

# Needle $\delta^{13}\text{C}$ and mobile carbohydrates in *Pinus koraiensis* in relation to decreased temperature and increased moisture along an elevational gradient in NE China

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**Abstract** A tree's crown interacts with atmospheric variables such as  $\text{CO}_2$ , temperature, and humidity. Physioecology of leaves/needles (e.g.  $\delta^{13}\text{C}$ , mobile carbohydrates, and nitrogen) is, therefore, strongly affected by microclimate in and surrounding a tree crown. To understand the physiological responses of leaves to changes in air temperature and moisture, we measured  $\delta^{13}\text{C}$ , soluble sugars, starch, and total nitrogen (N) concentrations in current year and 1-yr-old needles of *Pinus koraiensis* trees, and compared the growing season air temperature and relative humidity within and outside *P. koraiensis* crowns along an elevational gradient from 760 to 1,420 m a.s.l. on Changbai Mountain, NE China. Our results indicated that needle N and mobile carbohydrates concentrations, as well as needle  $\delta^{13}\text{C}$  values changed continuously with increasing elevation, corresponding to a continuous decrease in air temperature and an increase in relative

humidity. Needle carbon and nitrogen status is highly significantly negatively correlated with temperature, but positively correlated with relative humidity. These results indicate that increases in air temperature in combination with decreases in relative humidity may result in lower levels of N and mobile carbohydrates in *P. koraiensis* trees, suggesting that future climate changes such as global warming and changes in precipitation patterns will directly influence the N and carbon physiology at *P. koraiensis* individual level, and indirectly affect the competitive ability, species composition, productivity and functioning at the stand and ecosystem level in NE China. Due to the relatively limited range of the transect (760–1,420 m) studied, further research is needed to explain whether the present results are applicable to scales across large elevational gradients.

**Keywords** Altitudinal gradient · Nitrogen contents · Non-structural carbohydrates · Pine · Soluble sugars · Stable carbon isotope · Starch · Within-crown microclimate

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## Introduction

A tree crown is the tree's food factory. Leaves in a crown are the main site of photosynthesis, where sugars are made from  $\text{H}_2\text{O}$  and  $\text{CO}_2$ , using sunlight energy. Photosynthesis-related physioecology of leaves/needles (e.g.  $\delta^{13}\text{C}$ , mobile carbohydrates, and nitrogen) in a tree crown is, therefore, strongly affected by atmospheric variables such as  $\text{CO}_2$ , temperature, and humidity in and around the crown.

Environmental variables change continuously with altitude. Changes in temperature (Panek and Waring 1995), precipitation (Miller et al. 2001), and vapour pressure deficit (VPD) (Meinzer 2003; Sellin and Kupper 2004) affect leaf traits (Li et al. 2006a), leaf carbon gain and loss

(Körner 2003), and tree growth (Li et al. 2003; Li and Yang 2004) along elevational gradients. Lots of research works investigated changes in physiology of trees along altitudinal gradients, to understand the physiological mechanisms determining the reduction of tree growth at higher elevations (e.g. Hoch and Körner 2005; Shi et al. 2006; Li et al. 2008a, b).

Previous studies associated changes in  $\delta^{13}\text{C}$  in plant tissues along altitudinal gradients with air temperature (Körner et al. 1991; Marshall and Zhang 1994; Sparks and Ehleringer 1997). Hultine and Marshall (2000) measured variations in leaf  $\delta^{13}\text{C}$  in four evergreen species (*Pinus contorta*, *Pseudotsuga menziesii*, *Abies lasiocarpa*, and *Picea engelmannii*) in the USA and found that  $\delta^{13}\text{C}$  values increased at different rates (ranging from  $+0.91\text{‰ km}^{-1}$  for *A. lasiocarpa* to  $+2.68\text{‰ km}^{-1}$  for *P. contorta*) along elevational gradients. But Van de Water et al. (2002) observed opposite trends in leaf  $\delta^{13}\text{C}$  contents with increasing elevation for several  $\text{C}_3$  and  $\text{C}_4$  species in desert and woodlands in southeastern Utah and south-central New Mexico. Morecroft and Woodward (1990) indicated, however, that leaf  $\delta^{13}\text{C}$  of *Nardus stricta* did not significantly vary with elevation.

Shi et al. (2006) found that concentrations of non-structural carbohydrates (NSC) in leaves of four woody species (*Quercus aquifolioides*, *Abies faxoniana*, *Rhododendron fabri* subsp. *prattii*, and *Sorbus rufopilosa*) increased with increasing elevation from 3,150 to 3,740 m a.s.l. at the eastern edge of the Tibetan Plateau. Bansal and Germino (2008) found that needles of the upper treeline trees (*Abies lasiocarpa*, *Pseudotsuga menziesii* at 3,000 m a.s.l.) had up to 50 % greater NSC concentrations compared to needles of the low-elevation trees (2,450 m) in the Rocky Mountains. Sveinbjörnsson et al. (1992) found that foliage NSC concentrations in birch did not vary with increasing elevation ranging from the valley (360 m a.s.l.) to the treeline (550 m) on a northerly slope on Mount Luovare, Swedish Lapland.

Some studies have found that nitrogen (N) accumulation decreased with increasing elevation (Barrick and Schoettle 1996; Li et al. 2008b). However, some other studies found that N accumulation and conservation increased with increasing elevation as adaptive responses which enhance metabolic capacity in energy-limited systems at low temperature, or did not change along elevational gradients (Richardson et al. 2001; Shi et al. 2006).

