutrients (Luo et al. 2004). Thus, the NSC fixed by needles were translocated to underground organs to support the root activity and growth needed for nutrient acquisition (Kobe et al. 2010). Interestingly, starch, rather than soluble sugars, was mainly responsible for the increase in NSC accumulation in roots under $CO_2$ elevation. This result may be attributed to the different functions between starch and soluble sugars. Starch is used as a carbohydrate reserve and represents a relatively recalcitrant C pool (Hartmann and Trumbore 2016). Meanwhile, soluble sugars are usually used for immediate functions (e.g., substrates for respiration, osmoregulation and transport compounds) and represent a short-term pool (Du et al. 2020). Therefore, part of the photosynthate allocated to roots was converted to starch for future use, resulting in starch accumulation. Plants exchange soluble sugars, especially glucose, for nutrients with symbionts under $CO_2$ elevation; thus, soluble sugars are maintained at a critical level (Nehls et al. 2010; Smith and Smith 2011; Martínez-Vilalta et al. 2016).

Interestingly, $CO_2$ elevation resulted in the preferential allocation of NSC content to roots instead of to aboveground organs. The allocation of structural biomass to aboveground organs vs roots, however, did not change in response to $CO_2$ elevation. The promotion of plant growth under $CO_2$ elevation may result in nutrient limitation, especially nitrogen and phosphorus (Oren et al. 2001; Norby et al. 2010), because nitrogen and phosphorus concentration in Chinese fir saplings decreased when $CO_2$ was elevated (Fig. S3). According to the optimal partitioning theory, biomass is preferentially allocated to roots to increase the absorptive surface area of roots as the main mechanism for plants to capture limited sources (Bloom et al. 1985; Zak et al. 2000; Luo et al. 2004). However, the present study found that only the NSC reserves of roots increased under nutrient limitation. Similarly, Kobe et al. (2010) found that enhanced NSC storage contributed more than increased structural biomass to the change in root
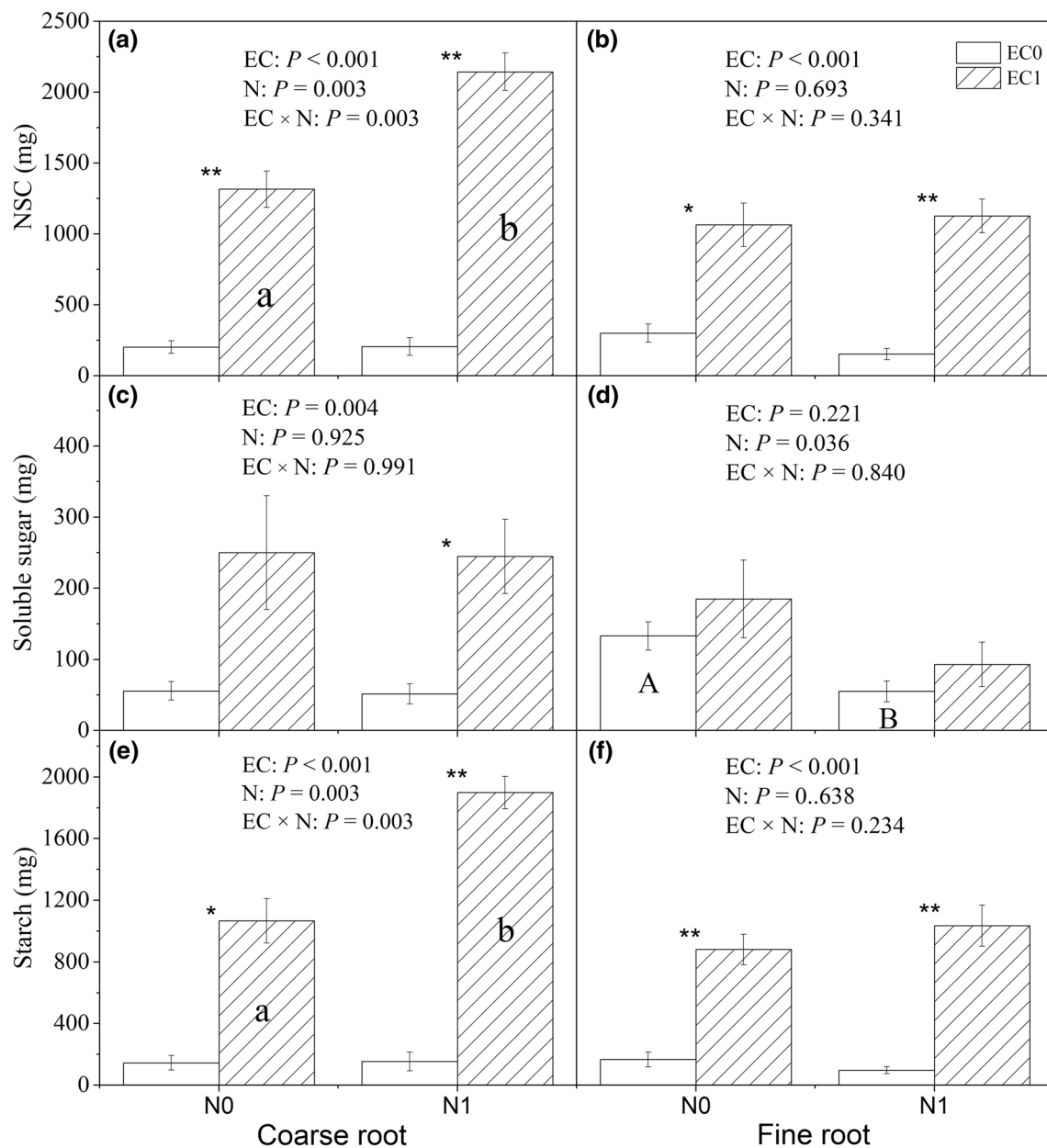
**Fig. 6** NSC (**a**, **b**), soluble sugars (**c**, **d**), and starch (**e**, **f**) contents in coarse (**a**, **c**, **e**) and fine roots (**b**, **d**, **f**) from Chinese fir saplings under CO₂ elevation and N addition. EC0: ambient air CO₂ concentration; EC1: elevated CO₂ concentration; N0: no N; and N1: N addition. Different uppercase letters denote significant differences between EC0N0 and EC0N1 ($P < 0.05$). Different lowercase letters denote significant differences between EC1N0 and EC1N1 ($P < 0.05$). Significant differences between EC0N0 and EC1N0 or between EC0N1 and EC1N1 are denoted by asterisks: *$P < 0.05$, **$P < 0.01$. Values are expressed as means ± SE ($n = 3$)

