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**The species-specific responses of nutrient resorption and carbohydrate accumulation in leaves
and roots to nitrogen addition in a subtropical mixed plantation**

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Abstract:

Ephemeral tissues such as leaves and fine roots are sensitive to nutrient alteration. Whether nutrient addition can influence the linkage between nutrient resorption and carbohydrate accumulation in leaves and roots is not clear. We measured nitrogen (N) and phosphorus (P) concentrations, and non-structural carbohydrates (NSC) of the <one- and >one-year old leaves, absorptive and transportive roots in two species of a mixed plantation during the dormant and growing seasons within a nitrogen addition experiment. N addition decreased N and P resorption efficiencies (NRE and PRE, respectively) in leaves of Chinese fir and increased PRE in absorptive roots of Chinese fir, but did not alter either efficiency in any tissues of Chinese sweetgum. N addition increased starch accumulation efficiency (STAE) in >one-year-old leaves of Chinese fir, but decreased soluble sugar accumulation efficiency (SSAE) in absorptive roots of Chinese sweetgum. Both NRE and PRE were negatively correlated with SSAE, STAE and NSC accumulation efficiency (NSCAE) in >one-year-old leaves of Chinese fir, but this pattern was not found in leaves of Chinese sweetgum. Our study indicates that nitrogen addition can influence the linkage between nutrient resorption and non-structural carbohydrate in leaves and roots, and this response to nutrient availability is species dependent.

Key words:

Nitrogen enrichment, Non-structural carbohydrates, Nutrient resorption, plant tissues; Subtropical tree species

61 Introduction

62 Nutrient and carbon economy are important determinants of tree function in forest ecosystems.
63 One key mechanism influencing a tree's nutrient economy is resorption—withdrawal of nutrients from
64 senesced organs and subsequent transport to storage or growing organs (Killingbeck 1996). Resorption
65 is an important nutrient conservation strategy, ensuring requisite nutrient availability for tree growth
66 and reducing dependence on external nutrients (Chen et al. 2015; Hu et al. 2011). However, the relative
67 importance of nutrient resorption and acquisition of nutrients from the soil depends on the energy
68 consumption required by each strategy (Vergutz et al. 2012). The acquisition of nutrients via resorption
69 usually involves lower energetic cost relative to acquisition from nutrient-poor soil (Wang et al. 2014),
70 and the opposite pattern may be shown in nutrient-rich soil (Vergutz et al. 2012). For either strategy,
71 energy is derived from non-structural carbohydrates (NSC), such as soluble sugar and starch (Dietze et
72 al. 2014). While the coupling relationship between nutrient resorption and NSC accumulation in
73 response to nutrient availability has not yet been clarified, improved understanding of this relationship
74 may in turn shed light on plant adaptation strategies with respect to nutrient and carbon economy under
75 the pressure of global change in forest ecosystems (Zhang et al. 2015).

76 Anthropogenic nitrogen (N) enrichment of the terrestrial biosphere from atmospheric
77 sources—following N emission to the atmosphere from agricultural, industrial, and urban sources—is a
78 key factor influencing tree growth and forest productivity in rapidly developing regions such as China,
79 Europe and North America (Chen et al. 2015; Liu et al. 2011). Ephemeral tissues such as leaves and
80 fine roots are sensitive to environmental change and often used to observe the response of plant
81 nutrients to N addition (Chen et al. 2017; Kou et al. 2017). Nutrient resorption helps predict the effects
82 of N input on tree growth since internal nutrient fluxes are generally larger than the external nutrient
83 fluxes associated with leaching and gaseous emissions, even under high exogenous N inputs (Gundale
84 et al. 2014). Tree N resorption generally decreases due to increased N input (Van Heerwaarden et al.
85 2003), while P resorption might show positive, negative or neutral responses to N input, with the
86 response mostly depending on plant species (Lu et al. 2016; van Heerwaarden et al. 2003). Although
87 studies of how N addition affects tree nutrient resorption are relatively abundant (Minocha et al. 2015),
88 the effects of N addition on non-structural carbohydrates accumulation in leaves and fine roots remain
89 uncertain (Guo et al. 2016). Kou et al. (2017) found different responses of carbon sources (leaves) and
90 carbon sinks (roots) to N addition. As we know, NSC is the main form of stored tree energy, and the

primary source of energy for metabolism to support plant growth (Dietze et al. 2014; Trumbore et al. 2015; Wiley and Helliker 2012). Therefore, improved understanding of NSC accumulation and translocation in tissues undergoing development and senescence would help explain plant nutrient resorption in response to N addition (Invers et al. 2004). Although a recent meta-analysis from 52 studies demonstrated only weak linkages between changes in nutrient availability and NSC, they also showed that: NSC with more than 20 observations was most significantly changed by N addition and the effects of N addition on C chemical composition varied among different plant types (Liu et al. 2016). Therefore, the assessment of the linkage between nutrient resorption and NSC accumulation might offer an unrevealed mechanism of tree adaptation to environmental change (Fajardo and Piper 2017).