All those studies used elevation as a substitute for the complexity of local environmental elements to study the elevational effects on tree physiology, but did not exactly measure the microclimate (e.g. temperature, humidity) along an elevational gradient studied (e.g. Li et al. 2008a, b; Zhu et al. 2012). Other studies have investigated the effects of within-crown irradiance and  $\text{CO}_2$  gradient on

foliage physiology (e.g. Frak et al. 2001; Le Roux et al. 2001; Takeuchi et al. 2001; Han et al. 2003; Koike et al. 2004), but did not investigate the differences between the within-crown microclimate and the outside crown climate, and the effects of those differences on leaf physiology within a tree crown. Hence, we measured needle  $\delta^{13}\text{C}$ , NSC, and total N concentrations of *Pinus koraiensis* trees grown along an elevational gradient from 760 to 1,420 m a.s.l. on Changbai Mountain, NE China, to examine the responses of those physiological parameters to changes in temperature (i.e. higher temperature at lower elevations vs. lower temperature at higher elevations) and moisture, in order to understand the effects of future climate changes on leaf or crown physiology. We also compared the growing season air temperature and relative humidity within and outside *P. koraiensis* crowns along that gradient, to explore whether the leaf carbon and nitrogen status is determined mainly by within-crown microclimate or by climate outside or surrounding the tree crown.

## Materials and methods

### Study sites

The study transect across the *P. koraiensis* distribution (760–1,420 m a.s.l.) was located on the north-facing slope of the Changbai Mt. ( $42^{\circ}5'–42^{\circ}6'\text{N}$ ,  $128^{\circ}5'–128^{\circ}6'\text{E}$ ), northeastern China. The mean annual temperature is  $3.2\text{°C}$  and average annual precipitation ranges from 600 to 900 mm (data collected since 1979, at the Research Station of Changbai Mountain Forest Ecosystem,  $42^{\circ}24'\text{N}$ ,  $128^{\circ}5'\text{E}$ , 738 m a.s.l.). The average temperature in January and July is  $-15.6\text{°C}$  and  $19.7\text{°C}$ , respectively. The transect was covered by forests dominated by Korean pine (*P. koraiensis*) (Table 1). Soils along the transect vary from dark brown forest soil (760–1,130 m), to albic and dark brown forest soil (1,130–1,260 m), and mountain brown forest soil (1,260–1,420 m) (Cheng 1981).

### Sample trees and needle sampling

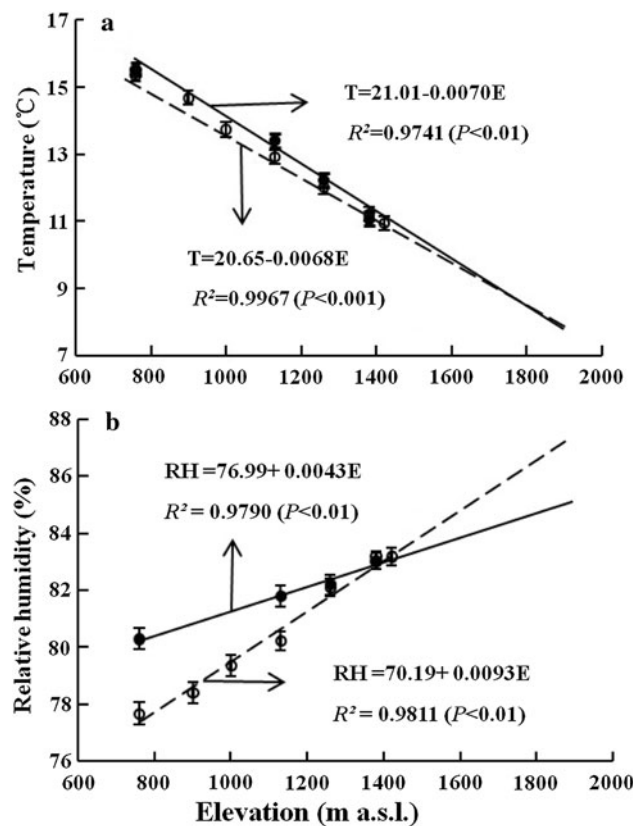
Nine healthy *P. koraiensis* trees ( $n = 9$ ,  $>30$  m apart from each other) were selected from the canopy layer as sample trees at each elevation (760, 900, 1,000, 1,130, 1,260, 1,380, and 1,420 m) of the transect. Diameter at breast height (DBH), tree height, crown length and width (S–N, E–W) were recorded and are summarised in Table 1. Needle longevity of *P. koraiensis* trees is 2 years and rarely reaches 3 years (Zhou et al. 2011, 2012). Hence, the present study sampled the current year needles and 1-year-old needles only.

