J. Mt. Sci. (2019) 16(7): 1546-1558 e-mail: jms@imde.ac.cn http://jms.imde.ac.cn
https://doi.org/10.1007/s11629-018-5344-0

Age-related variations of needles and twigs in nutrient, nonstructural carbon and isotope composition along altitudinal gradients

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Citation: Tang GT, Peng GQ, Zhang S (2019) Age-related variations of needles and twigs in nutrient, nonstructural carbon and isotope composition along altitudinal gradients. Journal of Mountain Science 16(7). https://doi.org/10.1007/s11629-018-5344-0

Abstract: The biochemical and physiological properties of alpine woody plants responding to elevation are associated with needles and twigs age. However, the interactions with elevation were not well studied. In this study, we investigated age-related (current, one-year and two-year old) functional traits of *Abies faxoniana* in needles and twigs with elevation (2500 m, 2750 m, 3000 m, 3250 m, and 3500 m a.s.l) at the eastern edge of the Tibetan Plateau. The macro-elements (C, N, P, K, Ca, and Mg), nonstructural carbons (soluble sugar, sucrose, and starch) and isotope composition ($\delta^{13}C$) were measured in needles and twigs of adult *A. faxoniana* trees (breast height diameter about 30 cm). There were significant age, elevation and their interaction effects on these traits (except for $\delta^{13}C$). Compared with two-year-old needles and twigs, the current and one-year-old tissues possessed higher concentrations of P and K, lower Ca concentrations, as well as a lower $\delta^{13}C$ values and C: P and N: P ratios in needles. The current-year twigs generally had higher sucrose concentration and sucrose: starch ratio than the old ones. This study suggested that more nutrients were invested to young needles and twigs to cope with elevation for *A. faxoniana* than the old ones.

Keywords: Fir; Elevation; Nutrient; Age; Needle and twig; Sub-alpine

Received: 16-Dec-2018
Revised: 16-Feb-2019
Accepted: 17-Feb-2019

Introduction

Needles and twigs are important tissues for assimilation and transportation for conifers. Lifespan of needles and twigs is a comprehensive trait that reflects the adaptive strategy to the environment for plants to achieve maximum photosynthetic production and maintain efficient nutrient utilization (Eckstein et al. 1999). It has been reported that plant tissues show kinds of physiological changes associated with age. Comparing with juvenile needles, the mature needles of *Pinus koraiensis* accumulated more nonstructural carbon (NSC) due to a higher photosynthetic capacity (Yan et al. 2012). However, younger tissues possessed higher nutrient concentrations than the old, *e.g*. nitrogen (N) and phosphorus (P), particular for coniferous and broad-leaved species (Escudero and Mediavilla

2003; Li et al. 2009a). Leaf age also had an impact on $\delta^{13}C$ values. Previous study has found a higher value of $\delta^{13}C$ in younger leaves than in older leaves (Vitoria et al. 2016). These age-related variations of plant functional traits may be a reflection of growth adapting strategy to resources utilization (Tegischer et al. 2002). Although these plant functional traits associated with lifespan had been found to show a similar tendency within or across plant species, environmental variations will shift their relationships (Day et al. 2001).

Declining temperature with an increasing elevation in the alpine regions has dramatic and multifactorial impacts on plant functional traits (Fajardo et al. 2017). For example, the pool size of NSC in plants, reflecting the balance between carbon gain by photosynthesis and demand by growth and metabolism, closely related to elevation (Garcia Lino et al. 2017; Zhu et al. 2012). Foliar N and P concentrations and $\delta^{13}C$ value generally increased with elevation (Fajardo et al. 2017; Hultine and Marshall 2000). Previous studies figured out that lifespan have a pronounced effect on the variation of plant functional traits along elevation. The younger leaves of *Rhododendron agglutinatum* showed more obvious changes than the old ones (Wang et al. 2017). Moreover, Peng et al. (2012) has reported that leaf characteristics showed significant differences with elevation between juvenile and mature trees of *Abies faxoniana*. However, such scarce knowledge was adverse to comprehend the plant functional traits and their correlation across the lifespan along elevation in subalpine regions (Li et al. 2009; Wang et al. 2017; Huang et al. 2018). Understanding these variations can reveal the plant adaptive strategies to environmental variation, and contribute to predict their responses to future climate changes.

Abies faxoniana Rehder & E.H, an evergreen subalpine conifer, is widely distributed in the eastern margin of Tibetan Plateau (Taylor and Qin 1988). It has been shown that the growth, spatial pattern and population structure of *A. faxoniana* are closely related to elevation. Zhao et al. (2015) has found that there is a unimodal pattern of plant functional traits along elevation, including stomata parameters, specific leaf area and C: N ratio of *A. faxoniana*. However, we still do not know whether there are age-related changes in *A. faxoniana* along elevation or whether young tissues are more sensitive to elevation. In this study, nutrient concentrations, NSC concentrations, and $\delta^{13}C$ in the needles and twigs of *A. faxoniana* were measured to reveal the changes of age-related functional traits from 2500 m to 3500 m a.s.l. We will answer the following two questions: (1) are there distinct differences in plant traits among age cohorts? (2) do these age-related characters affect by elevation?

1 Materials and Methods

1.1 Study site

This study was conducted in the Wanglang Natural Reserve (32°49'–33°02'N, 103°55'–104°10'E) at the eastern edge of the Tibetan Plateau, Sichuan Province of China. The natural elevation range of distribution of *A. faxoniana* was from 2500 m to 3500 m in Wanglang Natural Reserve. Five elevations were selected with an interval of 250 m (2500 m, 2750 m, 3000 m, 3250 m, and 3500 m, respectively) for tree stands, as shown in Table 1 and Figure 1. The mean annual air temperature (MAT) was from 5.04°C to 1.52°C, which measured by TP-2200usb loggers (digital sensor of CMOSens, Beijing Anfu electronic technique Co., China) from May of 2008 to September of 2009. Both rainfall and tree growth season was from April to October every year (Xu et al. 2013). The forest soils of the study sites were mountain brown soils. The main soil nutrients are listed in Table 2.