biomass under nutrient limitation. This result is probably related to the fact that, in our study high NSC reserves provide more advantages than just an increase in absorptive surface area to gain more nutrients. First, root NSC provide energy to roots and mycorrhizae for nutrient uptake and are a carbon source in rhizosphere exudates that prime microbial populations to mineralize organic matter and obtain nutrients (Rothstein et al. 2000; Dijkstra and Cheng 2007). Second, NSC storage, not the immediate construction of

fine roots, favors plant survival when nutrients are scarce because newly formed fine roots consume energy required for respiration (Kobe et al. 2010).

## Effect of N addition on the NSC concentration and content of different organs

Various factors could contribute to the decreased starch and NSC concentrations in needles under N addition (Fig. 1).
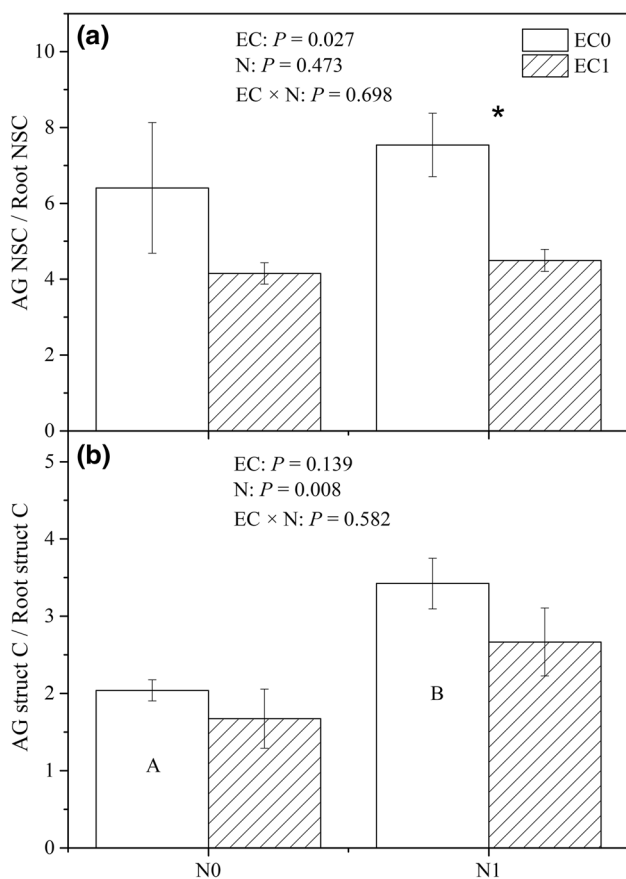
**Fig. 7** Ratio of the sum of NSC content of aboveground (AG) organs to the NSC content in roots (**a**) and ratio of the sum of structural (struct) C content of aboveground organs to the structural C content in roots (**b**) from Chinese fir saplings under $CO_2$ elevation and N deposition. EC0: ambient air $CO_2$ concentration; EC1: elevated $CO_2$ concentration; N0: no N; and N1: N addition. Different uppercase letters denote significant differences between EC0N0 and EC0N1 ($P < 0.05$). Different lowercase letters denote significant differences between EC1N0 and EC1N1 ($P < 0.05$). Significant differences between EC0N0 and EC1N0 or between EC0N1 and EC1N1 are denoted by asterisks: *$P < 0.05$. Values are expressed as means ± SE ($n = 3$)