**Table 1** Characteristics of stands and sample trees of *Pinus koraiensis* along an altitudinal gradient on a N-facing slope of Changbai Mountain, NE China

Forest type	Mixed broad-leaved and coniferous forest		Mixed coniferous forests		Dark coniferous forests		
	760	900	1,000	1,130	1,260	1,380	1,420
Elevation (m a.s.l.)	250–320	250–320	250–320	200–300	200–300	200–280	200–250
Age (year) of sample trees	30.88 ± 0.32	29.79 ± 0.10	27.44 ± 0.19	26.84 ± 0.11	25.48 ± 0.21	42.2 ± 0.22	36.0 ± 0.26
Height ± SE (m)	65.5 ± 0.23	63.0 ± 0.21	61.7 ± 0.15	58.8 ± 0.19	49.0 ± 0.31	7.76 ± 0.18	6.94 ± 0.24
DBH ± SE (cm)	14.91 ± 0.17	14.20 ± 0.08	13.59 ± 0.13	11.56 ± 0.17	9.30 ± 0.22	5.5 ± 0.11	5.1 ± 0.10
Crown length ± SE (m)	10.17 ± 0.23	9.56 ± 0.16	8.30 ± 0.22	7.21 ± 0.17	6.32 ± 0.20	70 ± 0.21	70 ± 0.11
Crown width ± SE (m)	90 ± 0.10	89 ± 0.11	87 ± 0.14	78 ± 0.22	74 ± 0.13		
Canopy cover ± SE (%)							

DBH diameter at breast height

Given the large seasonal variation in mobile carbohydrates in *Pinus* species (Fischer and Holl 1991; Li et al. 2001, 2002; Ludovici et al. 2002), we decided to take needle samples at the end of the growing season (1–5 October 2010), as suggested by Shi et al. (2006), to have comparable samples of mature needles. In each sample tree, current year and 1-year-old needles were collected from shoots located on the middle part of leading branches at the bottom crown level (i.e. within crown) of the south-facing crown side. Within 5 h of sampling, samples were treated in a microwave at 600 W for 45–60 s to stop the physiological activity, and then oven-dried to a constant weight at 65 °C for 72 h. The dried samples were ground into fine powder (passed through 100 meshes) for chemical analysis. Projected needle area and dry mass were measured (Yan et al. 2012) to calculate the specific leaf area (SLA) per unit leaf dry mass. SLA was then used to calculate the concentrations of carbohydrates and N on a projected needle area basis.



**Fig. 1** Microclimate (mean values ± 1SE, n = 3) outside crowns and within-crowns of *Pinus koraiensis* along an elevational gradient in NE China. Mean air temperature (a) and mean relative humidity (b) during 2010 growing season (1st April to 1st October). Symbols: open circle, dashed line within crown and filled circle, solid line outside crown

## Climate measurements

Within-crown temperature (WCT) and relative humidity (WCH) were measured for three trees ( $n = 3$ ) out of the nine sample trees at each elevation, i.e. at 760, 900, 1,000, 1,130, 1,260, 1,380, and 1,420 m a.s.l., respectively. A temperature/humidity data logger ( $-20$  to  $+85$  °C and 0–100 % humidity, DS1923 iButton, Maxim, USA) was placed on the north-facing stem side at the mid-crown level of the three trees, respectively. The loggers were fully shaded to protect from direct sunshine and rainfall. Outside crown air temperatures (OCT) and relative humidity (OCH) were measured at the height of 200 cm above ground in three gaps ( $n = 3$ ; each with a gap radius of  $>1$  time the height of the surrounding trees) at four elevations (760, 1,130, 1,260, and 1,380 m; data measured by the Research Station of Changbai Mountain Forest Ecosystem). OCT and OCH at other elevations (900, 1,000, and 1,420 m) were calculated using the equations given in Fig. 1a, b, respectively. The temperature and humidity were continuously recorded at hourly intervals from 1 April to 1 October 2010.

## Stable carbon isotope analysis

Needle  $\delta^{13}\text{C}$  measurements were performed with the procedure in which the dry sample powder (approx. 2 mg) was put into tin foil cup and then combusted in an elemental analyser (Flash EA-1112, Carlo Erba Thermoquest, Italy) interfaced via ConFlo II (Thermo Finnigan, Bremen, Germany) to an isotope ratio mass spectrometer (DELTA plus XL, Thermo Finnigan, Bremen, Germany).

Carbon isotope composition was reported by the following conventional  $\delta^{13}\text{C}$  values notion relative to Vienna Pee Dee Belemnite international standard (Farquhar et al. 1989):

$$\delta^{13}\text{C}(\text{‰}) = (R_{\text{sa}}/R_{\text{sd}} - 1) \times 1,000$$

where  $R = {}^{13}\text{C}/{}^{12}\text{C}$ , and  $R_{\text{sa}}$  and  $R_{\text{sd}}$  are the molecular abundance ratios of carbon isotope ( ${}^{13}\text{C}/{}^{12}\text{C}$ ) of the sample and the standard, respectively. The overall precision of the replicate sample measurements was estimated to be better than  $\pm 0.2$  ‰ standard deviation.

## Total soluble sugars and starch analyses

The powdered material (0.1 g) was put into a 10 ml centrifuge tube, where 5 ml of 80 % ethanol was added. The mixture was incubated at 80 °C in a water bath shaker for 30 min and then centrifuged at 4,000 rpm for 5 min. The pellets were extracted two more times with 80 % ethanol. The supernatants were retained, combined, and stored at  $-20$  °C for soluble sugar determinations. The soluble

sugar fraction was measured. Soluble sugars in the collected extracts were determined using the anthrone method. An aliquot of the extract was hydrolysed in 5 ml of 0.4 % anthrone solution (4 g anthrone in 1,000 ml 95 %  $\text{H}_2\text{SO}_4$ ) in a boiling water bath for 15 min. After cooling, the sugar concentration was determined spectrophotometrically (ultraviolet–visible spectrophotometer 752S, Cany Precision Instruments Co., Ltd., Shanghai, China) at 620 nm (Li et al. 2008a). Glucose was used as a standard. The sugar concentration was calculated on a dry mass basis ( $\text{SU}_{\text{mass}}$ , % d.m.) and on a projected needle area basis ( $\text{SU}_{\text{area}}$ ,  $\text{g m}^{-2}$ ), respectively.