In subtropical China, the forests suffer from dual pressures of soil P limitation and atmospheric N deposition (Hietz et al. 2011; Liu et al. 2013). Increased anthropogenic N addition is expected to increase P demand and limitation, since trees would need more P in the face of increased foliar N concentration to maintain stoichiometric homeostasis (Chen et al. 2015; Huang et al. 2016). Although it is assumed that increased N availability would lead to a more conservative usage of P in terrestrial ecosystems through stimulated plant growth (Vitousek et al. 2010), empirical evidence remains rare. Ecological stoichiometry has recently been suggested as a potential indicator of nutrient limitation in trees, including tissue N/P ratios (Sardans et al. 2012), and N/P resorption ratios (Reed et al. 2012). Moreover, Reed et al. (2012) uncovered linkages between soil nutrient status and plant N/P resorption ratios along local and regional gradients in tropical ecosystems. As aforementioned, trees may develop two non-mutually exclusive advantageous strategies to adapt to nutrient alteration, including nutrient acquisition regulation (acquisition species) through root nutrient uptake and nutrient conservation adjustment (conservative species) through nutrient resorption in order to maintain their stoichiometric homeostasis (Kou et al. 2017). The tradeoff between acquisition and conservation in tree nutrient economy depends on the relative energy consumption of these two processes—which process is energetically ‘cheaper’ (Wright and Westoby 2003). As these strategies may depend on tree species, how subtropical trees differ in response to N addition among species shall be intensively studied. Our previous study found that N deposition alters the supplies of N and P, and the stability of organic carbon in soils, and the effect of N deposition differs among tree species with divergent mechanisms, such as evergreen versus deciduous tree species of the subtropics (Wang et al. 2016). There are two

principle species (Chinese fir, *Cunninghamia lanceolata* and Chinese sweetgum, *Liquidambar formosana*) for mixed afforestation in South China. Chinese fir (A evergreen tree species) usually grows in rich soil with higher nutrient requirement (Chen et al. 2015), while Chinese sweetgum (A deciduous tree species) tends to be planted in poor soil and is usually considered as a pioneer tree species (Fang et al. 2015). Nitrogen and P concentrations, and N/P ratio were higher for Chinese fir than Chinese sweetgum in roots, although generally reversed in leaves. Moreover, there are distinct traits of the two species with different leaf phenologies and specific root length (Liu et al. 2010). Previous studies have reported that trees associated with higher specific root length generally show strong nutrient and water acquisition and can grow well on nutrient-poor soils (Liu et al. 2010; Ma et al. 2018). Therefore, we expected that the physiological linkage between nutrient resorption and carbohydrate accumulation in plant tissues in response to changes in nutrient availability may differ between these two species. In this study, we examined nutrient and NSC concentrations and their resorption and accumulation efficiencies in leaves and fine roots of these two dominant trees in a subtropical mixed forest plantation, in order to explore interspecific differences in coupling responses of nutrient and carbon economies to N addition. We hypothesized that: (I) N addition increases N concentration, P resorption efficiency (PRE), and N:P ratio, while decreasing N resorption efficiency (NRE), P concentration, and NRE:PRE ratio in the leaves and fine roots (Kou et al. 2017; Wang et al. 2014); (II) How can N addition influence the linkage between nutrient resorption efficiency and NSC /NSC accumulation efficiency (NSCAE) in the leaves and fine roots, since plant nutrient resorption requires consumption of NSC as nutrient retranslocation energy (Dietze et al. 2014); (III) N addition can influence the linkage between nutrient resorption efficiency and carbohydrate accumulation in leaves and roots, and these linkages in response to changes in nutrient availability are species dependent, since Chinese fir and Chinese sweetgum may develop two different strategies.

Materials and methods

Study site

The study site is located at the Qianyanzhou Ecological Research Station, Chinese Academy of Sciences, Taihe County, Jiangxi Province, China (26°44' N, 115°04' E) (Fig. S1). The site has a subtropical moist monsoonal climate, with a warm, dry summer and a cool, wet winter. The average annual precipitation is 1600 mm with about 50% of the total precipitation occurring from April to June.

Maximum and minimum average monthly temperatures are 29.7 °C in July and 6.5 °C in January, and the average annual temperature is 17.9 °C. The soil is the typical Hapludult Ultisols (locally "red soil"), which was developed from red sandstone and covers over 60% of 1.14 million km² of total land area in southeastern China. The elevation ranges from 30 to 200 m above sea level. The soil is acid with a pH value of about 4.5 and low P availability (Chen et al. 2015). Soil texture is sandy loam with 68% sand and 15% clay (Xiong et al. 2015). With industrialization, intensive agricultural management, and human population growth, atmospheric N deposition has reached 30 kg⁻¹ ha⁻¹ in most parts of southern China over the past several decades (Kou et al. 2017).

Experimental design

At the end of 2011, we applied N to a 15-year-old mixed forest plantation of Chinese fir and Chinese sweetgum (see Table S1 and Fig. S1) with 10 g N m⁻² yr⁻¹. Chinese fir is an evergreen coniferous tree species with an average needle lifespan of 2.5 years (unpublished observed data), and widely planted in subtropical China (Huang et al. 2013). Chinese sweetgum is a broadleaved tree species with an average leaf lifespan of 7.5 months (unpublished observed data). Both tree species were planted with seedlings from a local nursery after clear-cut harvesting and slash burning of a mature forest in 1996 (Chen et al. 2015). Four pairs of 20 m × 20 m plots were laid out along a discontinuous hilly slope (~15°), and randomly assigned N addition and control treatments within each plot. The initial properties of tree and soil between the N addition treatment and control plots were not significantly different (Table S1). Nitrogen was added four times each year in March (30%), June (30%), September (20%) and December (20%) in the form of NH₄NO₃. The fertilizer was mixed with 8 kg of clean and dry fine sand, and then applied to each treatment plot on a sunny day. The application dates were chosen based on the daily weather forecast to ensure no rainfall within the next several days. The control plots received the same amount of fine sand without N addition. To ensure well-distributed of N applications within a plot, we divided each plot into 16 5×5 m subplots, where the fertilizer could be equally spread.

