Leaf non-structural carbohydrates regulated by plant functional groups and climate: Evidences from a tropical to cold-temperate forest transect

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ABSTRACT

Non-structural carbohydrates (NSCs), e.g., glucose and starch, play important roles in metabolic processes of plants and represent important functional traits in plant's adaptation to external environment. To explore the variations in leaf NSCs among species and communities at a large scale and their influencing factors, we investigated the contents of leaf NSCs among 890 plant species in nine typical forests along the north–south transect of eastern China. The results showed that the contents of leaf soluble sugars, starch, and NSCs (sugars + starch) were highly variable among different plant species on the site scale, and their mean values for the 890 plant species were 45.7 mg g^{-1} , 47.5 mg g^{-1} , and 93.2 mg g^{-1} , respectively. All three metrics varied markedly across plant functional groups in the order of trees < shrubs < herbs. Weak latitudinal patterns of leaf soluble sugars, starch, and NSCs were observed from tropical to cold-temperate forests at the levels of species and plant functional groups. The contents of leaf soluble sugars, starch, and NSCs decreased with increasing temperature and precipitation which supports the growth limitation hypothesis at a large scale. In trees, leaf soluble sugars, starch, and NSCs increased with increasing photosynthetic active radiation (PAR); and were positively correlated with specific leaf area (SLA). The spatial patterns of leaf NSCs in forests along the north–south transect of eastern China and their relationships with temperature, precipitation, PAR, and SLA illustrate an important adaptation of plant communities to environmental changes at the continental scale.

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1. Introduction

Carbohydrates are the primary photosynthate and the key energy source of plant metabolism, and therefore play vital roles in plant's life processes (Volenc et al., 1996; Ögren et al., 1997; Ögren, 2000; Bouma, 2005). Carbohydrates in plants are generally classified as structural carbohydrates or non-structural carbohydrates (NSCs) (Li et al., 2002, 2008). The structural carbohydrates, such as lignin and cellulose, are primarily used for the construction of plant structural support tissues or long-term reserves (Li et al., 2002; Pan et al., 2002; Yin et al., 2009). NSCs, mainly as glucose and starch, are used in metabolic processes of photosynthesis, respiration, and production (Koch, 1996; van den Ende et al., 1999). The content of NSCs and their composition (ratio of glucose:starch) reflect the balance between carbon sources and sinks in plants (Mooney, 1972; Chapin et al., 1990; Hoch et al., 2003; Würth et al.,

2005) and indicate the adaptive strategies of plants to changing environments (Trom et al., 1989; Koch, 1996; Loewe et al., 2000). Gough et al. (2009) have demonstrated that, in temperate deciduous forests, 55% of the annual net carbon assimilation in plant canopy is firstly allocated to labile carbon production (main NSCs) rather than to structural carbon production, where labile carbon produced during late summer supported the structural growth and respiration in dormant season. The content of NSCs and their allocation ratios in leaves, roots, and stems are considered as an eco-physiological attribute to evaluate the carbon budgets and adaptive strategies of plant species (Chapin et al., 1990; Li et al., 2001; Myers and Kitajima, 2007; Poorter and Kitajima, 2007).

Many factors may influence leaf NSCs content (Volaire and Gaudin, 1996; Gaudet et al., 1999; Nanamori et al., 2004). To date, studies on factors influencing leaf NSCs have focused on nutrient elements (Nanamori et al., 2004), temperature (Gaudet et al., 1999; Gough et al., 2010), precipitation (Schellenbaum et al., 1999), and phenology (Gough et al., 2010). Less information is available regarding factors such as light intensity, leaf traits, leaf life forms (e.g., needle-leaf and broad-leaf), and plant functional groups

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(PFGs, trees, shrubs, and herbs). Furthermore, most of the studies related to leaf NSCs have mainly conducted on basis of controlled experiments regarding the responses of leaf NSCs to changing environment, or focused on the variations of leaf NSCs at the individual level, or along altitudinal gradients (Mooney and Billings, 1965; Hoch et al., 2002). To date, it remains unknown the level of variation of leaf NSCs among a large number of plant species at a large scale.