The ethanol-insoluble pellet was used for starch extraction. Ethanol was removed by evaporation. Starch in the residue was released in 2 ml distilled water for 15 min in a boiling water bath. After cooling to room temperature, 2 ml of 9.2 mol/l  $\text{HClO}_4$  was added. Starch was hydrolysed for 15 min. 4 ml distilled water was added to the samples. Samples were then centrifuged at 4,000 rpm for 10 min. The pellets were extracted one more time with 2 ml of 4.6 mol/l  $\text{HClO}_4$ . The supernatants were retained, combined, and filled to 20 ml. The glucose produced by the hydrolysis of starch was measured spectrophotometrically (ultraviolet–visible spectrophotometer 752S) at 620 nm using anthrone reagent, and starch concentration was calculated by multiplying glucose concentrations measured by the conversion factor of 0.9 (Li et al. 2008a). Glucose was used as a standard. The starch concentration was described on a dry mass basis ( $\text{ST}_{\text{mass}}$ , % d.m.) and on a projected needle area basis ( $\text{ST}_{\text{area}}$ ,  $\text{g m}^{-2}$ ).

## Total nitrogen analysis

The total nitrogen content was determined in finely ground oven-dried samples by the micro Kjeldahl procedure, using  $\text{CuSO}_4$ ,  $\text{K}_2\text{SO}_4$ , and  $\text{H}_2\text{SO}_4$  for digestion, and  $\text{NH}_3$  was determined on an auto-analyser, using the indophenol-blue colorimetric method (Seifter et al. 1950). The N concentration was expressed both on a dry mass basis ( $\text{N}_{\text{mass}}$ , % d.m.) and on a projected needle area basis ( $\text{N}_{\text{area}}$ ,  $\text{g m}^{-2}$ ).

## Statistical analysis

NSC expressed on a mass basis ( $\text{NSC}_{\text{mass}}$ , % d.m.) and a needle area basis ( $\text{NSC}_{\text{area}}$ ,  $\text{g m}^{-2}$ ) is defined as the sum of the starch ( $\text{ST}_{\text{mass}}$ ,  $\text{ST}_{\text{area}}$ ) plus the total soluble sugars ( $\text{SU}_{\text{mass}}$ ,  $\text{SU}_{\text{area}}$ ) for each sample within each category (Chapin et al. 1990; Li et al. 2001). In case that mass-based and area-based concentrations had the same result, concentration data were, then, not noted using “mass” or “area”. NSC–N ratio is defined as the ratio of NSC to N concentration for each sample (Li et al. 2008a, b; Yan et al. 2012). All data ( $\delta^{13}\text{C}$ , NSC, starch, total soluble sugars,

and N contents) were checked for normality by Kolmogorov–Smirnov tests. To test the effects of both elevation and needle age on the variables, two-way ANOVAs were performed with needle age (current year needles vs. 1-year-old needles) and altitudinal gradient (7 levels from 760 to 1,420 m) as factors. Pearson’s correlation coefficients were used to detect the relationships between the physiological variables and microclimate factors (i.e. WCT, OCT, WCH, and OCH). All statistical analyses were performed using SPSS 11.5 for Windows.

**Results**

Within- and outside crown temperature and relative humidity in relation to elevation.

Both mean WCT ( $T = 20.65 - 0.0068E$ ) and OCT ( $T = 21.01 - 0.0070E$ ) decreased significantly (both  $p < 0.01$ ) with increasing elevation during the growing season (Fig. 1a). From the lowest elevation of 760 m to the highest elevation of 1,420 m a.s.l. within the study transect, the mean OCT was 0.21 °C (760 m)–0.08 °C (1,420 m) higher than WCT. The mean OCT and WCT decreased by 0.70 °C and 0.68 °C with 100 m increase in elevation (Fig. 1a), respectively, and hence the negligible difference (<0.21 °C) between OCT and WCT will disappear at 1,800 m a.s.l. Beyond that elevation, the mean WCT will be higher than OCT (Fig. 1a).

Conversely, WCH ( $RH = 70.19 + 0.0093E$ ) and OCH ( $RH = 76.99 + 0.0043E$ ) increased significantly (both  $p < 0.01$ ) with increasing elevation during the growing season (Fig. 1b). The mean WCH and OCH increased by 0.93 and 0.43 % with 100 m increase in elevation during the growing season, respectively. The mean WCH was

lower than OCH at lower elevations, but was higher than OCH at higher elevations, with an *intersection* point at the elevation of 1,360 m for the WCH and OCH functions (Fig. 1b).