Sample collection and tree monitoring

In November of 2011, tree characteristics including stand density (number of individuals per plot), diameter at breast height (DBH), and height were measured in the 8 (i.e., randomly selected 4 N addition and 4 control) plots. Understory plant biomass was measured in 8 random 1×1 m quadrats within each 20 m × 20 m plot. In addition, soil (0–10 cm) was collected from 36 random points using

181 soil cores of 2.5 cm diameter; subsamples were mixed to provide a single sample for each plot. These
182 samples were immediately brought back to the laboratory and air-dried after the removal of roots and
183 all organic debris for analyses of soil general properties.

184 Rhizosphere soil, root, and leaf were sampled from three standard trees for each species in the
185 eight plots. In August 2015, living roots (<2 mm) and adhering rhizosphere soil under the tree canopy
186 were carefully sampled along north-south transects. Soil adhering to living roots after gentle shaking
187 was defined as rhizosphere soil. All samples were kept cold in the field and refrigerated at 4 °C until
188 processing. The fresh soil samples were immediately used for the determination of available nutrients
189 (See [Wang et al. 2016](#)). The roots were refrigerated at 4 °C and further divided into absorptive and
190 transportive roots according to their different absorptive functions within a week (Gu et al. 2016; Hu et
191 al. 2017). The biomass ratio of absorptive roots to transportive roots was about 0.68, with much shorter
192 lifespan for absorptive than transportive roots. The samples of absorptive roots and transportive roots
193 were mixed to obtain a composite sample for each plot.

194 Meanwhile, the living leaves from one first order branch with full sunlight were collected from
195 the standard trees. The needles of Chinese fir were divided into <one-year-old and >one-year-old
196 needles based on their locations with respect to branch orders ([Chen et al. 2015](#)), while the Chinese
197 sweetgum only had <one-year-old leaves.

198 In addition, aboveground litters of Chinese fir and Chinese sweetgum were collected in March
199 2016 and October 2015 under the canopies of three standard trees within each plot to calculate nutrient
200 resorption and NSC accumulation. The leaves and twigs of Chinese fir generally grow and senesce at
201 the same time, and fall together in the form of whole branches, but the dead leaves and twigs usually
202 stay on the stem for several years before falling. Thus, leaf litters of three dead hanging branches from
203 different trees were collected in each plot and mixed as a composite sample for <one-year-old
204 versus >one-year-old needle litters by the previously described protocols([Chen et al. 2015](#)).

205 For the sampling of root litter, we divided dark, black roots with hard and brittle texture into dead
206 roots, these dehydrated or dry roots with soft and resilient texture into dormant roots, and normal color
207 roots with soft and resilient texture as normal living roots in dormant season. We considered that there
208 is a nutrient resorption process from normal living root to dormant root, and then from normal living
209 root to dead root. Only the normal living root was sampled for analysis in August 2015, while these
210 dead roots and dormant roots were mixed as a composite sample of root litters in January 2016,

because it was difficult to collect enough samples of dead roots. These root samples were also divided into absorptive and transportive roots to represent corresponding root litters. The samples of each plant tissue were mixed to form a composite for each plot, rinsed with deionized water, oven dried at 105 °C for 15 minutes to stop all enzymatic activity (Woodruff and Meinzer, 2011), and then oven dried to a constant mass at 60 °C. After being ground and passed through a sieve (<0.2 mm), the samples were stored in sealed bags at room temperature before nutrient analyses.

Chemical analysis

All variables in soils were determined by the methods provided by Allen (1989), and the data were shown in our previous study (Wang et al. 2016, Tables S2 and S3). Organic C was determined using the Walkley–Black wet oxidation method (Allen 1989). Total N and total P in plant tissues were determined by the Kjeldahl method and by the molybdenum-antimony colorimetric method after the samples were digested with H₂SO₄ (Allen 1989). Plant soluble sugars were extracted from tree tissues in 80% v/v ethanol and starch was extracted from the ethanol-insoluble fraction with 55% v/v sulphuric acid (Guo et al. 2016). Soluble sugars and starch were determined colorimetrically using the anthrone-sulphuric acid reagent method and the absorbance was determined in the wavelength of 625 nm with a spectrophotometer (Yemm and Willis 1954). Non-structural carbohydrates (NSC, g kg⁻¹) are defined here as the sum of soluble sugars and starch.