Currently, two bio-physiological hypotheses have been developed from these treeline studies to explain the variation in leaf NSCs with temperature: carbon limitation hypothesis (Schulze et al., 1967; Stevens and Fox, 1991; Wardle, 1993) and direct growth limitation hypothesis (Körner, 1998). According to the carbon limitation hypothesis, leaf NSCs content is expected to decrease with decreasing temperature because of the reduced photosynthetic carbon assimilation (Stevens and Fox, 1991; Wardle, 1993). In contrast, according to the growth limitation hypothesis, if tissue formation is limited with decreasing temperature, the content of leaf NSCs should increase because of the decreased demand for structural carbon with no consideration of the limitation of nitrogen, phosphorus, and others (Körner, 1998). These two hypotheses provide contradictory predictions and have not yet to be verified using the data from forests across the latitudinal transect at a large scale.

Leaf NSCs are mainly composed of soluble sugars and starch. The soluble sugars in leaves are primarily glucose, sucrose, and fructose (Hoch et al., 2003; Würth et al., 2005). The quantity of soluble sugars may increase under conditions of low temperature and drought, because soluble sugars are not only involved in the osmotic adjustment of cells but are also signal substances that enable adaptation to changing environment (Pan et al., 2002). Starch is used as a relatively long-term energy storage in plants (Zhang et al., 2006), and it's storage in leaves is negatively related to photosynthesis (Li et al., 2008). Highly soluble sugars are beneficial for amylolysis, and their accumulation is triggered by extreme day–night temperature differences (Trethewey and Aplees, 1994). Spatial variations in leaf NSCs across multiple climatic zones have been scarcely reported, preventing the testing of the above-mentioned hypotheses in terrestrial ecosystems at a large scale.

The north–south transect of eastern China (NSTEC) as the 15th standard transect of the International Geosphere-Biosphere Programme, covering multiple climatic zones and including almost all forest types except for boreal forest in Northern Hemisphere, provide an ideal setting for investigating the geographical patterns of leaf NSCs at a large scale. Herein, we measured the contents of leaf NSCs in 890 plant species collected from nine typical forests along the NSTEC. The main objectives of the present study were: (1) to investigate the variations in leaf NSCs among species, plant functional groups, and communities at a large geographic scale, (2) to explore the spatial patterns of leaf NSCs (the content of sugars and starch, NSCs, and the allocation ratio of sugar:starch) and their influencing factors (temperature, precipitation, plant functional groups, and others), and (3) to test the above-mentioned hypotheses to better characterize the underlying physiological mechanisms for the variations in leaf NSCs.

2. Materials and methods

2.1. Site description

Nine typical forests were selected along the 3500 km north–south transect of eastern China (NSTEC), which is an unique belt of vegetation formed by the heat gradients (Fig. 1) (Zhang and Yang, 1995). The growing season of plants in the north is from approximately April to October, whereas the plants

Fig. 1. The selected nine forest ecosystems along the north–south transect of eastern China (NSTEC, yellow frame). JF, Jiangfeng; DH, Dinghu; JL, Jiulian; SN, Shennong; TY, Taiyue; DL, Dongling; CB, Changbai; LS, Liangshui; HZ, Huzhong. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

in the south grow year-round. The mean annual temperature at these sites ranges from -3.67 to 23.15 °C, and the mean annual precipitation ranges from 473.0 to 2265.8 mm (Table 1).

The nine forests thus span cold-temperate, temperate, subtropical, and tropical forests. For each forest, field investigations were conducted on plots setup within the national nature reserves of China to avoid strong disturbances (e.g., fire, cutting, or plantation); these nature reserves are strongly representative of each region. Detailed information regarding the selected forests is provided in Table 1.

2.2. Field sampling

Field sampling was conducted from July 2013 to August 2013. We randomly set up four plots ($30\text{ m} \times 40\text{ m}$) in each selected forest. Firstly, we investigated the community structure of trees, shrubs, and herbs in each plot. Subsequently, we sampled the leaves of trees, shrubs, and herbs in these plots from 10:00 a.m. to 3:00 p.m. For each tree species, we randomly selected healthy and mature trees in each plot and then sampled the leaves from the fully sunlit upper crown using a long chain saw or artificial climbing trees. For each shrub species, we collected the upper leaves; for each herb species, a mixed sample of fresh and healthy leaves was taken. Leaf samples for a given plant species were mixed together in each plot as a replicate. After field sampling, leaf samples were immediately stored in a cool box with ice, and upon transfer to the laboratory, they were minimally cleaned and stored immediately in a refrigerator (-20 °C).