Needle physiological parameters in relation to elevation

Needle age had significant effects on both mass-based and area-based concentrations of N and mobile carbohydrates (NSC, soluble sugars, and starch), but no effects on  $SU_{mass}$ ,  $ST_{area}$ , and  $\delta^{13}C$  (Table 2). Elevation significantly affected all studied parameters including  $\delta^{13}C$ , total N, mobile carbohydrates, and NSC–N ratio, except for  $NSC_{area}$  (Table 2). The elevational effects on needle  $SU_{mass}$  and  $ST_{mass}$  were dependent upon needle age (significant interaction for both; Table 2), showing that the two elevational trend lines for  $SU_{mass}$  (Fig. 2c) and  $ST_{mass}$  (Fig. 2e) in current year and 1-year-old needles crossed (i.e. interaction), respectively. SLA was significantly affected by needle age, elevation, and their interaction (Table 2), showing a decreased trend with both increased needle age and elevation (data not shown).

The elevational patterns of changes in mass-based and area-based concentrations of N and mobile carbohydrates were highly similar within each parameter (Fig. 2), except for NSC in current year needles (Fig. 2a vs. b).  $\delta^{13}C$  values (Fig. 2i) and concentrations of  $NSC_{mass}$  (Fig. 2a), sugars (Fig. 2c, d), and nitrogen (Fig. 2g, h) in needles increased with increasing elevation.  $\delta^{13}C$  values (Fig. 2i) were higher (less negative), but NSC (Fig. 2a, b) and N concentrations (Fig. 2g, h), as well as  $SU_{area}$  (Fig. 2d) were lower in current year needles than in 1-year-old needles at any given elevation. The 1-year-old needles had higher  $SU_{mass}$  compared to the current year needles at lower elevations,

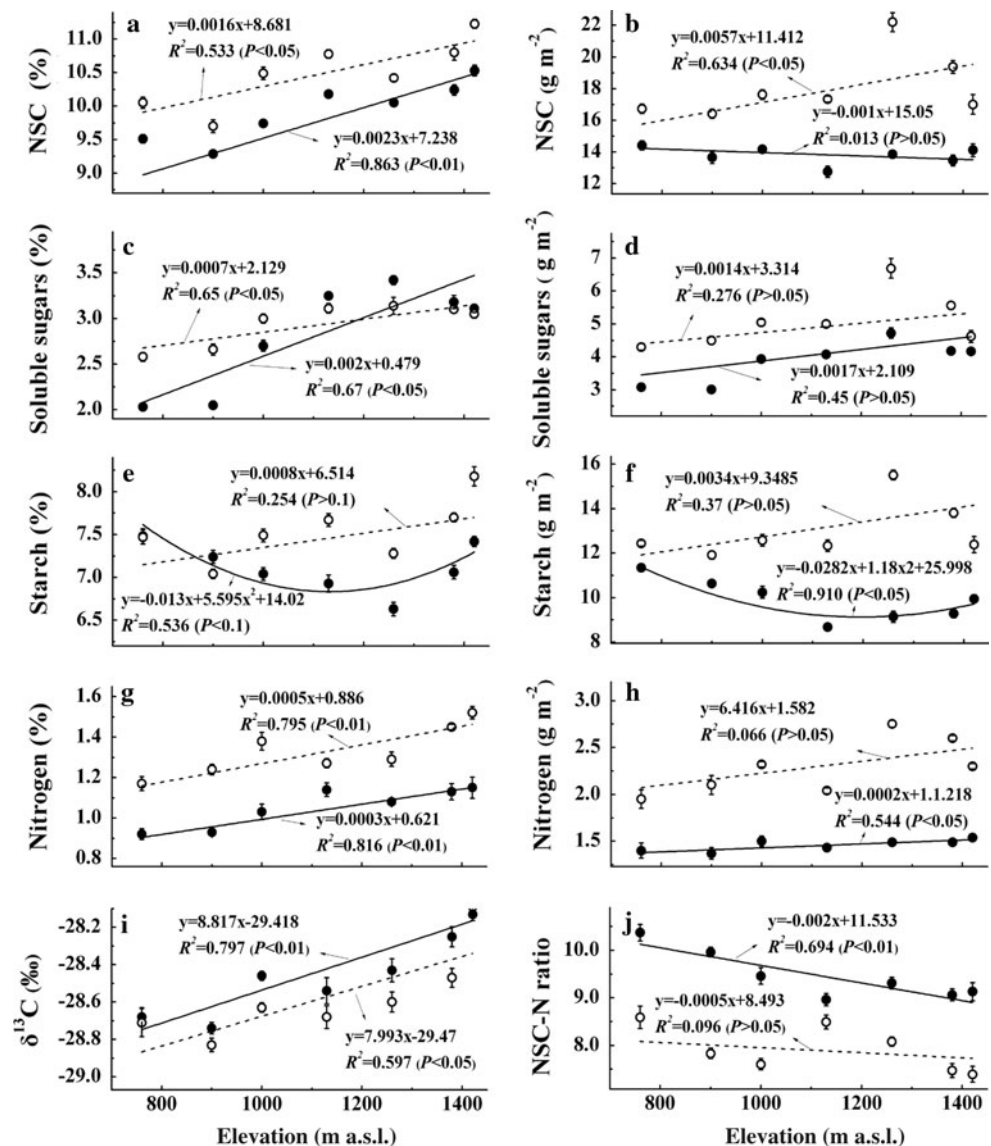
**Table 2** Effects of needle age (current year needles vs. 1-year-old needles), and elevation on non-structural carbohydrates (NSC), soluble sugars, starch, and total nitrogen expressed on a dry mass basis (dM-based) and on a projected area basis (pA-based), as well as