Calculation and statistical analysis

Nutrient resorption was calculated on a mass basis (mg nutrient per unit mass) and does not account for mass loss during senescence (Aerts 1996; Chen et al. 2012). Nutrient resorption efficiency was the proportion of nutrients resorbed prior to rapid turnover organ abscission and was calculated using the formula of Aerts (1996), i.e., nutrient resorption efficiency = [(nutrient in living tissue – nutrient in the litter tissue)/(nutrient in living tissue)] × 100%. In this study, NRE and PRE were determined separately for <one-year-old and >one-year-old leaves (as available) or for absorptive and transportive roots. Likewise, soluble sugar, starch and NSC accumulation efficiencies (SSAE, STAE and NSCAE) were the proportions of soluble sugar, starch and NSC accumulated prior to rapid turnover organ abscission, respectively. For example, STAE=[(starch in litter tissue –starch in the living tissue)/(starch in the living tissue)] × 100%.

The data were tested for homogeneity of variances (Levene's test) before statistical analysis. Multiple-way analysis of variance (ANOVA) was used to determine the effect of N addition and its

interaction with other factors such as tree species or tissue age/order on nutrient variables for each of sampling time. Pearson's correlation analysis was used to determine the relationship among nutrients and NSC parameters within a same tissue for each tree species. All statistical analyses were performed with the SPSS 11.5 for Windows statistical software package (SPSS 2007). The statistical tests were considered significant at the $\alpha = 0.05$ level.

Results

Tree nutrient dynamics and their resorptions

N addition increased total N concentration in >one-year-old leaves and transportive roots of Chinese fir by 20% and 26%, respectively, but not for tissues of Chinese sweetgum. Total P concentration was not altered by N addition in any tissues of either species (Fig. 1). Total N and P concentrations were the highest in leaves, followed by absorptive roots, and the lowest in transportive roots for both tree species (Fig. 1). Meanwhile, both N and P concentrations of leaves were lower in Chinese fir than Chinese sweetgum, but were reversed in roots (Fig. 1).

The N/P ratio increased by 14% and 29% under N addition in leaves of Chinese fir and Chinese sweetgum, respectively, but was not altered in roots of either species. The N/P ratio of leaf was lower in Chinese fir than Chinese sweetgum, but was reversed in roots (Fig. 1). In addition, the C/N and C/P ratios also varied with N addition, tree tissue, and species (Table S4).

Except NRE in <one-year-old leaves, both NRE and PRE decreased by about 15% with N addition in leaves of Chinese fir. PRE in absorptive roots increased by 38% with N addition for Chinese fir. However, both NRE and PRE were not altered by N addition in leaves and roots of Chinese sweetgum (Fig. 2). NRE and PRE were higher in leaves than roots for both tree species (Fig. 2). In addition, N addition significantly increased NRE/PRE ratio in <one-year-old leaves from 1.01 ± 0.13 (Mean $\pm$ SE, $n=4$) to 1.54 ± 0.66 for Chinese fir and from 0.56 ± 0.23 to 1.08 ± 0.32 in leaves of Chinese sweetgum, respectively.

Tree non-structural carbohydrates dynamics and accumulations

Soluble sugar concentration was not altered by N addition in any observed tissues of either tree species, while N addition decreased starch concentration by 40% in >one-year old leaves of Chinese fir, but increased 96% and 64% of starch concentration, respectively in absorptive and transportive roots of Chinese sweetgum. The NSC decreased with N addition only in >one-year old leaves of

Chinese fir (Fig. 3). In addition, the ratios of starch and NSC to organic carbon decreased with N addition in >one-year old leaves of Chinese fir during the growing season, while the ratio of starch to organic carbon increased with N addition in absorptive and transportive roots of Chinese sweetgum (Table S4).

SSAE, STAE and NSCAE were not altered by N addition except that SSAE and STAE increased in absorptive roots of Chinese sweetgum and in >one-year old leaves of Chinese fir (Fig. 4). SSAE, STAE and NSCAE generally were lower in aboveground tissues than belowground tissues for both tree species. Meanwhile, SSAE was significantly lower than STAE in aboveground and belowground tissues, respectively (Fig. 4).

Linkages among nutrients and non-structural carbohydrates

Leaf NRE was only negatively ($p < 0.05$) related to N concentration in leaf litters for Chinese fir and with organic carbon in leaf litters for Chinese sweetgum. Leaf PRE was positively and negatively related to NSC and N/P, respectively in living leaves, and negatively related with N and P in leaf litters for Chinese fir, while leaf PRE only negatively related with N/P in living leaves and P in leaf litters for Chinese sweetgum. Leaf NSCAE was negatively related to NSC and NSC/OC in living leaves, but positively related to N, NSC and NSC/OC in leaf litters for Chinese fir. The correlations between leaf NSCAE and all measured variables in living leaves and leaf litters were not found in Chinese sweetgum (Table 1).

Root NRE was positively related to NSC and N/P in roots during the growing season and negatively related to N in roots during the dormant season for Chinese fir, while positively related to N and P in roots during the growing season for Chinese sweetgum. Root PRE was only positively related to N/P in roots during the dormant season for Chinese fir, as well as P in roots during the growing season for Chinese sweetgum. Root NSCAE was negatively related to NSC and NSC/OC in roots during the growing season and positively related to NSC, N/P and NSC/OC in roots during the dormant season for Chinese fir. For Chinese sweetgum, root NSCAE was positively related to NSC and NSC/OC in roots during the dormant season (Table 1).