First, the allocation of NSC for N assimilation needed to synthesize proteins likely increased under N addition. Kraemer et al. (1997) reported that high N assimilation rates is associated with high N contents. N addition increased foliar N concentration in our study (Fig. S3) and in others (Hicks et al. 2000; Mo et al. 2008; Lü et al. 2016). Therefore, NSC consumption goes up due to the increased demand for C skeletons and energy for N assimilation (Foyer et al. 2003; Cheng et al. 2004; Invers et al. 2004). Second, because N addition promotes plant growth (Cechin and Fumis 2004; Mo et al. 2008), photosynthate allocation to structural biomass increased after N addition, resulting in the decrease in needle NSC concentration (Liu et al. 2016). This interpretation is well supported by the increase in sapling structural biomass under N addition in our study (Fig. S4).

Branch and stem NSC concentrations decreased under N addition, whereas NSC content increased or remained stable. Similar to the decrease in needle NSC concentration, the decrease in branch and stem NSC concentrations may be attributed to increased use of NSC for N assimilation, as indicated by the increase in the total N concentrations of branches and stems under N addition (Fig. S3). Moreover, the promotion of branch and stem growth after N addition was responsible for the reduction in NSC concentration. This interpretation is in line with the increases in branch (Fig. S1) and stem (Fig. S2) biomass under N addition. The NSC contents in different organs were calculated as organ-specific concentrations multiplied by organ-specific biomass. Therefore, the decrease in NSC concentration under N addition may be used to promote growth of branch and stem and result in increased or constant NSC contents.

N addition decreased the NSC concentration of fine roots, but the NSC concentration of coarse roots was not responsive to N addition. The different physiological functions of coarse and fine roots may account for the different responses in NSC concentrations after N addition. Coarse roots play an important role in resource reserves and transport (Guo et al. 2004; Hartmann and Trumbore 2016). Thus, stable NSC reserves in coarse roots favor the survival of plants during environmental change. Fine roots are mainly responsible for nutrient uptake (Mei et al. 2015). Based on the optimal partitioning theory, N addition alleviates nutrient limitation, so that plants do not have to increase fine root production and promote mycorrhizal infection (Zak et al. 2000; Luo et al. 2004). Thus, the allocation of NSC to fine roots is reduced (Kobe et al. 2010). Another explanation is that the N content in fine roots is higher than in coarse roots (Fig. S3) (Guo et al. 2004). Root respiration rate is strongly and positively correlated with N content (Burton et al. 2012; Mei et al. 2015). Thus, compared with coarse roots, the much higher level of NSC in fine roots is likely used to maintain respiration.

The proportion of the NSC content in aboveground organs to the NSC content of roots was not responsive to N addition. However, we found that N addition increased structural biomass more in aboveground organs than in root, possibly because the increase in aboveground structural biomass rather than an increase in NSC reserve was conducive to alleviating the C limitation caused by N addition. Previous studies have demonstrated that the stimulation of plant growth and N assimilation by N addition could result in a C limitation (Invers et al. 2004; Monson et al. 2006; Liu et al. 2016). Bloom et al. (1985) reported that plants may maximize their net photosynthetic rates through the timely reinvestment of carbohydrates into the continuous production of new leaves, so that the plant can enhance photosynthate acquisition. Similarly, in our study, photosynthates were mainly used for needle production, branch elongation,

and stem growth to optimize light harvest by maximizing spatial occupation rather than being stored as NSC (Imaji and Seiwa 2010).