1.2 Field sampling

Table 1 Description of the sample stands along an altitudinal gradient in Wanglang Natural Reserve

Sample stands	E1	E2	E3	E4	E5
Elevation (m a.s.l.)	2500±50	2750±50	3000±50	3250±50	3500±50
Longitude	104°05' E	104°02' E	104°01' E	104°01' E	104°01' E
Latitude	32°58' N	32°59' N	32°59' N	32°59' N	32°59' N
Slope (°)	37	38	37	30	40
Mean annual temperature (°C)	5.04±0.36	3.91±0.31	2.87±0.29	2.13±0.25	1.52±0.23

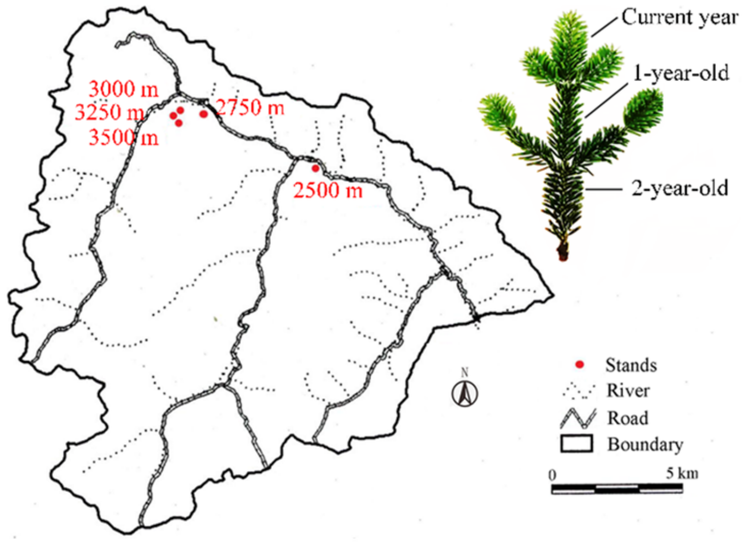

Figure 1 Distribution of stands in Wanglang Natural Reserve and the morphology of the current-year, one-year and two-year old needles and twigs of *A. faxoniana*.

Fifteen stands were selected from 2500 m to 3500 m a.s.l (three sub-stands for each elevation). For each elevation, nine *A. faxoniana* adult trees with breast height diameter of 30.0±2.00 cm were selected for sample collection in early September, 2008. The three sub-stands of each elevation were at least 200 m away and the sampled trees were more than 25 m away from each other within a sub-stand. The trees from each site were according to the following criterions: (1) the *A. faxoniana* forest coverage ranges from 70% to 90%, and the tree height reaches 30 m within the study area; (2) were away from the edge of the forest and there was little evidence of human disturbance; (3) were at a similar slope and aspect.

For each individual tree, a one-meter long and fully expanded twig was identified and cut by a telescoping pole. The sun-exposed tissues (needles and twigs) of current year, 1-year-old and 2-year-old were collected, respectively (Figure 1). Afterwards, the needles and twigs were bagged and labeled, and then stored in a cooler for transportation. To eliminate the effects of irradiance and tissue temperature on diurnal NSC concentrations, sample collection was carried out between 10:00 and 14:00 (Li et al. 2008). All of the plant samples were dried at 105°C for 40 min and then at 75°C to constant weight in a force-air stove. The selected samples were ground into fine powder for further analyses. At the downslope direction of the sampled trees, forest soils were collected up to a depth of 20 cm using a soil-corer. Nine soil samples were naturally dried at the room temperature.

1.3 Chemical analysis

1.3.1 Determination of nutrients in plant and soil

Two hundred milligram of dry powder of plants or soils was used for nutrient determination. The carbon (C) concentration was determined using a rapid dichromate oxidation technique (Nelson and Sommers 1982). The concentrations of N and P were determined by the semi-micro Kjeldahl method and induced plasma emission spectroscopy, following with Mitchell (1998), Lotscher and Hay (1997), respectively. The concentrations of calcium (Ca), potassium (K), and magnesium (Mg) of plant needles and twigs were analyzed by atomic absorption spectroscopy (Agilent 710 ICP-OES) after HNO_3 digestion.

1.3.2 Non-structural carbohydrates (NSCs)

NSCs defined as the sum of soluble sugar and starch in this study. The extraction process of NSC was according to Hoch et al. (2003). The resolution of total soluble sugar was detected colorimetrically at 625 nm following the anthrone-sulfuric acid method (Yemm and Willis 1954). The sucrose

Table 2 Soil nutrient concentrations of *A. faxoniana* along an altitudinal gradient

Stands (m)	C ($g·kg^{-1}$)	N ($g·kg^{-1}$)	P ($g·kg^{-1}$)	K ($g·kg^{-1}$)	C: N	C: P	N: P
2500	87.92±7.64a	6.53±0.43ab	0.83±0.07b	15.23±0.68ab	13.32±0.38a	110.80±12.77a	8.25±0.85ab
2750	132.76±14.94a	9.17±0.82a	0.86±0.05b	10.58±0.76c	14.37±0.93a	151.82±11.90a	10.64±0.79a
3000	116.20±14.01a	8.03±0.82ab	1.14±0.08a	17.73±1.51a	14.33±0.33a	104.32±11.06a	7.20±0.66b
3250	109.24±17.18a	7.15±0.83ab	0.86±0.05b	13.91±0.77bc	14.86±0.61a	128.36±17.22a	8.43±0.88ab
3500	91.04±9.03a	6.13±0.39b	0.87±0.05b	15.93±0.54ab	14.66±0.73a	107.77±13.79a	7.19±0.63b
P:Fa	0.115	0.026	0.007	0.000	0.490	0.097	0.020

Note: *P: Fa*, altitude effect. Values followed by the different letters in the same column are significantly different at the p<0.05 level according to Tukey's test. Each value is the mean±SE (n=9).

concentration was detected colorimetrically at 480 nm following the resorcinol method with little modification (Murata et al. 1968). Residues left in the tubes after extraction were used to starch determination (Green et al. 2010).