For Chinese fir, NRE and PRE were negatively related to SSAE, STAE and NSCAE in >one-year old leaves, while positive relations were only found between NRE and STAE in <one-year old leaves. NRE was only negatively related to STAE in absorptive roots, while PRE was only negatively related to SSAE in transportive roots of Chinese fir. In contrast, NRE and PRE were only negatively related to

SSAE in absorptive roots of Chinese sweetgum, and no significant correlations between nutrient resorption and NSCAE were found in leaves of Chinese sweetgum (Table 2).

Discussion

Our study mainly demonstrates that N addition can influence the physiological linkage between nutrient resorption efficiency and non-structural carbohydrate (NSC) accumulation in leaves and roots, and that the response of the physiological relationship to changes in nutrient availability is species dependent in a subtropical plantation.

Nitrogen addition effects on nutrient dynamics of leaves and fine roots

Some NREs were altered by changes in nutrient availability in a species-specific manner, with different organs responding in different species. Partially consistent with our hypothesis I, N addition increased N concentrations in >one-year old leaves, transportive roots of Chinese fir, and N/P ratios in leaves of both species, while decreased NRE in >one-year old leaves of Chinese fir. Several mechanisms, including the differences between N and P biogeochemistry, soil acidification, and stoichiometric homeostasis (Long et al. 2016), could help explain the relatively rapid changes in N and P status in ecosystems as a consequence of N addition. In contrast, N concentration and NRE in <one-year-old Chinese fir leaves showed an insignificant response to N addition, which was inconsistent with our expectations. Since new organs may acquire nutrients from storage organs, such as twigs and stems (Aerts 1996), young tissues (<one-year-old leaves) should be less dependent on soil nutrient supply than old tissues (>one-year-old leaves). PRE increased in absorptive roots but decreased in leaves of Chinese fir due to N addition. In contrast, N and P concentrations, NRE, and PRE were not affected by N addition in any sampled organs of Chinese sweetgum. Variability in nutrient concentration and resorption efficiency was generally driven by changes in source (i.e., nutrient supply in soil pools) and sink (i.e., nutrient demand for growth), and their relative strength. Kobe et al. (2005) found that plant nutrient resorption efficiency generally decreased with increasing nutrient availability in soils. However, Aerts (1996) examined 24 species of evergreen trees and shrubs and found that only five species significantly increased resorption efficiencies under low nutrient conditions. Deng et al. (2016) conducted a 4-year N-addition experiment in larch (*Larix principis-rupprechtii*) plantations, and found that PRE exhibited little plasticity since the tree activated a variety of P-acquisition pathways to alleviate the P-deficiency caused by N deposition. Therefore, we

suggested that Chinese sweetgum, with low nutrient plasticity, has a stronger internal mechanism than Chinese fir with high nutrient plasticity to maintain homeostasis in organs nutrients.

We also found that NRE/PRE and N/P ratios in <one-year-old leaves for Chinese fir and Chinese sweetgum increased under N addition. Clearly, the single coupling between tree N and P internal cycles in response to a shift in nutrient limitation driven by N addition cannot provide a satisfactory explanation to simultaneous increases in N/P and NRE/PRE ratios. More active nutrient economy response of Chinese fir than Chinese sweetgum to N addition indicated that fertilization might be a more effective practice to improve tree nutrients and growth in Chinese fir plantations (Zhou et al. 2016).

Nitrogen addition effects on non-structural carbohydrate dynamics of leaves and fine roots

Some NSC efficiencies were altered by changes in nutrient availability in a species-specific manner, with different organs responding in different species. NSC is the primary source of energy for metabolism to support plant growth (Dietze et al. 2014; Trumbore et al. 2015; Wiley and Helliker 2012). Our study found that starch, NSC concentrations, and STAE decreased with N addition in old leaves of Chinese fir, associated with increases in N concentrations and decreases in NRE. Starch serves as an important pool to balance resource utilization and storage and has been found to decline in favorable environments (Fajardo and Piper 2017; Ribeiro et al. 2012). Thus, the decreased starch concentration and STAE in old leaves of Chinese fir was associated with increased N concentrations and decreased NRE as an effect of N addition.

We also identified that SSAE, STAE, and NSCAE were negatively related to NRE and PRE in old leaves of Chinese fir. These results were partly consistent with our hypothesis II. Likewise, negative correlations were found between NSC and NRE in absorptive and transportive roots, between STAE and NRE in absorptive roots, and between SSAE and PRE in transportive roots for Chinese fir, although root NSC, N, P concentrations, NSC accumulation and nutrient resorption efficiencies were not altered by N addition. Clearly, coupling between nutrient and NSC changes driven by N addition occurs in different leaves and fine roots via a nutrient acquisition-conservation tradeoff and homeostatic mechanisms controlling the carbohydrate source-sink balance for Chinese fir, an evergreen tree species.

In contrast, soluble sugar, starch, NSC, and their accumulation efficiencies of leaves were not altered by N addition for Chinese sweetgum, while starch concentration increased in absorptive and

transportive roots, and SSAE increased in absorptive roots. Meanwhile, we found that NSC in Chinese sweetgum roots during the dormant season was positively related to NSCAE, and SSAE were negatively related to NRE and PRE in absorptive roots. Interestingly, Chinese sweetgum leaves showed insignificant response to N addition and positive linkages between nutrients and NSC variables were not observed. In contrast to Chinese fir, the coupling between C and N metabolism mainly occurred in fine roots via a tradeoff between nutrient acquisition and carbohydrates storage in Chinese sweetgum. These observations together with relatively stable root nutrient composition following N addition, lead us to deduce that Chinese sweetgum, a deciduous tree species, increased starch level in absorptive and transportive roots and maintained nutrient stability in leaves and fine roots to adapt to N enrichment.