In total, samples of 1112 plant species were collected in the nine forests (four replicates for each species). Since some plant species were present in more than one forests, the collected plant species comprised 890 plant species, 491 genera, and 156 families. Of the 890 plant species sampled, there were 282 tree species,

Table 1

Selected information for nine forest ecosystems along the north–south transect of eastern China (NSTEC).

Sampling sites	Longitude (E)	Latitude (N)	MAT ^b (°C)	MAP (mm)	Vegetation type	Dominant species	Soil type
JF ^a	108°51'26"	18°44'18"	23.15	2265.8	Tropical mountain rain forest	<i>Schoepfia jasminodora</i> Sieb., <i>Ficus vasculosa</i> Wall., <i>Madhuca hainanensis</i> Chun.	Lateritic yellow earth
DH	112°32'14"	23°10'25"	21.83	1927	South subtropical evergreen broad-leaved forest	<i>Schima superba</i> Gardn., <i>Cryptocarya chinensis</i> Hemsl., <i>Pinus massoniana</i> Lamb.	Laterite
JL	114°26'28"	24°35'05"	18.22	1769.93	Subtropical evergreen broad-leaved forest	<i>S. superba</i> Gardn., <i>Castanopsis fargesii</i> Franch., <i>Castanopsis carlesii</i> Hayata	Red earth
SN	110°29'43"	31°19'15"	8.50	1446.71	North subtropical evergreen deciduous mixed forest	<i>Fagus engleriana</i> Seemen., <i>Quercus serrata</i> Thunb., <i>Cyclobalanopsis oxyodon</i> Oerst.	Yellow-brown earth
TY	112°04'39"	36°41'43"	5.98	644.38	Warm temperate deciduous and broadleaf mixed forest	<i>Quercus wutaishanica</i> Mayr., <i>Pinus tabulaeformis</i> Carr., <i>Populus davidiana</i> Dode.	Cinnamon soil
DL	115°25'24"	39°57'27"	6.55	539.07	Warm temperate deciduous and broadleaf mixed forest	<i>P. tabulaeformis</i> Carr., <i>Q. wutaishanica</i> Mayr., <i>Larix principis-rupprechtii</i> Mayr.	Brown soil
CB	128°05'27"	42°24'16"	2.79	691.00	Temperate conifer broadleaf mixed forest	<i>Pinus koraiensis</i> Siebold., <i>Larix gmelinii</i> Rupr., <i>Quercus mongolica</i> Fisch.	Dark brown soil
LS	128°53'51"	47°11'06"	0.01	648.34	Temperate conifer broadleaf mixed forest	<i>P. koraiensis</i> Siebold., <i>L. gmelinii</i> Rupr., <i>Betula platyphylla</i> Suk.	Dark brown soil
HZ	123°01'12"	51°46'48"	−3.67	472.96	Cold temperate coniferous forest	<i>L. gmelinii</i> Rupr., <i>Pinus sylvestris</i> L., <i>B. platyphylla</i> Suk.	Gray forest soil

^a JF, Jiangfeng; DH, Dinghu; JL, Jiulian; SN, Shennong; TY, Taiyue; DL, Dongling; CB, Changbai; LS, Liangshui; HZ, Huzhong.^b MAT, mean annual temperature; MAP, mean annual precipitation.

250 shrub species, and 260 herb species. Among the 282 tree species sampled, 191 were evergreen and 91 were deciduous. Additionally, 13 species were conifers and 269 were broadleaf tree species.

It is necessary to point out that our data and analyses did not account for the influence of forest age, phenology, and season on the contents of leaf NSCs (Cruz and Moreno, 2001; Palacio et al., 2007; Scartazza et al., 2013) because of the economic and human cost of

Table 2

Descriptive statistics regarding the contents of leaf soluble sugars, starch, and non-structural carbohydrates (NSCs) from nine forest ecosystems along the north–south transect of eastern China (NSTEC).