## Conclusions

In our study of organ-specific NSC responses to CO$_2$ elevation and N addition, CO$_2$ elevation promoted NSC accumulation in all organs, and NSC contents were preferentially allocated to roots rather than to aboveground organs. This allocation pattern may be related to nutrient limitation experienced by plant under CO$_2$ elevation and the increased use of NSC as an energy source by roots to increase nutrient absorption. N addition reduced NSC concentrations in needles, branches, stems, and fine roots and increased the NSC contents in branches and coarse roots. On a whole-tree basis, structural biomass was preferentially partitioned to aboveground organs instead of underground roots under N addition. This partitioning pattern indicates that timely reinvestment of carbohydrate in growth rather than in NSC reserves helps alleviate C limitation under N addition. Our results indicated that different trade-offs between structural growth and NSC storage help ameliorate resource limitations under CO$_2$ elevation and N addition in Chinese fir. Our results thus show that it is important to separate biomass into structural biomass and NSC storage when analyzing the effect of environmental change on biomass allocation.

## References

Arp WJ, Drake BG (1991) Increased photosynthetic capacity of *Scirpus olneyi* after 4 years of exposure to elevated CO$_2$. Plant Cell Environ 14:1003–1006

Bloom AJ, Chapin FS, Mooney HA (1985) Resource limitation in plants: an economic analogy. Annu Rev Ecol Syst 16:363–392

Burton AJ, Jarvey JC, Jarvi MP, Zak DR, Pregitzer KS (2012) Chronic N deposition alters root respiration-tissue N relationship in northern hardwood forests. Glob Chang Biol 18:258–266

Canham CD, Kobe RK, Latty EF, Chazdon RL (1999) Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. Oecologia 121:1–11

Carbone MS, Czimczik CI, Keenan TF, Murakami PF, Pederson N, Schaberg PG, Xu X, Richardson AD (2013) Age, allocation and availability of nonstructural carbon in mature red maple trees. New Phytol 200:1145–1155

Cechin I, Fumis TdF (2004) Effect of nitrogen supply on growth and photosynthesis of sunflower plants grown in the greenhouse. Plant Sci 166:1379–1385

Cheng L, Ma F, Ranwala D (2004) Nitrogen storage and its interaction with carbohydrates of young apple trees in response to nitrogen supply. Tree Physiol 24:91–98

Curtis PS, Wang X (1998) A meta-analysis of elevated CO$_2$ effects on woody plant mass, form, and physiology. Oecologia 113:299–313

De Souza AP, Gaspar M, Da Silva EA, Ulian EC, Waclawovsky AJ, Nishiyama MY Jr, Dos Santos RV, Teixeira MM, Souza GM, Buckeridge MS (2008) Elevated CO$_2$ increases photosynthesis, biomass and productivity, and modifies gene expression in sugarcane. Plant Cell Environ 31:1116–1127

Dietze MC, Sala A, Carbone MS, Czimczik CI, Mantooth JA, Richardson AD, Vargas R (2014) Nonstructural carbon in woody plants. Annu Rev Plan Biol 65:667–687

Dijkstra FA, Cheng W (2007) Interactions between soil and tree roots accelerate long-term soil carbon decomposition. Ecol Lett 10:1046–1053

Dong J, Hunt J, Delhaize E, Zheng SJ, Jin CW, Tang C (2021) Impacts of elevated CO$_2$ on plant resistance to nutrient deficiency and toxic ions via root exudates: a review. Sci Total Environ 754:142434

Du Y, Lu R, Xia J, Martin A (2020) Impacts of global environmental change drivers on non- structural carbohydrates in terrestrial plants. Funct Ecol 34(8):1525–1536

Evans JR, Terashima I (1988) Photosynthetic characteristics of spinach leaves grown with different nitrogen treatments. Plant Cell Physiol 29:157–165

Foyer CH, Parry M, Noctor G (2003) Markers and signals associated with nitrogen assimilation in higher plants. J Exp Bot 54:585–593

Granath G, Strengbom J, Breeuwer A, Heijmans MM, Berendse F, Rydin H (2009) Photosynthetic performance in *Sphagnum* transplanted along a latitudinal nitrogen deposition gradient. Oecologia 159:705–715