Nitrogen plays a complex role in regulating carbon metabolism especially starch storage and usage level (Invers et al. 2004). The immediate products of photosynthetic carbon assimilation are partitioned between sucrose (immediately available for growth) and starch, which accumulates in the leaf during the daytime (Fajardo and Piper 2017; Yin et al. 2014), is used during the nighttime for plant respiration and growth (Fajardo and Piper 2017; Ribeiro et al. 2012). In our study, soluble sugar concentrations in leaves and roots varied only slightly, mostly because soluble sugars serve as building blocks for tissue formation and signaling molecules, and/or as gene expression regulators thereby contributing to tree cell proliferation (Guo et al. 2016; Wiley and Helliker 2012). Our study found that starch concentrations were coupled with the N concentrations in both leaves and roots of Chinese fir, but only roots of Chinese sweetgum, which might reflect differing carbon allocation strategies when confronted with soil N enrichment and growth demands (Trumbore et al. 2015).

Linkages between nutrients and non-structural carbohydrates differed in species and tissues

The linkages between NRE and NSC efficiencies were also found to vary by species and tissues. Our results generally supported our hypothesis III that the coupling model between nutrients and NSC changes in response to N addition differed by tree species and by organ. The mechanism controlling this coupling may depend on the relative contribution of N redundancy and P limitation to organ development when forced by N addition (Lu et al. 2013). Our results found that Chinese fir showed a stronger response than Chinese sweetgum to N addition. This is consistent with the habit of trees that Chinese fir usually grows in rich soil with higher nutrient requirement (Chen et al. 2015), while Chinese sweetgum is planted in poor soil considered a pioneer tree species (Fang et al. 2015). Nitrogen and P concentrations, and N/P ratio were higher for Chinese fir than Chinese sweetgum in roots,

although generally reversed in leaves. The distinct traits of the two species with different leaf phenologies and specific root length (Liu et al. 2010) were supported by our findings that N concentrations in leaves and roots increased for Chinese fir but not Chinese sweetgum as an outcome of N addition. Moreover, NRE and PRE decreased in Chinese fir leaves, since exogenous N input not only accelerated N uptake but also might help improve P acquisition (Vitousek et al. 2010).

We expected that Chinese fir would have weaker internal mechanisms for maintaining stoichiometric homeostasis compared to sweetgum, and therefore need more P if foliar N increased. In contrast to the priori expectation, our results demonstrated that leaf PRE of Chinese fir decreased due to N addition. Actually, various effects of N fertilization on P resorption have been reported (Lu et al. 2013; Yuan and Chen 2015) due to the differences of tree species and soil condition. The significant synchronous decrease of starch and NSC in old leaves of Chinese fir indicated that more carbohydrate compounds produced by leaf photosynthesis were transported to roots following N addition in order to increase root biomass, exudates, and P uptake (Deng et al. 2016). In this study, we did not measure root biomass. However, previous studies have reported that plants invest more energy into root growth to overcome P limitation (Kou et al. 2017; Long et al. 2016). Consistent with our expectations, absorptive root PRE of Chinese fir increased due to N addition, which indicated that rapid turnover roots tended to withdraw more P prior to their abscission when subjected to intensified P limitation driven by N addition. Due to the fundamental difference between Chinese fir, which experienced enhanced P limitation due to N addition, and Chinese sweetgum, which experienced increasing N redundancy due to N addition, it was not surprising to see that the coupling model between nutrients and NSC changes differed in tree species. Recently, Zhang et al. (2015) found that the growth rates of tropical plant species are mainly dependent on N supply and unrelated to limiting P availability, even though weathered tropical soils are P deficient. This finding supports our results that the growth rate of Chinese fir slightly increased due to N addition even in a presumably seriously P limited ecosystem.

The negative relationships between NRE or PRE and SSAE, STAE and NSCAE only occurred in >one-year-old leaves, but disappeared in <one-year-old leaves of Chinese fir, since nutrients in both soil and storage tissues preferentially meet the growth of new leaf tissue, but old leaf tissue mostly reflects ecosystem (soil) nutrient supply (Chen et al. 2015). A study reported that evergreen species with a long leaf life span, nutrient and NSC concentrations tended to decrease with leave age (Chen et al. 2015) because light availability generally decreased vertically downwards through the tree canopy

(Wright et al. 2006). Meanwhile, fine root turnover speed is commonly rapid especially for absorptive roots (life span <2.5-year old) than for transportive roots. Therefore, NRE and PRE were negatively related to SSAE only in absorptive roots, but not in transportive roots of Chinese sweetgum potentially due to greater responsiveness of absorptive roots to nutrient alteration (Yin et al. 2014).