Sampling sites	Indicators (mg g ^{−1})	Min	Max	Mean	Median	SD	CV	Kurtosis	Skewness	K–S Z ^b	P ^b	n ^b
JF ^a	Soluble sugars	9.49	118.98	39.05	34.79	19.31	0.49	0.49	−0.05	0.60	0.87	154
	Starch	12.81	200.41	42.87	40.53	20.88	0.49	1.22	0.13	0.63	0.82	154
	NSCs	29.28	236.94	81.92	76.85	32.08	0.39	0.59	0.21	0.46	0.99	154
DH	Soluble sugars	5.30	106.80	33.72	27.46	20.61	0.61	0.09	0.02	0.56	0.91	172
	Starch	8.35	140.91	34.86	28.04	21.39	0.61	0.03	0.29	0.82	0.51	172
	NSCs	19.47	174.42	68.58	59.32	32.82	0.48	−0.35	0.01	0.66	0.78	172
JL	Soluble sugars	6.48	156.93	37.56	31.33	23.07	0.61	0.23	−0.11	0.52	0.95	175
	Starch	11.94	162.64	34.93	32.36	17.09	0.49	0.76	0.42	0.47	0.98	175
	NSCs	22.39	203.08	72.49	65.70	30.94	0.43	0.09	0.09	0.95	0.33	175
SN	Soluble sugars	7.62	162.20	43.45	38.40	24.61	0.57	0.32	−0.19	0.64	0.81	138
	Starch	7.56	137.32	46.68	39.97	24.83	0.53	0.43	−0.17	0.71	0.70	138
	NSCs	23.35	203.34	90.13	84.64	39.38	0.44	0.06	−0.18	0.47	0.98	138
TY	Soluble sugars	12.10	122.82	47.00	44.10	22.23	0.47	−0.18	−0.18	0.60	0.86	82
	Starch	17.84	104.09	49.53	45.60	18.89	0.38	−0.34	−0.09	0.38	0.99	82
	NSCs	33.91	203.99	96.52	92.19	33.15	0.34	−0.05	−0.20	0.60	0.86	82
DL	Soluble sugars	13.87	169.99	52.92	50.86	25.26	0.48	0.52	−0.26	0.83	0.50	80
	Starch	13.06	134.40	48.95	48.93	24.98	0.51	−0.67	−0.28	1.14	0.15	80
	NSCs	35.74	222.94	101.87	98.54	36.68	0.36	0.22	−0.39	0.50	0.96	80
CB	Soluble sugars	16.42	210.63	77.80	71.26	44.02	0.57	−0.44	−0.43	0.81	0.53	114
	Starch	7.39	201.82	75.93	68.26	42.11	0.55	0.66	−0.63	0.78	0.58	114
	NSCs	32.45	337.51	153.72	141.74	72.19	0.47	−0.39	−0.38	0.78	0.58	114
LS	Soluble sugars	13.97	141.57	56.25	49.56	27.34	0.49	−0.32	−0.27	0.55	0.4	105
	Starch	12.68	225.55	65.07	60.48	32.23	0.50	1.30	−0.36	0.87	0.43	105
	NSCs	33.69	301.75	121.32	119.36	48.97	0.40	0.11	−0.20	0.68	0.75	105
HZ	Soluble sugars	13.52	218.45	55.31	49.85	31.35	0.57	−0.32	−0.14	0.91	0.37	92
	Starch	8.46	161.27	55.83	46.14	34.63	0.62	−0.23	−0.17	0.48	0.97	92
	NSCs	33.80	379.72	111.14	102.59	58.15	0.52	−0.47	0.02	0.65	0.79	92

^a JF, Jiangfeng; DH, Dinghu; JL, Jiulian; SN, Shennong; TY, Taiyue; DL, Dongling; CB, Changbai; LS, Liangshui; HZ, Huzhong.^b K–S Z, Kolmogorov–Smirnov Z; P, P-value for the Kolmogorov–Smirnov test; n, number of plant species.

repeated investigations on all of these factors at a large scale. Therefore, since leaf collection was conducted during the peak growing season for each forest we minimized the influences of phenology and seasonal differences on the content of leaf NSCs.