1.3.3 Determination of carbon isotope composition

The determination of $\delta^{13}C$ values in needle and twig samples followed Hubick et al. (1986). The ^{13}C: ^{12}C ratio was analyzed by Isotope Ratio Mass Spectrometer (DELTA V Advantage; Thermo Fisher Scientific, Inc., USA). The $\delta^{13}C$ value was expressed relative to the standard Pee Dee Belemnite (PDB) and expressed as:

$$\delta^{13}C(‰) = \left[\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right] \times 1000,$$

where R_{sample} is the ^{13}C: ^{12}C ratio of the samples and R_{standard} is that of the standard material.

1.4 Statistical analyses

To detect the difference within these functional traits (the concentrations of C, N, P, K, Ca and Mg and the ratios of C: N, C: P, and N: P) and NSCs (the soluble sugar, sucrose, and starch) concentrations and the ratio of sucrose: starch among samples, two-factor ANOVA performed with age and elevation as fixed factors. One-way ANOVA was applied to analyze the variations in soil nutrients along an altitudinal gradient. Further, Tukey's HSD tests were carried out as post hoc tests to clarify the significances. Pearson's correlation coefficients were calculated to express the relationships among variables. In addition, the relationships between elevations and plant traits were investigated by regression analyses with Origin 8.5. All statistical analyses were conducted with SPSS 16.0 for windows statistical software package (SPSS Inc., Chicago, IL, USA).

2 Results

2.1 Variation of nutrient concentrations in soils

As shown in Table 2, the concentrations of soil N, P, K, and N: P ratio showed significant differences among elevations ($p<0.05$). There was a higher N concentration and N: P ratio at 2750 m than 3500 m, and a higher P concentration at 3000 m than that of at other elevations. Potassium concentration showed a lowest value at 2750 m (10.58 g·kg^{-1}). However, C, C: N ratio and C: P ratio showed less variation among elevations.

2.2 Variation of nutrients, NSCs and $\delta^{13}C$ among ages

As shown in Tables 3 and 4, the effects of age, elevation and their interaction on C, N, P, K, Ca, Mg, and NSCs concentrations were significant ($p<0.05$) in both needles and twigs of *A. faxoniana*. The $\delta^{13}C$ values were significantly affected by age and elevation but not by their interaction either in needles or in twigs (Figure 2). Compared among three age cohorts, the current-year needles and twigs had the highest P and K

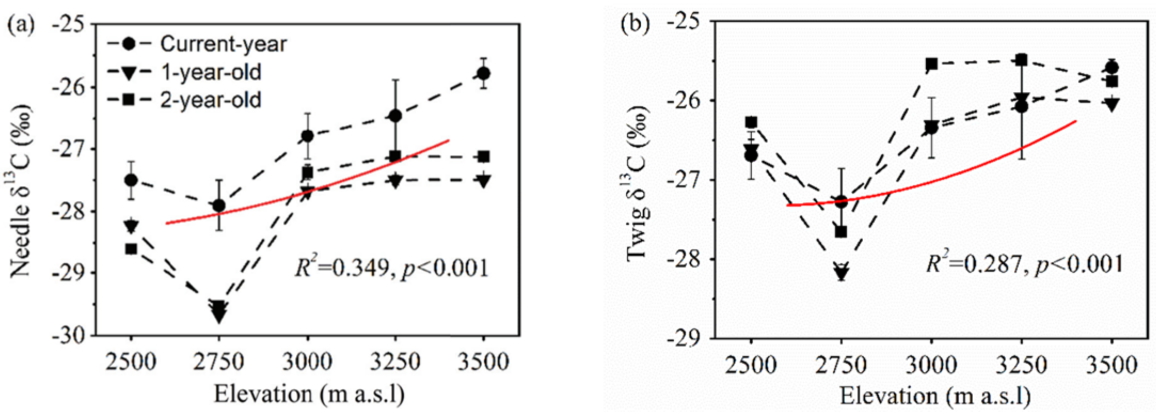

Figure 2 Variations of $\delta^{13}C$ in *A. faxoniana* needles (a) and twigs (b) along an altitudinal gradient. *P: Fy*, age effect; *P: Fa*, altitude effect; *P: Fy×a*, age and altitude interaction effect. The lower cases indicates difference in needles or twigs with the same age along an altitudinal gradient, capitals indicates difference in the same elevation among different ages at the $p < 0.05$ level according to Tukey's test. Each value is the mean ± SE (n=9).

Table 3 The concentration of nutrients in needle of *A. faxoniana* along an altitudinal gradient