Guo DL, Mitchell RJ, Hendricks JJ (2004) Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. Oecologia 140:450–457

Guo HX, Xu B, Wu Y, Shi FS, Wu C, Wu N (2016) Allometric partitioning theory versus optimal partitioning theory: the adjustment of biomass allocation and Internal C-N balance to shading and nitrogen addition in *Fritillaria unibracteata* (Liliaceae). Polish J Ecol 64:189–199

Hamilton JG, DeLucia EH, George K, Naidu SL, Finzi AC, Schlesinger WH (2002) Forest carbon balance under elevated CO$_2$. Oecologia 131:250–260

Hartmann H, Trumbore S (2016) Understanding the roles of nonstructural carbohydrates in forest trees–from what we can measure to what we want to know. New Phytol 211:386–403

Hättenschwiler S, Körner C (1997) Biomass allocation and canopy development in spruce model ecosystems under elevated CO$_2$ and increased N deposition. Oecologia 113:104–114

Hicks WK, Leith ID, Woodin SJ, Fowler D (2000) Can the foliar nitrogen concentration of upland vegetation be used for predicting atmospheric nitrogen deposition? Evidence from field surveys. Environ Pollut 107:367–376

Huang JG, Bergeron Y, Denneler B, Berninger F, Tardif J (2007) Response of forest trees to increased atmospheric CO$_2$. Crit Rev Plant Sci 26(5–6):265–283

Idso SB, Kimball BA (1992) Effects of atmospheric CO$_2$ enrichment on photosynthesis, respiration, and growth of sour orange trees. Plant Physiol 99:341–343

Imaji A, Seiwa K (2010) Carbon allocation to defense, storage, and growth in seedlings of two temperate broad-leaved tree species. Oecologia 162:273–281

Invers O, Kraemer GP, Pérez M, Romero J (2004) Effects of nitrogen addition on nitrogen metabolism and carbon reserves in the temperate seagrass *Posidonia oceanica*. J Exp Mar Biol Ecol 303:97–114

Kobe RK, Iyer M, Walters MB (2010) Optimal partitioning theory revisited: Nonstructural carbohydrates dominate root mass responses to nitrogen. Ecology 91:166–179

Kozlowski TT (1992) Carbohydrate sources and sinks in woody plants. Bot Rev 58(2):107–222

Kraemer GP, Mazzella L, Alberte RS (1997) Nitrogen assimilation and partitioning in the mediterranean seagrass *Posidonia oceanica*. Mar Ecol 18:175–188

Li MH, Cherubini P, Dobbertin M, Arend M, Xiao WF, Rigling A (2013) Responses of leaf nitrogen and mobile carbohydrates in different *Quercus* species/provenances to moderate climate changes. Plant Biol 15:177–184

Li RS, Yang QP, Zhang WD, Zheng WH, Wang SL (2018a) Response of nonstructural carbohydrates to thinning and understory removal in a Chinese fir [*Cunninghamia lanceolata* (Lamb.) Hook] plantation. Trees 32:801–808

Li WB, Hartmann H, Adams HD, Zhang HX, Jin CJ, Zhao CY, Guan DX, Wang AZ, Yuan FH, Wu JB (2018b) The sweet side of global change-dynamic responses of non-structural carbohydrates to drought, elevated $CO_2$ and nitrogen fertilization in tree species. Tree Physiol 38:1706–1723

Liu XJ, Zhang Y, Han WX, Tang AH, Shen JL, Cui ZL, Vitousek P, Erisman JW, Goulding K, Christie P, Fangmeier A, Zhang FS (2013) Enhanced nitrogen deposition over China. Nature 494:459–462

Liu J, Wu NN, Wang H, Sun JF, Peng B, Jiang P, Bai E (2016) Nitrogen addition affects chemical compositions of plant tissues, litter and soil organic matter. Ecology 97:1796–1806

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants face the future. Annu Rev Plant Biol 55:591–628

Lü XT, Reed SC, Yu Q, Han XG (2016) Nutrient resorption helps drive intra-specific coupling of foliar nitrogen and phosphorus under nutrient-enriched conditions. Plant Soil 398:111–120

Luo YQ, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, Murtrie REM, Oren R, Parton WJ (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience 54:731–739