2.3. Measurements

We used the standard anthrone colorimetric method to measure the content of leaf NSCs, given its advantage for rapid analysis of large samples (Dubois et al., 1956; Hewitt, 1958; Kabeya and Sakai, 2003). Specifically, for each sampling, 0.2 g of fresh cut leaves was placed into a 20-ml test tube and mixed with distilled water. The samples were then boiled twice for 30 min in distilled water to extract the soluble sugars. Filtration of each sample followed by dilution with distilled water yielded a final soluble sugar solution. Subsequently, the solid residues from the filtrate were transferred into a 50-ml flask and boiled with 15 ml of distilled water for 15 min. After cooling to room temperature, 2 ml of 9.2 M HClO₄ was added to hydrolyze the starch in the residue. After 15 min, the solution was filtered with distilled water to obtain the final starch solution. Sugar and starch concentrations in this solution were determined from the absorbance of the solutions measured at 630 nm using a UV-vis spectrophotometer (UV-1700, Shimadzu Corp., Suzhou, China). The concentrations of soluble sugars and starch were then transformed to dry matter ratio (mg g⁻¹) using leaf water content (Wang et al., 2015).

2.4. Other data collected or measured

Meteorological data (temperature, precipitation, and photosynthetic active radiation [PAR]) were obtained from the long-term monitoring data collected from 722 meteorological stations in China from 1961 to 2010 by spatial interpolation incorporating elevation and topography (Gao et al., 2013). Considering that the leaf samples were collected during the growing season, we also used the warm index as a climatic factor (Xu, 1985) to discuss the influence of climate on leaf NSCs. The warm index is a seasonal mean temperature, where the season is defined by the number of months with a monthly mean temperature above 5 °C.

Furthermore, specific leaf area (SLA) was measured simultaneously with the measurements of NSCs. In practice, we randomly selected 10 fresh leaves from each plant species and scanned them using a scanner (CanoScan LiDE 110, Canon, Tokyo, Japan); leaf area was measured using Photoshop CS (Adobe Systems, San Jose, CA, USA). The leaves were oven-dried at 70 °C for 48 h and then weighed to calculate SLA (m² kg⁻¹) (Wang et al., 2015).

2.5. Data analysis

The contents of soluble sugars, starch, and NSCs, as well as the sugar:starch ratio, were expressed on a mass basis (mg g⁻¹). NSCs

Fig. 2. Histograms of leaf soluble sugars (A), starch (B), and non-structural carbohydrates (C, NSCs) contents in nine forests along the north-south transect of eastern China. Histogram values are log₁₀-transformed, and statistics are shown as untransformed values.

were defined as the sum of soluble sugars and starch (NSCs = soluble sugars + starch). To further compare leaf NSCs content among different plant functional groups, we classified plant species into trees, shrubs, and herbs (Han et al., 2005), with further classification of the

Table 3

Variations in the contents of leaf soluble sugars, starch, and non-structural carbohydrates (NSCs) among plant functional groups in nine forest ecosystems.

Functional groups	n	Soluble sugars (mg g ⁻¹)		Starch (mg g ⁻¹)		NSCs (mg g ⁻¹)		r ^a	p ^b
		Average	SD	Average	SD	Average	SD		
Herb	260	49.89	32.69	60.12	34.58	110.00	56.94	0.45	<0.001
Shrub	250	45.15	25.43	44.90	22.47	90.05	39.77	0.43	<0.001
Tree	282	43.63	25.00	40.19	20.10	83.81	38.22	0.42	<0.001
Evergreen tree	191	37.80	18.39	37.06	16.71	74.86	28.10	0.31	<0.001
Deciduous tree	91	55.85	31.84	46.76	24.66	102.62	48.65	0.54	<0.001
Conifer tree	13	62.88	27.43	40.51	19.19	103.39	41.69	0.53	0.06
Broad leaf tree	269	42.69	24.55	40.18	20.18	82.87	37.87	0.42	<0.001
Total	890	45.70	27.44	47.50	27.22	93.20	46.26	0.44	<0.001

^a Spearman's rank correlation coefficient (r) between soluble sugars and starch content of leaves (n, number of species).

^b P, test value.

Fig. 3. Contents of leaf soluble sugars, starch, and non-structural carbohydrates (NSCs) among different plant functional groups in forests. Black points show the means and bars represent the standard deviation.

tree species into evergreen and deciduous trees, and into conifers and broadleaf trees.