Conclusions

Our results support that N addition can influence the linkage between nutrient resorption and non-structural carbohydrates in leaves and roots, and that responses to changes in nutrient availability are species dependent. Nitrogen addition increased tree uptake of N and P, and starch accumulation for Chinese fir, with the aboveground-belowground functional coordination revealed by the negative relations between nutrients resorption and NSC accumulation in leaves and roots. For Chinese sweetgum, N addition mainly increased starch in roots and maintained nutrient stability in leaves and fine roots mostly due to organism homeostasis. These results indicate some acquisition species may alter the balance of nutrient acquirement and energy consumption across leaves and roots under nitrogen deposition and some conservation species may keep the balance due to organism homeostasis. The different adaptation strategies may affect species competition, especially in mixed plantation with acquisition species and conservative species. N deposition may be in favor of the growth and expansion of acquisition species in the mixed plantation, particularly on nutrient-poor sites.

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Table 1. The correlation co-efficients among nutrients and non-structural carbohydrate variables of Chinese fir and Chinese sweetgum in a mixed forest plantation

Tree Species	Chinese fir			Chinese sweetgum		
Tissues	NRE	PRE	NSCAE	NRE	PRE	NSCAE
Living leaves						
N concentration	0.03 ^{ns}	-0.34 ^{ns}	-0.40 ^{ns}	0.38 ^{ns}	-0.56 ^{ns}	0.28 ^{ns}
P concentration	0.11 ^{ns}	0.17 ^{ns}	-0.08 ^{ns}	-0.16 ^{ns}	0.60 ^{ns}	-0.29 ^{ns}
OC concentration	0.33 ^{ns}	0.12 ^{ns}	-0.13 ^{ns}	-0.20 ^{ns}	-0.43 ^{ns}	-0.14 ^{ns}
NSC concentration	0.50 ^{ns}	0.50*	0.86**	-0.48 ^{ns}	-0.11 ^{ns}	0.69 ^{ns}
N/P	-0.05 ^{ns}	-0.57*	-0.44 ^{ns}	0.38 ^{ns}	-0.75*	0.34 ^{ns}
NSC/OC	0.42 ^{ns}	0.45 ^{ns}	0.83**	-0.39 ^{ns}	0.02 ^{ns}	0.66 ^{ns}
Leaf litters						
N concentration	-0.57*	-0.65**	-0.55*	-0.60 ^{ns}	-0.51 ^{ns}	0.21 ^{ns}
P concentration	-0.46 ^{ns}	-0.77**	-0.47 ^{ns}	-0.20 ^{ns}	-0.77*	0.17 ^{ns}
OC concentration	0.40 ^{ns}	0.13 ^{ns}	-0.06 ^{ns}	-0.85**	0.07 ^{ns}	0.13 ^{ns}
NSC concentration	0.01 ^{ns}	-0.24 ^{ns}	-0.72**	-0.53 ^{ns}	0.35 ^{ns}	-0.56 ^{ns}
N/P	-0.48 ^{ns}	-0.24 ^{ns}	-0.41 ^{ns}	-0.58 ^{ns}	0.10 ^{ns}	0.08 ^{ns}
NSC/OC	-0.16 ^{ns}	-0.27 ^{ns}	-0.68**	-0.16 ^{ns}	0.35 ^{ns}	-0.71 ^{ns}
Roots in growing season						
N concentration	0.14 ^{ns}	-0.03 ^{ns}	0.17 ^{ns}	0.67**	0.46 ^{ns}	0.29 ^{ns}
P concentration	-0.48 ^{ns}	0.05 ^{ns}	0.13 ^{ns}	0.70**	0.68**	0.39 ^{ns}
OC concentration	0.48 ^{ns}	0.14 ^{ns}	0.04 ^{ns}	-0.04 ^{ns}	0.13 ^{ns}	-0.16 ^{ns}
NSC concentration	0.50*	-0.17 ^{ns}	0.55*	-0.09 ^{ns}	-0.03 ^{ns}	-0.07 ^{ns}
N/P	0.80**	-0.13 ^{ns}	-0.02 ^{ns}	-0.05 ^{ns}	-0.42 ^{ns}	-0.19 ^{ns}
NSC/OC	0.35 ^{ns}	-0.28 ^{ns}	0.66**	-0.08 ^{ns}	-0.17 ^{ns}	0.07 ^{ns}
Roots in dormant season						
N concentration	-0.68**	0.05 ^{ns}	-0.15 ^{ns}	-0.06 ^{ns}	-0.26 ^{ns}	0.19 ^{ns}
P concentration	-0.39 ^{ns}	-0.50 ^{ns}	0.28 ^{ns}	-0.01 ^{ns}	-0.29 ^{ns}	0.17 ^{ns}
OC concentration	-0.12 ^{ns}	0.22 ^{ns}	-0.08 ^{ns}	-0.43 ^{ns}	-0.25 ^{ns}	0.02 ^{ns}
NSC concentration	0.22 ^{ns}	0.17 ^{ns}	-0.54*	-0.18 ^{ns}	-0.17 ^{ns}	-0.81**
N/P	-0.23 ^{ns}	0.78**	-0.59*	-0.11 ^{ns}	0.13 ^{ns}	-0.03 ^{ns}
NSC/OC	0.25 ^{ns}	0.14 ^{ns}	-0.58*	-0.09 ^{ns}	-0.13 ^{ns}	-0.87**

Note: OC=organic carbon, NSC=non-structural carbohydrates, NRE=nitrogen resorption efficiency, PRE=phosphorus resorption efficiency, NSCAE=non-structural carbohydrates accumulation efficiency; n=16 for living leaves of Chinese fir and n=8 for other tissues. ns=not significant, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$.