Age	Elevation (m)	C (g·kg-1)	N (g·kg-1)	P (g·kg-1)	K (g·kg-1)	Ca (g·kg-1)	Mg (g·kg-1)	C: N	C: P	N: P
Current-year	2500	510.15±4.16f	11.71±0.17g	1.16±0.03b	7.52±0.10b	4.36±0.04fh	0.78±0.04cd	43.57±0.50b	441.48±10.26g	10.14±0.16fg
	2750	520.37±2.30cde	12.30±0.25ef	1.34±0.03a	9.45±0.26a	3.72±0.06i	0.75±0.04cde	42.34±0.85bc	389.79±8.42h	9.22±0.29h
	3000	519.78±1.21de	12.25±0.20ef	1.29±0.04a	7.19±0.12c	4.14±0.09h	0.72±0.02def	42.45±0.62bc	402.96±10.87h	9.50±0.20gh
	3250	524.58±1.67bcde	13.08±0.10b	1.33±0.04a	7.48±0.19bc	3.19±0.15j	0.70±0.06defg	40.09±0.38f	395.70±12.90h	9.87±0.24fgh
	3500	528.42±1.08bc	13.62±0.10a	1.29±0.05a	7.17±0.13c	2.59±0.06k	0.62±0.04h	38.80±0.29g	410.12±16.24h	10.57±0.50f
1-year-old	2500	517.36±3.26e	12.42±0.12ef	0.97±0.00d	5.41±0.04e	10.60±0.27b	0.90±0.01a	41.65±0.24cde	531.89±3.84e	12.77±0.15e
	2750	522.21±1.47cde	12.84±0.16bcd	0.98±0.02d	6.26±0.03d	7.05±0.05e	0.80±0.02bc	40.67±0.50ef	533.66±11.65de	13.12±0.44de
	3000	525.77±1.08bcd	13.99±0.11a	1.07±0.02c	3.66±0.08h	7.61±0.07d	0.87±0.01ab	37.59±0.22g	491.17±10.64f	13.07±0.30de
	3250	537.60±4.30a	13.86±0.07a	0.97±0.01d	4.83±0.00f	5.88±0.09f	0.69±0.01efgh	38.78±0.33g	555.83±7.06de	14.33±0.08b
	3500	532.66±3.43ab	12.93±0.07bc	0.97±0.02d	4.25±0.04h	5.41±0.10g	0.63±0.01gh	41.18±0.31cdef	548.02±11.29de	13.31±0.37cde
2-year-old	2500	522.75±1.11cde	12.08±0.09fg	0.93±0.01de	4.77±0.05f	15.03±0.09a	0.87±0.00ab	43.29±0.34b	562.72±5.64cd	13.00±0.11de
	2750	531.63±2.40ab	11.79±0.09g	0.86±0.01efg	4.71±0.04f	7.20±0.07e	0.65±0.02fgh	45.11±0.15a	618.31±6.63b	13.71±0.18bcd
	3000	526.36±2.60bcd	12.63±0.12cde	0.90±0.00def	3.52±0.04h	9.34±0.08c	0.67±0.01efgh	41.67±0.40cde	584.45±3.00c	14.02±0.09bc
	3250	526.59±1.47bcd	12.86±0.05bcd	0.84±0.01fg	4.26±0.04g	7.01±0.04e	0.54±0.01i	40.94±0.22def	626.89±5.49ab	15.31±0.14a
	3500	528.19±1.99bc	12.51±0.14de	0.82±0.00g	4.37±0.04g	6.15±0.03f	0.51±0.01i	42.24±0.47bcd	646.79±5.16a	15.31±0.17a
	P:Fy	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	P:Fa	0.000	0.000	0.014	0.000	0.000	0.000	0.002	0.000	0.000
	P:Fy×a	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4 The concentration of nutrients in twigs of *A. faxoniana* along an altitudinal gradient

Age	Elevation (m)	C (g·kg-1)	N (g·kg-1)	P (g·kg-1)	K (g·kg-1)	Ca (g·kg-1)	Mg (g·kg-1)	C: N	C: P	N: P
Current-year	2500	502.48±3.31c	10.76±0.12c	2.13±0.06a	12.24±0.13a	4.44±0.22def	1.18±0.03cd	46.70±0.25fg	235.65±6.34e	5.05±0.15i
	2750	515.04±2.80b	10.46±0.28c	1.87±0.04b	11.65±0.13b	3.34±0.10h	1.25±0.03bc	49.26±1.44de	274.81±7.13d	5.58±0.27hi
	3000	530.64±2.53a	10.75±0.05c	1.81±0.05bc	8.87±0.19de	4.12±0.10fg	1.13±0.02d	49.38±0.36de	293.44±9.87cd	5.94±0.17h
	3250	513.74±2.53b	13.05±0.35a	1.78±0.07bc	9.49±0.16c	3.94±0.17g	1.31±0.05ab	39.37±1.20i	288.02±9.83cd	7.31±0.43ef
	3500	518.70±3.45b	11.50±0.21b	1.70±0.08c	8.75±0.20e	3.00±0.21i	1.12±0.05de	45.10±0.99g	305.40±17.61c	6.77±0.26fg
1-year-old	2500	490.99±2.06de	9.52±0.06de	1.42±0.01d	8.22±0.06f	6.61±0.07b	1.27±0.02abc	51.59±0.52cd	346.10±3.39b	6.71±0.07g
	2750	501.08±1.08c	8.89±0.09f	1.47±0.01d	9.11±0.08d	4.65±0.05de	1.22±0.00bc	56.34±0.53b	341.97±2.50b	6.07±0.09h
	3000	497.47±1.09cd	9.81±0.05d	1.42±0.02d	7.13±0.09h	4.32±0.05ef	1.35±0.01a	50.69±0.30cd	349.41±5.10b	6.89±0.08fg
	3250	501.99±1.16c	10.37±0.07c	1.17±0.00e	7.76±0.01g	3.86±0.03g	1.19±0.01cd	48.42±0.39ef	429.50±1.96a	8.87±0.08b
	3500	497.33±2.78cd	11.86±0.06b	1.49±0.01d	6.79±0.03i	4.14±0.04fg	1.03±0.01ef	41.92±0.23h	334.88±4.09b	7.99±0.08cd
2-year-old	2500	489.41±2.92de	9.28±0.01ef	1.17±0.00e	5.75±0.07k	7.19±0.05a	1.31±0.03ab	52.75±0.29c	419.86±3.03a	7.96±0.02cd
	2750	497.63±1.56cd	8.45±0.11g	1.17±0.02e	7.58±0.03g	5.08±0.04c	0.98±0.04f	58.87±0.97a	426.72±7.36a	7.25±0.06efg
	3000	500.15±0.96c	9.09±0.07ef	1.19±0.03e	6.20±0.07j	4.78±0.04cd	1.11±0.02de	55.00±0.33b	420.69±10.94a	7.65±0.17de
	3250	510.91±5.17b	9.91±0.05d	1.17±0.02e	6.66±0.07i	4.44±0.17def	1.10±0.04de	51.53±0.66cd	435.32±13.21a	8.45±0.17bc
	3500	485.23±5.04e	10.56±0.15c	1.10±0.04d	5.99±0.08jk	3.90±0.03g	1.00±0.01f	45.97±0.91g	442.88±13.58a	9.63±0.28a
	P:Fy	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	P:Fa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	P:Fy×a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: *P:Fy,* age effect; *P:Fa,* altitude effect; *P:Fy×a,* age and altitude interaction effect. Values followed by the different letters in each column are significantly different at the $p<0.05$ level according to Tukey's test. Each value is the mean±SE (n=9).

concentrations but a lower Ca concentration than other cohorts. The ratios of C: P and N: P in both needles and twigs as well as δ^{13}C in needles were lower in the current-year needles than that of in other age cohorts. The starch concentrations in the current-year needles and sucrose in the current-year twigs were higher than other ages (Figures 3 and 4). The δ^{13}C values decreased significantly with ages in needles, in which the current-year needles showed significantly higher values than the one-year and the two-year old needles.