The data for the contents of soluble sugars, starch, and NSCs were first $\log_{10}$ -transformed for normality testing. Then, the untransformed data of soluble sugars, starch, and NSCs were compared among different plant functional groups (PFGs) using one-way analysis of variance with least significant difference test. To determine whether soluble sugars and starch are correlated, we calculated Spearman's rank correlation coefficients across all 890 plant species, and across plant functional groups. Furthermore, we used the regression analyses to explore the latitudinal patterns of leaf NSCs, and determined the best-fitting regression models using the Akaike information criterion. We used temperature (mean of July and August), precipitation (mean of July and August), WI, PAR, and SLA to explore the factors of NSCs. Statistical analyses were conducted using SPSS ver. 11.0 (IBM Corp., Armonk, NY, USA) and figures were produced using Origin ver. 8.0 (OriginLab Corp., Northampton, MA, USA). Significant differences were defined at $P=0.05$ level.

3. Results

3.1. Variation in leaf NSCs contents across plant species

The contents of soluble sugars, starch, and NSCs in leaves were log-normally distributed across all 890 plant species (Fig. 2; Table 2). The contents of soluble sugars, starch, and NSCs exhibited large variations at the species level. In detail, the soluble sugars content ranged from 6.48 to 218.45 mg g^{-1} with a mean of 45.70 mg g^{-1} , the starch content ranged from 7.56 to 200.41 mg g^{-1}

with a mean of 47.50 mg g^{-1} , and the NSCs content ranged from 19.47 to 379.72 mg g^{-1} with a mean of 93.20 mg g^{-1} (Fig. 2). The coefficients of variation (CV) of the soluble sugars, starch, and NSCs contents across all species were 0.60, 0.57, and 0.50, respectively.

3.2. Variation in leaf NSCs content across different PFGs

The contents of soluble sugars, starch, and NSCs in leaves varied markedly among different PFGs (Table 3; Fig. 3); they were highest in herbs, followed by shrubs and then trees for all three parameters

Fig. 4. Coefficient of variation (CV, %) for leaf soluble sugars, starch, and non-structural carbohydrates (NSCs) among different plant functional groups.

Fig. 5. Changes in sugar:starch ratio among different plant functional groups.

(Table S1). For trees, the contents of soluble sugars, starch, and NSCs were higher in deciduous trees than in evergreen trees and were higher in conifers than in broadleaf trees (Table 3; Fig. 3). Among different PFGs, the coefficient of variation of NSCs was smaller than those of starch and soluble sugars (Fig. 4), indicating a close relationship between starch and soluble sugars in leaves.

Spearman's rank correlation coefficients between leaf soluble sugars and starch showed a significant correlation for each PFGs (all

P 's < 0.001), except in conifers ($P > 0.05$) (Table 3). The sugar:starch ratio ranged from 0.99 to 1.42 in the nine forests, which was higher in trees and shrubs than in herbs (Fig. 5). Unexpectedly, the sugar:starch ratio was not significantly correlated with latitude, longitude, temperature, or precipitation, irrespective of statistical scales (data not shown).

3.3. Spatial patterns of leaf NSCs along the transect

Spatial patterns related to both latitude and longitude were observed for leaf soluble sugars, starch, and NSCs at the levels of species and PFGs (Fig. 6; Table S2). The contents of soluble sugars, starch, and NSCs were significantly correlated with both latitude and longitude (all P 's < 0.05, but lower values of R^2) (Fig. 6). Specifically, all three metrics increased with increasing latitude and longitude.

3.4. Relationships between leaf NSCs content and climatic factors

The contents of soluble sugars, starch, and NSCs in leaves were negatively correlated with the mean temperature and precipitation of July and August at the levels of species, PFGs (except in conifers) (all P 's < 0.05) (Fig. 7; Table S3). The contents of soluble sugars, starch, and NSCs were significantly and negatively correlated with warm index ($P < 0.05$) (Fig. 8; Table S4). The contents of leaf soluble sugars, starch, and NSCs in tree species increased

Fig. 6. Spatial patterns of the contents of leaf soluble sugars, starch, and non-structural carbohydrates (NSCs) along the north-south transect of eastern China. Data represent the mean for each species (see details in the Supplementary data file).