<0.01.

Table 2. The correlation co-efficients ($n=8$) among nutrient resorption and non-structural carbohydrate accumulation efficiencies in various tree tissues of Chinese fir and Chinese sweetgum in a mixed forest plantation

Tree Species	Chinese fir			Chinese sweetgum		
Tissues	SSAE	STAE	NSCAE	SSAE	STAE	NSCAE
NRE						
<one-year-old leaves	0.01 ^{ns}	0.85**	0.54 ^{ns}	-0.06 ^{ns}	0.10 ^{ns}	-0.02 ^{ns}
>one-year-old leaves	-0.76*	-0.82*	-0.69*	—	—	—
Absorptive roots	0.38 ^{ns}	-0.68*	-0.46 ^{ns}	-0.68*	0.01 ^{ns}	-0.39 ^{ns}
Transportive roots	0.38 ^{ns}	-0.55 ^{ns}	-0.47 ^{ns}	-0.39 ^{ns}	-0.01 ^{ns}	-0.08 ^{ns}
PRE						
<one-year-old leaves	-0.66*	-0.04 ^{ns}	-0.47 ^{ns}	0.49 ^{ns}	0.07 ^{ns}	0.30 ^{ns}
>one-year-old leaves	-0.67*	-0.80*	-0.62*	—	—	—
Absorptive roots	0.46 ^{ns}	-0.07 ^{ns}	0.13 ^{ns}	-0.65*	-0.03 ^{ns}	-0.42 ^{ns}
Transportive roots	-0.68*	0.39 ^{ns}	0.25 ^{ns}	-0.32 ^{ns}	-0.02 ^{ns}	-0.06 ^{ns}

Note: NRE=nitrogen resorption efficiency, PRE=phosphorus resorption efficiency, SSAE=soluble sugar accumulation efficiency, STAE=starch accumulation efficiency, NSCAE=non-structural carbohydrates accumulation efficiency; ns=not significant, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$.

Figure captions

Fig. 1. Nitrogen addition effect on nutrient concentrations in various tissues of Chinese fir and Chinese sweetgum in a mixed forest plantation.

Note: <1YL=<one-year-old leaves, >1YL=>one-year-old leaves, AR=absorptive roots, TR=transportive roots. Whiskers indicate standard errors. The * and capital letters indicate the differences ($p < 0.05$) between control (CK) and nitrogen addition (+N) treatments, and among the different tree tissues, respectively.

Fig. 2. Nitrogen addition effect on nitrogen and phosphorus resorption efficiencies (NRE and PRE) in various tissues of Chinese fir and Chinese sweetgum in a mixed forest plantation.

Note: <1YL=<one-year-old leaves, >1YL=>one-year-old leaves, AR=absorptive roots, TR=transportive roots. The bars indicate standard errors. The * and capital letters indicate the differences ($p < 0.05$) between control (CK) and nitrogen addition (+N) treatments, and among the different tree tissues, respectively.

Fig. 3. Nitrogen addition effect on non-structural carbohydrates (soluble sugar=SS and starch=ST) in various tissues of Chinese fir and Chinese sweetgum in a mixed forest plantation.

Note: <1YL=<one-year-old leaves, >1YL=>one-year-old leaves, AR=absorptive roots, TR=transportive roots. The whiskers indicate standard errors. The * and capital letters indicate the differences ($p < 0.05$) between control (CK) and nitrogen addition (+N) treatments, and among the different tree tissues, respectively.

Fig. 4. Nitrogen addition effect on soluble sugar and starch accumulation efficiencies (SSAE and STAE) in various tissues of Chinese fir and Chinese sweetgum in a mixed forest plantation. Note:

NSCAE=non-structural carbohydrates accumulation efficiency, <1YL=<one-year-old leaves, >1YL=>one-year-old leaves, AR=absorptive roots, TR=transportive roots. The bars indicate standard errors. The * and capital letters indicate the differences ($p < 0.05$) between control (CK) and nitrogen addition (+N) treatments, and among the different tree tissues, respectively.

Fig. 1.

Fig. 1. Nitrogen addition effect on nutrient concentrations in various tissues of Chinese fir and Chinese sweetgum in a mixed forest plantation.

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Fig. 2.

Fig. 2. Nitrogen addition effect on nitrogen and phosphorus resorption efficiencies (NRE and PRE) in various tissues of Chinese fir and Chinese sweetgum in a mixed forest plantation.

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Fig. 4.

Fig. 4. Nitrogen addition effect on soluble sugar and starch accumulation efficiencies (SSAE and STAE) in various tissues of Chinese fir and Chinese sweetgum in a mixed forest plantation. Note: NSCAE=non-structural carbohydrates accumulation efficiency, <1YL=<one-year-old leaves, >1YL=>one-year-old leaves, AR=absorptive roots, TR=transportive roots. The bars indicate standard errors. The * and capital letters indicate the differences ($p < 0.05$) between control (CK) and nitrogen addition (+N) treatments, and among the different tree tissues, respectively.