NSC–N ratio, SLA, and  $\delta^{13}C$  content values in needles of *Pinus koraiensis* grown at 760, 900, 1,000, 1,130, 1,260, 1,380, and 1,420 m a.s.l. on Changbai Mt., NE China

	NSC		Soluble sugars		Starch		Nitrogen		NSC–N ratio	SLA	$\delta^{13}C$
	dM-based	pA-based	dM-based	pA-based	dM-based	pA-based	dM-based	pA-based			
Needle age (A)											
$F_{(1,112)}$	223.336	54.151	2.420	23.646	27.823	1.906	81.035	107.233	60.911	70.713	3.459
$P$	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.131	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.178	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.073
Elevation (E)											
$F_{(6,112)}$	89.661	2.218	14.488	4.558	5.889	3.297	6.579	2.731	2.640	3.530	8.192
$P$	<b>&lt;0.001</b>	0.071	<b>&lt;0.001</b>	0.002	<b>&lt;0.001</b>	<b>0.014</b>	<b>&lt;0.001</b>	<b>0.032</b>	<b>0.037</b>	<b>0.01</b>	<b>&lt;0.001</b>
Needle age (A) × elevation (E)											
$F_{(6,112)}$	2.024	2.392	2.907	0.757	3.093	0.212	1.075	1.686	1.162	2.957	0.335
$P$	0.096	0.054	<b>0.025</b>	0.609	<b>0.019</b>	0.970	0.401	0.162	0.354	<b>0.023</b>	0.913

F- and P values are given (n = 9). Significant differences (P < 0.05) are highlighted in bold

**Fig. 2** Needle  $\delta^{13}\text{C}$  (‰), NSC–N ratios, and mass-based (a, c, e, g in % d.m.) and projected needle area-based (b, d, f, h in  $\text{g m}^{-2}$ ) concentrations (mean values  $\pm$  1SE,  $n = 9$ , sampled at the end of 2010 growing season) of nitrogen (N) and mobile carbohydrates in current year (filled circle, solid line) and 1-year-old needles (open circle, dashed line) of *Pinus koraiensis* grown along an elevational gradient in NE China. NSC Non-structural carbohydrates



but the trend was opposite at higher elevations (Fig. 2c). The current year needles seemed to have higher starch concentrations at lower elevations, but lower starch concentrations at higher elevations compared to the 1-year-old needles (Fig. 2e, f). NSC–N ratios decreased with increasing elevation, and current year needles had higher NSC–N ratios than the 1-year-old needles at any given elevation (Fig. 2j).

Needle  $\delta^{13}\text{C}$  and mobile carbohydrates in relation to within- and outside crown temperature and moisture

For both current year and 1-year-old needles, their  $\delta^{13}\text{C}$  values,  $\text{NSC}_{\text{mass}}$ ,  $\text{SU}_{\text{mass}}$ , and  $\text{N}_{\text{mass}}$  were significantly negatively correlated with both WCT and OCT, but significantly positively correlated with both WCH and OCH, respectively (Table 3). The correlation coefficients were somewhat

higher in case with WCT or WCH compared to with OCT or OCH, respectively (Table 3). The projected area-based concentrations were found to be less sensitive to temperature and moisture. Only  $\text{SU}_{\text{area}}$  and  $\text{N}_{\text{area}}$  in current year needles were significantly correlated with temperature (negative correlation with both WCT and OCT) and moisture (positive correlation with both WCH and OCH), respectively (Table 3). NSC–N ratio in current year needles was significantly positively correlated with temperature, but significantly negatively correlated with moisture (Table 3).

## Discussion

The present study found that needle mean  $\delta^{13}\text{C}$  values increased significantly with increasing elevation (Fig. 2i), which corresponded to the decreased crown air temperature

**Table 3** Coefficients between needle non-structural carbohydrates (NSC), soluble sugars, starch, total nitrogen expressed on a dry mass basis (dM-based) and on a projected area basis (pA-based), NSC–N ratio, SLA,  $\delta^{13}\text{C}$  content values, air temperature (Temp) and relative humidity (RH) within and outside crowns of *Pinus koraiensis* in NE China