Fig. 7. Relationships between the contents of leaf soluble sugars, starch, and non-structural carbohydrates (NSCs) and temperature (mean of July and August) and precipitation (mean of July and August). Data represent the average for each species (see details in the Supplementary data file).

with increasing PAR (Fig. 9); and also with increasing SLA ($P < 0.05$) (Fig. 10).

4. Discussion

4.1. The variation of leaf NSCs in forests

The average contents of leaf soluble sugars, starch, and NSCs of the 890 species were 45.70 mg g^{-1} , 47.50 mg g^{-1} , and 93.20 mg g^{-1} , respectively, with high variation among different plant species. These data corroborate Würth et al. (2005) who reported leaf NSC content of $60\text{--}70 \text{ mg g}^{-1}$ in 17 dominant plant species in tropical forests and Hoch et al. (2003) who reported the contents of leaf NSCs of 137.5 mg g^{-1} for the 10 dominant plant species in temperate forests. However, direct comparison of the data reported in the previous studies and in the present study is not unambiguous because of the apparent differences in scale and species number in different studies. The higher content of leaf NSCs in herbs compared to shrubs and trees is possibly due to higher N and P content (Han et al., 2005; Yin et al., 2009) or due to the lack of structural mass and carbon storage sources in herbs that required for reproduction or longevity. Furthermore, we found that the contents of leaf NSCs was higher in deciduous trees than in evergreen trees, because short-lived, fast-growing species tend to have a higher NSCs content than long-lived, slow-growing species. These findings were in line with previous stoichiometric studies that found that leaves in short-lived, fast-growing species had higher N and P content with

higher photosynthetic capability (Thompson et al., 1997; Gusewell, 2004); and these deciduous trees stored more NSCs in their leaves before autumn to supply energy for stem or root tissues through the winter (Würth et al., 2005; Gough et al., 2010). The leaf NSCs content in conifers was higher than in broadleaf trees (Table 3; Fig. 3), which was in agreement with the findings of Hoch et al. (2003).

4.2. Spatial patterns of leaf NSCs and its main influencing factors

The contents of leaf soluble sugars, starch, and NSCs showed a weak spatial pattern with latitude at the levels of plant species and PFGs. At the species level, the contents of leaf soluble sugars, starch, and NSCs increased with increasing latitude. At the level of PFGs, similar spatial patterns were also observed. Our findings indicate that the spatial patterns of leaf NSCs depend on changes in plant functional groups along the NSTEC. Importantly, the NSCs content, as a plant functional trait, provides valuable information for studies of the leaf economic spectrum because it reflects a mixture of direct and indirect causal relationships between traits (Wright et al., 2004).

Temperature and precipitation exert a strong effect on the contents of leaf NSCs (Stevens and Fox, 1991; Volenec et al., 1996; Grace et al., 2002). In our study, the contents of leaf soluble sugars, starch, and NSCs were negatively correlated with the temperature in July and August. Körner (2003) found that the contents of leaf NSCs in pines decreased as temperature increased from temperate to tropical zones. Our findings extend these conclusions

Fig. 8. Relationships between the contents of leaf soluble sugars, starch, and non-structural carbohydrates (NSCs) and warm index. Data represent the mean and standard error for all plant species in each forest.

across many species and plant functional groups, as well as in environments ranging from cold temperate to tropical forests. These findings provide a tentative support for the growth limitation hypothesis in forests at a large geographical scale (Körner, 1998). Low temperature appeared to directly limit the carbon sink activity and restricted the growth of structural tissues. Essentially, structural carbon demand was reduced in favor of NSCs accumulation in leaves because the physiological processes governing the formation of new tissue were more sensitive to low temperatures than the physiological processes governing photosynthesis (Hoch and Körner, 2003; Körner, 1998; Li et al., 2008).