2.3 Variation of nutrients, NSCs and δ^{13}C among elevations

The δ^{13}C values showed an increasing tendency with elevation in both needles and twigs with a lowest value in 2750 m a.s.l. (Figure 2). The concentration of C and N in needles as well as sucrose in twigs increased with elevation (Appendix 1). Moreover, the concentration of Mg in needles and Ca in twigs decreased with increasing elevation. The twig C: N ratio increased with elevation below 2750 m a.s.l., then declined (Appendix 1). Both soluble sugar and sucrose in the

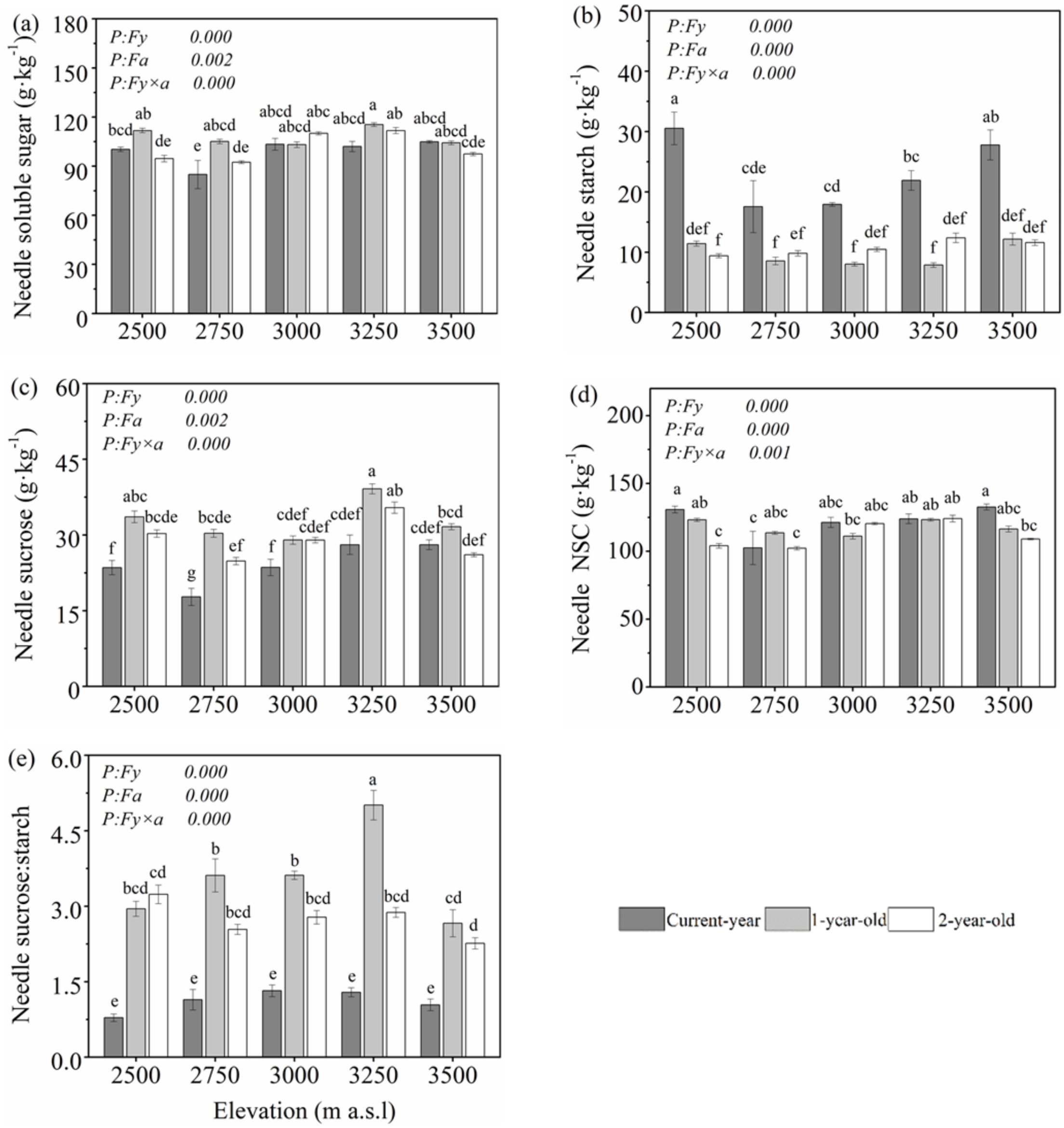

Figure 3 The concentrations of soluble sugar (a), starch (b), sucrose (c) and NSC (d) and the ratio of sucrose: starch (e) in the current, one-year and two-year old of *A. faxoniana* needles along an altitudinal gradient. *P: Fy*, age effect; *P: Fa*, altitude effect; *P: Fy*×*a*, age and altitude interaction effect. Values followed by different letters are significantly different at the p<0.05 level according to Tukey's test. Each value is the mean ± SE (n=9).