Luo ZB, Calfapietra C, Liberloo M, Scarascia-Mugnozza G, Polle A (2006) Carbon partitioning to mobile and structural fractions in poplar wood under elevated $CO_2$ (EUROFACE) and N fertilization. Global Change Biol 12:272–283

Martínez-Vilalta J, Sala A, Asensio D, Galiano L, Hoch G, Palacio S, Piper FI, Lloret F (2016) Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. Ecol Monogr 86:495–516

Mašková P, Radochová B, Lhotáková Z, Michálek J, Lipavská H (2017) Nonstructural carbohydrate-balance response to long-term elevated $CO_2$ exposure in European beech and Norway spruce mixed cultures: biochemical and ultrastructural responses. Can J for Res 47:1488–1494

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–739

Mei L, Xiong YM, Gu JC, Wang ZQ, Guo DL (2015) Whole-tree dynamics of non-structural carbohydrate and nitrogen pools across different seasons and in response to girdling in two temperate trees. Oecologia 177:333–344

Mo JM, Li DJ, Gundersen P (2008) Seedling growth response of two tropical tree species to nitrogen deposition in southern China. Eur J Forest Res 127:275–283

Monson RK, Rosenstiel TN, Forbis TA, Lipson DA, JaegerIII CH (2006) Nitrogen and carbon storage in alpine plants. Integr Comp Biol 46:35–48

Nakaji T, Fukami M, Dokiya Y, Izuta T (2001) Effects of high nitrogen load on growth, photosynthesis and nutrient status of *Cryptomeria japonica* and *Pinus densiflora* seedlings. Trees 15:453–461

Nehls U, Gohringer F, Wittulsky S, Dietz S (2010) Fungal carbohydrate support in the ectomycorrhizal symbiosis: a review. Plant Biol 12:292–301

Norby RJ, Hanson PJ, O'Neill EG, Tschaplinski TJ, Weltzin JF, Hansen RA, Cheng WX, Wullschleger SD, Gunderson CA, Edwards NT (2002) Net primary productivity of a $CO_2$-enriched deciduous forest and the implications for carbon storage. Ecol Appl 12:1261–1266

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE (2010) $CO_2$ enhancement of forest productivity constrained by limited nitrogen availability. Proc Natl Acad Sci USA 107:19368–19373

Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schäfer KVR, Mccarthy H, Hendrey G, Mcnulty SG (2001) Soil fertility limits carbon sequestration by forest ecosystems in a $CO_2$-enriched atmosphere. Nature 411:469–472

R Core Team (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Rothstein DE, Zak DR, Pregitzer KS, Curtis PS (2000) Kinetics of nitrogen uptake by *Populus tremuloides* in relation to atmospheric $CO_2$ and soil nitrogen availability. Tree Physiol 20:265–270

Sala A, Woodruff DR, Meinzer FC (2012) Carbon dynamics in trees: feast or famine? Tree Physiol 32:764–775

Smart D, Chatterton J, Bugbee B (1994) The influence of elevated $CO_2$ on nonstructural carbohydrate distribution and fructan accumulation in wheat canopies. Plant Cell Environ 17:435–442

Smith SE, Smith FA (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annu Rev Plant Biol 62:227–250

Tissue DT, Griffin KL, Turnbull MH, Whitehead D (2001) Canopy position and needle age affect photosynthetic response in field-grown *Pinus radiata* after five years of exposure to elevated carbon dioxide partial pressure. Tree Physiol 21:915–923

Wang QK, Zhang WD, Sun T, Chen LC, Pang XY, Wang YP, Xiao FM (2017) N and P fertilization reduced soil autotrophic and heterotrophic respiration in a young *Cunninghamia lanceolata* forest. Agr for Meteorol 232:66–73

Wurth MK, Pelaez-Riedl S, Wright SJ, Korner C (2005) Non-structural carbohydrate pools in a tropical forest. Oecologia 143:11–24

Yang QP, Zhang WD, Li RS, Xu M, Wang SL (2016) Different responses of non-structural carbohydrates in above-ground tissues/organs and root to extreme drought and re-watering in Chinese fir (*Cunninghamia lanceolata*) saplings. Trees 30:1863–1871

Zak DR, Pregitzer KS, King JS, Holmes WE (2000) Elevated atmospheric $CO_2$, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytol 147:201–222

Zha TS, Wang KY, Ryyppö A, Kellomäki S (2002) Impact of needle age on the response of respiration in Scots pine to long-term elevation of carbon dioxide concentration and temperature. Tree Physiol 22:1241–1248