In temperate and tropical forests, drought stress may affect plant growth more than photosynthesis, resulting in the accumulation of leaf NSCs (Guehl et al., 1993). Würth et al. (2005) found that leaf NSCs content in 17 tropical plant species was higher in the dry season than in the wet season, and Tissue and Wright (1995) reported similar results. Moreover, previous studies demonstrated that drought stress facilitated the accumulation of leaf NSCs (Bacelar et al., 2006; Newell et al., 2002), perhaps because drought stress restricted the structural utilization of carbon (Mooney and Billings, 1965; Tissue and Wright, 1995) but had less effect on photosynthesis (Bryant et al., 1983). Our results confirmed these findings, indicating that the contents of leaf soluble sugars, starch, and NSCs increased with the decrease in the mean precipitation level in July and August across a wide range of forest types and climates. The accumulation of leaf NSCs thus appears to be an adaptive

Fig. 9. Relationships between the contents of leaf soluble sugars, starch, and non-structural carbohydrates (NSCs) in trees and photosynthetic active radiation (PAR, mean of July and August). Data represent the mean for tree species within each forest (see details in the Supplementary data file).

strategy for plants to survive drought and changing temperature (Poorter and Kitajima, 2007; Sala et al., 2012), or to buffer plant during periods when the utilization of NSCs exceeds the uptake (Würth et al., 2005).

The zonal distribution of PAR has an apparent impact on leaf NSCs content by regulating photosynthesis and vegetation distribution at a large geographical scale. The contents of soluble sugars, starch, and NSCs in tree leaves increased with increasing PAR along the NSTEC. The contents of soluble sugars and NSCs were positively correlated with PAR ($P < 0.05$), but a similar correlation was not observed for starch, possibly because starch was mainly stored as energy and therefore was affected less by PAR. Furthermore, SLA is a key leaf trait and reflects the strategy of plant carbon absorption. A higher SLA is usually associated with a higher light capture area of leaves and a higher net photosynthetic rate (Wright et al., 2002). Therefore, the observed increases in soluble sugars, starch, and NSCs with increasing SLA possibly resulted from higher photosynthetic rates. It is therefore necessary to consider additional factors, such as phylogenetic relationships, climate, soil, leaf morphological traits, element composition, and others to further explore the factors determining leaf NSCs at large scale in the future.

4.3. Allocation strategies of leaf NSCs in forests

The soluble sugar content may increase under conditions of low temperature and drought because soluble sugars are not only

Fig. 10. Relationships between the contents of leaf soluble sugars, starch, and non-structural carbohydrates (NSCs) and specific leaf area (SLA). Data represent the means and standard error.

involved in the osmotic adjustment of cells but also serve as signal substances to enable adaptation to environmental changes. Starch has little effect on osmolality but has benefits for long-term energy storage in plants (Yan et al., 2014). The proportion of soluble sugars and starch in plants (i.e., the sugar:starch ratio) should thus reflect the response to environmental changes, and should be regulated by phenology or seasonality (Li et al., 2008; Gough et al., 2010). Possibly for this reason, some studies have found higher sugar:starch ratios in plants subjected to lower temperatures (Strand et al., 2003; Patton et al., 2007). These differences between the previous and current studies may be attributed to the higher temperature in sampled forests in this study, compared to those measured in Himalayan trees reported by Li et al. (2008); this higher temperature is expected to shift the allocation balance from soluble sugars toward starch, as discussed above. Additionally, the number of plant species was different. Li et al. (2008) reported a relatively narrow set of species. However, in this study, the expected relationship between leaf sugar:starch ratio and temperature was not observed across the transect. It is possible that the sugar:starch ratio could be amplified at very low temperatures, such as in the Himalayas, but decreased in more temperate and tropical forests. For example, we found that the sugar:starch ratio in tree leaves was higher than that of herbs, possibly because herbs may experience a higher

demand for soluble sugars than woody plants, or because woody plants tend to invest more in long-term energy storage than herbs.

In summary, the contents of soluble sugars, starch, and NSCs varied among plant species in Chinese forests, with the mean of 45.70 mg g⁻¹, 47.50 mg g⁻¹, and 93.20 mg g⁻¹, respectively. There is a weak latitudinal pattern of leaf soluble sugars, starch, and NSCs from tropical to cold-temperate forests at the levels of species and PFGs. The contents of leaf soluble sugars, starch, and NSCs decreased with increasing temperature, precipitation, and warm index, thus supporting the growth limitation hypothesis. Furthermore, the sugar:starch ratio was not correlated with temperature or precipitation. These findings provide new insights into the underlying mechanisms driving NSCs allocation at a large geographical scale, with implications for the adaptability of plant species to climate change scenarios.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2015.11.017>.

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