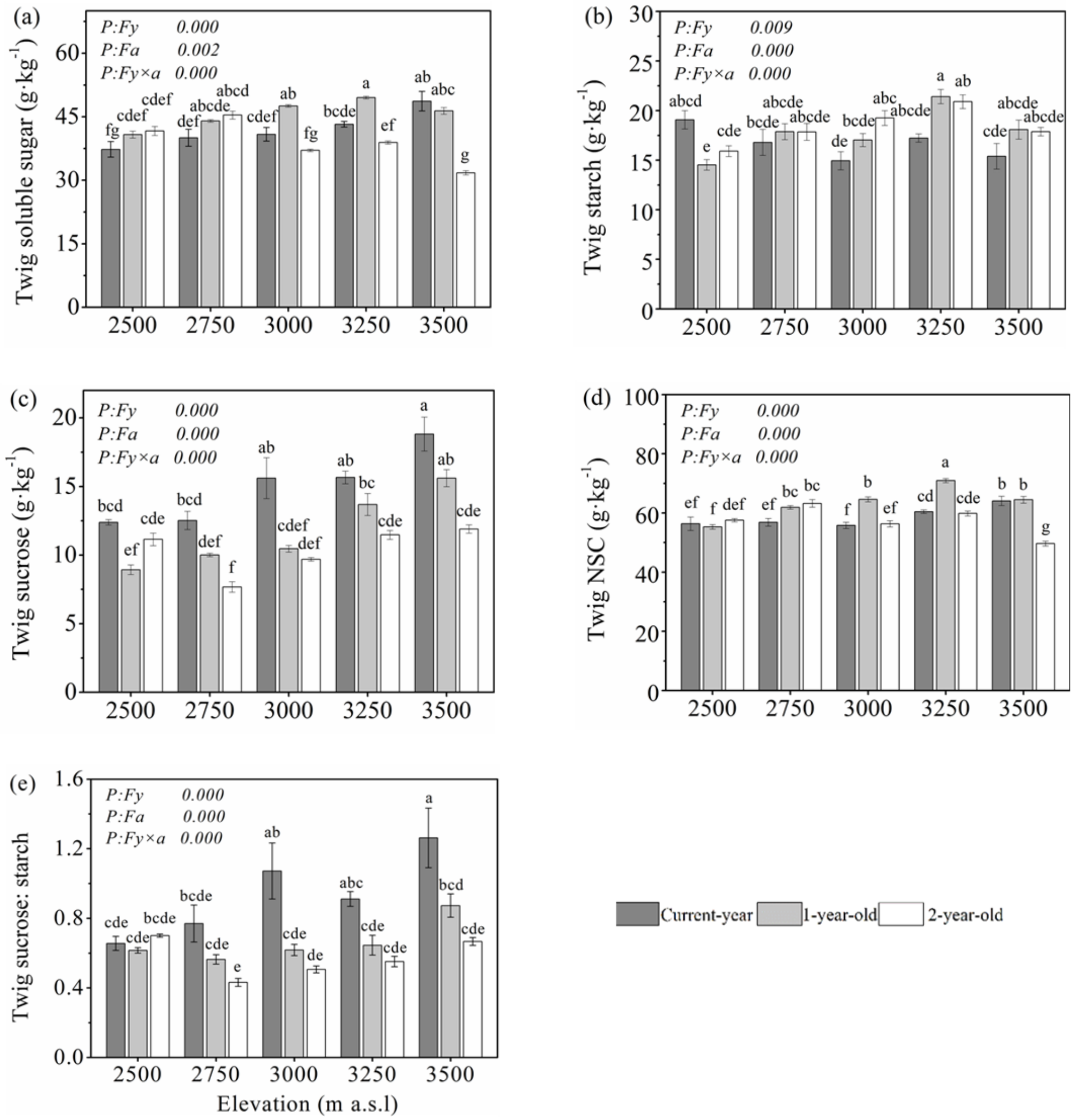

Figure 4 The concentrations of soluble sugar (a), starch (b), sucrose (c) and NSC (d) and the ratio of sucrose: starch (e) in the current, one-year and two-year old of *A. faxoniana* twigs along an altitudinal gradient. *P: Fy*, age effect; *P: Fa*, altitude effect; *P: Fy×a*, age and altitude interaction effect. Values followed by different letters are significantly different at the $p<0.05$ level according to Tukey's test. Each value is the mean ± SE ($n=9$).

current-year twigs increased with elevation (Figure 4). The effects of elevation were generally more profound in the current-year needles and twigs, suggesting a growth rate variation among three ages along elevation (Appendix 2).

2.4 The correlation among nutrients and NSCs in needles and twigs

In this study, we found not only the effects of age or/and elevation but also their interaction were significant for all the plant traits except for $\delta^{13}C$, indicating water may be not a growth limiting factor in our study sites. To better understand the relationship among these functional traits, partial correlation analysis was performed (Tables 5 and 6). In needles, the $\delta^{13}C$ values showed a significantly positive correlation ($p<0.01$) with the concentrations of N, P, starch and NSCs but significantly negative correlation with the concentrations of Ca and Mg and the ratios of C: N and sucrose: starch ($p<0.01$). Sucrose had a significantly positive correlation with the concentrations of C, N, Ca and soluble sugar and the ratios of C: P and N: P, but a significantly negative correlation with the concentrations of P, K and starch and C: N ratio. Additionally, C: N ratio was strongly negative correlated ($p<0.001$) with N concentration. In twigs (Table 6), $\delta^{13}C$ value was positively correlated ($p<0.05$) with the concentrations of N, sucrose and TNC and the ratios of N: P and sucrose: starch, but was negatively correlated ($p<0.01$) with K concentration and C: N ratio. Sucrose was positively correlated ($p<0.05$) with C, N, P and soluble sugar concentrations but negatively correlated ($p<0.01$) with Ca concentration and the ratios of C: N and C: P. Additionally, P had a strongly positive correlation ($p<0.001$) with C and N concentrations.

3 Discussion

3.1 Variation in nutrient concentration, NSCs and $\delta^{13}C$ associated with age

The concentration and distribution of soil nutrients have considerable effects on plant growth and nutrient use efficiency (Vondrackova et al. 2014), and the limited soil nutrient availability may translate into reduced leaf nutrient concentration (Sullivan et al. 2016). The ratio of soil C: N and C: P has been widely used to estimate the mineralization capability and nutrient availability (Dise et al. 1998; Sardans et al. 2012). In this study, a constant ratio of C: N and C: P at each elevation suggests less variation in the availability of soil C, N, and P for tree growth.