	NSC			Soluble sugars			Starch			Nitrogen			NSC–N ratio	SLA	$\delta^{13}\text{C}$
	dM-based		pA-based	dM-based		pA-based	dM-based		pA-based	DM-based		pA-based			
<b>Current year needles</b>															
Within-crown air temp	-0.922 ( <b>0.003</b> )	0.256 (0.579)	-0.870 ( <b>0.011</b> )	-0.833 ( <b>0.011</b> )	0.330 (0.470)	0.710 (0.074)	-0.921 ( <b>0.013</b> )	-0.816 ( <b>0.025</b> )	0.862 ( <b>0.013</b> )	-0.731 (0.062)	-0.923 ( <b>0.003</b> )				
Outside crown air temp	-0.888 ( <b>0.008</b> )	0.206 (0.658)	-0.799 ( <b>0.031</b> )	-0.763 ( <b>0.046</b> )	0.243 (0.600)	0.633 (0.127)	-0.871 ( <b>0.011</b> )	-0.784 ( <b>0.037</b> )	0.800 ( <b>0.031</b> )	-0.675 (0.096)	-0.921 ( <b>0.003</b> )				
Within-crown air RH	0.909 ( <b>0.007</b> )	-0.200 (0.668)	0.841 ( <b>0.018</b> )	0.819 ( <b>0.024</b> )	-0.321 (0.483)	-0.666 (0.102)	0.874 ( <b>0.010</b> )	0.801 ( <b>0.030</b> )	-0.806 ( <b>0.029</b> )	0.674 (0.097)	0.926 ( <b>0.007</b> )				
Outside crown air RH	0.906 ( <b>0.005</b> )	-0.282 (0.540)	0.834 ( <b>0.020</b> )	0.784 ( <b>0.037</b> )	-0.285 (0.536)	-0.693 (0.085)	0.871 ( <b>0.004</b> )	0.783 ( <b>0.037</b> )	-0.855 ( <b>0.014</b> )	0.735 (0.060)	0.920 ( <b>0.003</b> )				
<b>One-year-old needles</b>															
Within-crown air temp	-0.848 ( <b>0.016</b> )	-0.497 (0.256)	-0.852 ( <b>0.015</b> )	-0.520 (0.231)	-0.654 (0.111)	-0.472 (0.285)	-0.834 ( <b>0.020</b> )	-0.700 (0.08)	0.590 (0.129)	0.095 (0.839)	-0.816 ( <b>0.028</b> )				
Outside crown air temp	-0.808 ( <b>0.028</b> )	-0.457 (0.302)	-0.775 ( <b>0.041</b> )	-0.466 (0.291)	-0.647 (0.116)	-0.441 (0.322)	-0.846 ( <b>0.016</b> )	-0.687 (0.088)	0.637 (0.124)	0.074 (0.875)	-0.814 ( <b>0.026</b> )				
Within-crown air RH	0.854 ( <b>0.014</b> )	0.547 (0.204)	0.821 ( <b>0.024</b> )	0.552 (0.199)	0.683 (0.091)	0.532 (0.219)	0.845 ( <b>0.017</b> )	0.746 (0.054)	-0.599 (0.156)	-0.165 (0.723)	0.802 ( <b>0.03</b> )				
Outside crown air RH	0.835 ( <b>0.019</b> )	0.448 (0.313)	0.820 ( <b>0.023</b> )	0.472 (0.285)	0.656 (0.110)	0.423 (0.344)	0.850 ( <b>0.015</b> )	0.674 (0.097)	-0.623 (0.135)	-0.058 (0.902)	0.785 ( <b>0.037</b> )				

P values are given in brackets (n = 9). Significant differences (P < 0.05) are highlighted in bold

and increased crown relative humidity along the elevational gradient (Fig. 1; see also Table 3). Consistent with our results, previous studies have observed an increase in leaf  $\delta^{13}\text{C}$  values with increasing altitude (Körner et al. 1988, 1991; Vitousek et al. 1990; Marshall and Zhang 1994). Lower water potential leading to stomata closure may result in higher  $\delta^{13}\text{C}$  values at higher altitude with lower temperature. Morphological features also impose constraints on the physiological response to low temperature.  $\delta^{13}\text{C}$  value was positively correlated with LMA (leaf mass per unit leaf area) and LMA increased with increasing elevation (Hultine and Marshall 2000; Li et al. 2006a). Longer diffusive path in thicker leaves (higher LMA,) increased resistance to  $\text{CO}_2$  and thus decreased partial pressure, leading to increase in  $\delta^{13}\text{C}$  (Körner et al. 1988; Vitousek et al. 1990). The mean increase rate of needle  $\delta^{13}\text{C}$  with 1,000 m increase in elevation was 0.88 ‰ (current year needles) and 0.80 ‰ (1-year-old needles) (Fig. 2i) in the present study, which are a little larger than 0.7 ‰ per 1,000 m elevation increase found by Körner et al. (1988), but less than 0.91 ‰  $\text{km}^{-1}$  for *A. lasiocarpa* to 2.68 ‰  $\text{km}^{-1}$  for *P. contorta* reported by Hultine and Marshall (2000).

The present study showed that the mass-based concentrations of mobile carbohydrates and N were much more sensitive to temperature and moisture than the area-based concentrations (Table 3, see also Fig. 2). This insensitivity of the area-based concentrations may be caused by a combined effect of a microclimate-insensitive SLA (non-significant correlation between SLA and climate factors; Table 3) and a significant interaction of elevation  $\times$  needle age (Table 2). The latter means that the elevational effects were modified by ageing of needles. The mass-based concentrations of leaf carbon and nitrogen are highly significantly correlated with both within-crown microclimate and microclimate outside the tree crown (Table 3). We interpreted this similarity as a result to reflect the significantly positive correlation between within-crown microclimate and microclimate outside a tree crown (Fig. 1). The mass-based concentrations of soluble sugars and NSC were significantly negatively correlated with temperature, but positively correlated with humidity (Table 3; Fig. 1, Fig. 2a, c). Previous studies have documented that foliage sugars and NSC concentrations (both mass based) were not lower in trees at higher elevations compared to trees at lower elevations during the growing season (Hoch and Körner 2005; Shi et al. 2006; Li et al. 2008a, b), indicating also a negative correlation between mobile carbohydrates and temperature. Such higher NSC concentration in plants at high elevations may be regarded as contributing to active osmotic adjustment that is one of the crucial processes in plant adaptation to environmental stresses such as freezing temperature, to avoid intracellular ice formation (Beck et al.