Growth rate hypothesis suggests that organisms adjust their C: N: P ratio to adapt to growth rate variations (Main et al. 1997). Various data indicate that rapidly growing organisms commonly have low biomass C: P and N: P ratios (Elser et al. 2003). In this study, the increasing ratios of C: P and N: P with age (Tables 3 and 4) may attribute to increased allocation to P rich ribosomal RNA, as rapid protein synthesis by ribosomes is required to support fast growth (Elser et al. 2003). As known, the younger tissues generally tend to be more active in relation to the older. Moreover, it generally associates with higher photosynthetic rate, growth rate and competitiveness for resources that more N and P in leaves, while more foliar C suggests a strong defense against environmental variations (Lourens and Frans 2006; Wright et al. 2004). Hence, the higher concentration of starch in current-year needles may result from the accumulation of N, P and the lower C: P ratio (Figure 3 and Table 3). Moreover, the significant correlations between the P, C: P, N: P and starch also confirm their intensive relationship. It is commonly considered that N: P ratio >16 indicates a P limitation while a value <14 indicates N limitation (Koerselman and Meuleman 1996). The mean N: P ratios in the current-year, one-year and two-year needles were 9.86, 13.32 and 14.27, respectively. A similar foliar N concentration of *A. faxonana* in Wolong Natural Reserve (8.0~13.0 $g \cdot kg^{-1}$) and in Wanglang Natural Reserve (8.0~13.6 $g \cdot kg^{-1}$) have been reported respectively (Peng et al. 2012; Zhao et al. 2015). Considering the consistent N concentration along elevation, we inferred that a greater demand for N supply to the current-year needles than other cohorts due to its active physiological activity. In addition to the various demands for nutrients among ages, the nutrient status is also probably due to the resorption capacity from the senescent needles (Sohrt et al. 2018). Foliar resorption allows trees to reduce nutrient loss and to establish nutrient storage pools, which contributes faster shoot growth in spring and decreases the need for competitive nutrient uptake (See et al. 2015).

3.2 Variation in nutrient, NSC and $\delta^{13}C$ associated with elevation

Plants growing at high elevations have higher $\delta^{13}C$ values than that at low elevations (Korner et al. 1988). It has documented that the $\delta^{13}C$ values of plants increased with elevation both on a global scale and locally in humid climates, usually with an average of 1.2‰ km^{-1} (Korner et al. 1991). High carboxylation capacity in relation to the stomatal conductance of plants and reduction of CO_2 diffusivity due to the low temperature may be accounted for the increase of $\delta^{13}C$ at the high elevations. Interestingly, in this study, the $\delta^{13}C$ value decreased with elevation below 2750 m, then increased from 2750 to 3500 m. Similar results have been found in *Quercus aquifolioides* (Li et al. 2009a) and *A. faxoniana* (Zhao et al. 2015) at this region. This inflection point was interpreted

Table 5 Correlation coefficient among nutrients, NSCs and δ13C in needle of *A. faxoniana* along an altitudinal gradient

		C	N	P	K	Ca	Mg	C: N	C: P	N: P	Soluble sugar	Starch	Sucrose	NSC	Sucrose: starch	δ13C
(a)	C		0.494***	-0.275*	-0.429***	-0.049	-0.429***	-0.252	0.376**	0.451***	0.232	-0.380**	0.415***	-0.061	0.449***	0.108
	N	0.355**		0.113	-0.229	-0.22	-0.043	-0.964***	-0.091	0.196	0.415***	-0.140	0.465***	0.241	0.392**	0.358**
	P	0.102	-0.006		0.817***	-0.605***	0.205	-0.204	-0.984***	-0.942***	-0.228	0.623***	-0.487***	0.222	-0.609***	0.402**
	K	-0.077	-0.505***	0.310*		-0.575***	0.142	0.125	-0.812***	-0.862***	-0.401**	0.634***	-0.575***	0.091	-0.664***	0.173
	Ca	-0.004	0.386**	0.133	-0.399**		0.422***	0.229	0.548***	0.489***	0.061	-0.580***	0.333**	-0.328*	0.529***	-0.504***
	Mg	0.096	0.621***	0.004	-0.563***	0.649***		-0.067	-0.322*	-0.301*	0.008	-0.044	-0.003	-0.022	0.156	-0.396**
	C: N	-0.102	-0.964***	0.032	0.501***	-0.425***	-0.633***		0.208	-0.090	-0.408**	0.048	-0.404**	-0.295*	-0.298*	-0.374**
	C: P	0.023	-0.094	-0.943***	-0.111	-0.325*	-0.193	0.108		0.955***	0.198	-0.623***	0.460***	-0.246	0.577***	-0.357**
	N: P	0.073	0.505***	-0.834***	-0.409**	-0.018	0.219	-0.516***	0.791***		0.332**	-0.652***	0.593***	-0.158	0.686***	-0.252
	Soluble sugar	0.091	0.372**	-0.347**	-0.539***	0.237	0.312*	-0.387**	0.260*	0.467***		-0.057	0.750***	0.762***	0.398**	0.231
	Starch	-0.313*	-0.421***	0.027	0.214	-0.228	-0.344**	0.369**	0.026	-0.221	-0.004		-0.372**	0.603***	-0.815***	0.541***
	Sucrose	0.237	0.521***	-0.399**	-0.471***	0.423***	0.408**	-0.502***	0.301*	0.565***	0.758***	-0.199		0.357**	0.722***	0.042
	NSC	-0.087	0.096	-0.282*	-0.347**	0.082	0.085	-0.136	0.236	0.282*	0.851***	0.522***	0.542***		-0.211	0.536***
	Sucrose: starch	0.418**	0.694***	-0.288*	-0.466***	0.323*	0.507***	-0.621***	0.187	0.558***	0.432***	-0.715***	0.692***	-0.008		-0.406**
	δ13C	-0.184	-0.065	0.136	-0.083	0.237	-0.119	0.017	-0.174	-0.148	0.188	0.484***	0.083	0.414**	-0.313*	
(b)	C		0.256	-0.331*	-0.384**	0.330*	-0.048	0.022	0.391**	0.397**	0.122	-0.488***	0.355**	-0.227	0.546***	-0.350**
	N	0.575***		0.139	-0.134	0.096	0.631***	-0.959***	-0.178	0.079	0.351**	-0.199	0.418**	0.146	0.480***	0.022
	P	0.080	-0.007		0.836***	-0.703***	0.292*	-0.233	-0.988***	-0.969***	-0.235	0.623***	-0.500***	0.228	-0.609***	0.504***
	K	-0.282*	-0.599***	0.288*		-0.811***	-0.014	0.028	-0.814***	-0.855***	-0.369**	0.658***	-0.553***	0.144	-0.68***	0.386**
	Ca	-0.426***	-0.184	0.096	0.008		0.099	-0.018	0.696***	0.725***	0.217	-0.649***	0.536***	-0.259*	0.611***	-0.288*
	Mg	-0.385**	-0.075	0.005	-0.078	0.831***		-0.665***	-0.364**	-0.196	0.263*	-0.022	0.236	0.194	0.218	0.063
	C: N	-0.365**	-0.970***	0.029	0.589***	0.089	-0.014		0.291*	0.025	-0.344**	0.077	-0.345**	-0.223	-0.342**	-0.122
	C: P	0.178	0.080	-0.909***	-0.201	-0.412**	-0.324*	-0.042		0.963***	0.181	-0.630***	0.452***	-0.275*	0.580***	-0.523***
	N: P	0.338**	0.638***	-0.733***	-0.514***	-0.351**	-0.221	-0.630***	0.798***		0.292*	-0.681***	0.571***	-0.220	0.707***	-0.507***
	Soluble sugar	0.213	0.433***	-0.337**	-0.575***	-0.008	0.031	-0.445***	0.308*	0.513***		-0.069	0.735***	0.749***	0.411**	0.113
	Starch	-0.217	-0.312*	0.026	0.177	-0.200	-0.275*	0.294*	0.040	-0.167	0.010		-0.392**	0.609***	-0.816***	0.648***
	Sucrose	0.331*	0.555***	-0.388**	-0.515***	0.113	0.088	-0.545***	0.347**	0.599***	0.772***	-0.179		0.324*	0.743***	-0.124
	NSC	0.071	0.210	-0.275*	-0.400**	-0.109	-0.114	-0.230	0.283*	0.353**	0.859***	0.520***	0.568***		-0.215	0.520***
	Sucrose:starch	0.332*	0.565***	-0.288*	-0.429***	0.231	0.349**	-0.538***	0.178	0.487***	0.418***	-0.715***	0.670***	-0.009		-0.506***
	δ13C	0.282*	0.337**	0.101	-0.306*	-0.342**	-0.543***	-0.313*	0.051	0.217	0.295*	0.401**	0.222	0.457***	-0.240	