2007). This may also imply that trees at higher elevations (lower temperature) invest less mobile carbohydrates to structural growth compared to trees at lower elevation (higher temperature), because lower temperature limits growth rate associated with rates of cell division and elongation (sink intensity) rather than photosynthesis (source intensity) (Körner 1998, 2003). Classic works, however, indicated that effects of elevated temperature on mobile carbohydrates were positive for tropical grasses, but negative for temperate grasses (Wilson and Bailey 1971; Wilson and Haydock 1971). Li et al. (2012) did not find any species- or provenance-specific responses of N and NSC to air warming for *Quercus* spp.

Needle N content increased with increasing elevation (Fig. 2g, h). In line with our result, previous studies have shown that leaf N increased with increasing altitude (Körner 1989; Hoch and Körner 2005; Li et al. 2008b). Weih and Karlsson (2001) found that a decrease in air temperature led to an increase in leaf  $N_{\text{mass}}$  content in *Betula pubescens* plants. Way and Sage (2008) reported that *Picea mariana* grown in high temperature (30°/24 °C day/night temperature) had lower needle  $N_{\text{area}}$  (−26 ‰) compared to trees grown in low temperature (22°/16 °C). Higher N concentrations at high elevation have important implications for resource use efficiency and photosynthesis of trees in a harsh environment (Li et al. 2008b). High leaf N content was proposed to compensate for lower efficiency of biochemical and physiological processes in low temperature conditions (Reich and Oleksyn 2004).

At lower elevations up to 1,360 m a.s.l. (OCH–WCH intersection point), the mean OCH (measured at 2 m above the ground surface) was higher than WCH (measured at a height of >10 m) (Fig. 1b), which is consistent with data measured on a flux tower located at 740 m a.s.l. within the study forest (Han SJ, personal communication). The mean OCT was somewhat higher than WCT up to an elevation of 1,800 m (OCT–WCT intersection point; Fig. 1a), and beyond that elevation WCT will be higher than OCT (Fig. 1a). But the present study was carried out along a transect, ranging from 760 to 1,420 m a.s.l. only, where the species *P. koraiensis* exists. The study transect just covered the WCH–OCH intersection point at 1,360 m, but did not cover the WCT–OCT intersection point at 1,800 m (Fig. 1a), which resulted in a limitation of the present study. Hence, further research is still needed to explain whether the relationships shown in Table 3 are applicable to elevations beyond the intersection points. Despite this limitation, the present study has important implications for further research using meteorological data as proxy of within-crown microclimate to study physiology from individual leaf level to tree crown and stand canopy level.

A tree's geometrical structure, especially its crown architecture, determines solar radiation interception



associated with crown (e.g. surface) temperature and moisture. Large and complex crowns exhibit a remarkable heterogeneity of light and CO<sub>2</sub> environments created by the crowns themselves (Frak et al. 2001; Le Roux et al. 2001; Takeuchi et al. 2001; Han et al. 2003; Koike et al. 2004), which in turn influence the rates of whole plant photosynthesis, leaf ageing, and acclimation (Hikosaka et al. 1993, 1994; Kull et al. 1995; Kull and Jarvis 1995). During a clear summer day, leaf temperature in water-stressed plants with low stomatal conductance may rise to values that exceed the critical temperature for the stability of PSII (Valladares and Pearcy 1997) and ultimately lead to leaf necrosis. Interactions between light microenvironment, nitrogen allocation, and photosynthetic characteristics generate a remarkable functional heterogeneity within the foliage of a complex crown (Li et al. 2001; Yan et al. 2012). For example, net photosynthetic rates have been found to be higher on the south-facing crown side and at the top crown level than on the north-facing side and at the bottom crown level (Sala and Tenhunen 1996; Johnson et al. 1996; Gonzalez-Real and Baille 2000; Peters et al. 2008). England and Attiwill (2005) reported that sun-exposed *Pinus canariensis* needles at the upper crown level had higher net photosynthetic rate and stomatal conductance than needles which were shaded. Single leaf area, length and thickness, and stomatal parameters were also found to vary with crown position (Maguire and Batista 1996; Niinemets et al. 2001; Niinemets and Kull 2003). Significant azimuthal effects on both leaf thickness and leaf density within *Fagus crenata* crowns were also reported, with greater leaf thickness and density on the N-facing than the S-facing crown side (Iio et al. 2005). Yan et al. (2012) found that needle N concentration significantly decreased, but needle starch and  $\delta^{13}\text{C}$  significantly increased with increasing vertical crown levels of *P. koraiensis*. All those studies indicated that the leaf or tree crown physiology is highly affected by the microclimate within and around the tree crown.

The contents of needle  $\delta^{13}\text{C}$ , N and mobile carbohydrates (except for starch) changed continuously with increasing elevation (Fig. 2), corresponding to a continuous decrease in air temperature and an increase in moisture in and around the tree crowns (Fig. 1). This result indicates that increases in air temperature in combination with decreases in relative humidity (Fig. 1) may result in lower levels of N and mobile carbohydrates in *P. koraiensis* trees (Fig. 2), which suggests that future global climate changes such as temperature increase and changes in precipitation patterns will directly influence the N and carbon physiology at *P. koraiensis* individual level, and indirectly affect the competitive ability (Zhang et al. 2008), species composition (Li et al. 2006b), productivity, and functioning (Pettorelli et al. 2012) at the stand and ecosystem level in NE China.

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