Note: (a) Partial correlation coefficients on removing the effects of both age and altitude (upper triangle). The correlation coefficients without removing the effects of age and altitude (lower triangle).
(b) Partial correlation coefficients on removing the effects of age (upper triangle). The correlation coefficients without removing the effects of altitude (lower triangle).
*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Table 6 Correlation coefficient among nutrients, NSC and $\delta^{13}C$ in twig of *A. faxoniana* along an altitudinal gradient

		C	N	P	K	Ca	Mg	C: N	C: P	N: P	Soluble sugar	Starch	Sucrose	NSC	Sucrose: starch	$\delta^{13}C$
(a)	C		0.364**	0.523***	0.442***	-0.519***	0.036	-0.162	-0.443***	-0.404**	0.202	-0.152	0.543***	0.378**	0.549***	0.040
	N	-0.303*		0.508***	0.297*	-0.529***	0.035	-0.967***	-0.508***	0.043	0.074	-0.035	0.760***	0.652***	0.581***	0.383**
	P	-0.005	0.234		0.873***	-0.359**	0.272*	-0.433***	-0.978***	-0.817***	0.011	-0.237	0.424***	0.215	0.420***	-0.213
	K	-0.011	-0.084	0.405**		-0.355**	0.268*	-0.216	-0.831***	-0.784***	-0.002	-0.079	0.205	0.128	0.193	-0.394**
	Ca	-0.213	0.475***	-0.175	-0.699***		0.256*	0.452***	0.306*	0.045	-0.147	-0.169	-0.560***	-0.614***	-0.40**	-0.180
	Mg	-0.144	0.133	-0.307*	-0.380**	0.296*		-0.043	-0.324*	-0.333**	0.250	-0.234	-0.010	-0.169	0.075	-0.141
	C: N	0.557***	-0.931***	-0.219	0.102	-0.487***	-0.180		0.446***	-0.132	-0.010	0.000	-0.704***	-0.627***	-0.511***	-0.426***
	C: P	0.270*	-0.260*	-0.858***	-0.158	-0.036	0.165	0.293*		0.826***	-0.073	0.304*	-0.406**	-0.153	-0.424***	0.244
	N: P	-0.261*	0.510***	-0.608***	-0.210	0.347**	0.295*	-0.540***	0.633***		-0.070	0.323*	-0.013	0.209	-0.148	0.519***
	Soluble sugar	0.032	-0.308*	-0.470***	-0.338**	0.135	0.287*	0.331*	0.308*	0.010		-0.121	0.325*	0.206	0.334**	-0.115
	Starch	-0.021	0.048	0.150	0.523***	-0.320*	-0.066	-0.059	0.024	0.036	-0.093		-0.213	0.495***	-0.587***	0.092
	Sucrose	0.148	0.203	-0.046	-0.351**	0.382**	0.068	-0.179	0.112	0.209	0.172	-0.319*		0.743***	0.902***	0.366**
	NSC	0.088	0.194	0.110	0.244	-0.028	-0.014	-0.187	0.104	0.187	0.037	0.723***	0.425***		0.400**	0.388**
	Sucrose:starch	0.206	0.007	-0.146	-0.492***	0.346**	0.060	0.026	0.124	0.082	0.195	-0.747***	0.835***	-0.105		0.258*
	$\delta^{13}C$	0.021	0.340**	-0.025	-0.292*	0.252	0.174	-0.357**	0.105	0.331*	-0.210	-0.070	0.272*	0.132	0.209	
(b)	C		0.320*	0.577***	0.547***	-0.539***	0.127	-0.074	-0.496***	-0.628***	0.180	-0.198	0.544***	0.348**	0.527***	-0.071
	N	-0.04		0.750***	0.628***	-0.257*	0.355**	-0.953***	-0.757***	-0.401**	-0.012	-0.187	0.646***	0.456***	0.484***	0.118
	P	-0.103	-0.153		0.878***	-0.654***	0.217	-0.654***	-0.977***	-0.880***	0.040	-0.206	0.663***	0.456***	0.538***	-0.137
	K	-0.149	-0.476***	0.548***		-0.831***	0.144	-0.520***	-0.830***	-0.761***	0.054	-0.007	0.529***	0.503***	0.372**	-0.262*
	Ca	-0.312*	-0.326*	0.219	0.095		-0.031	0.139	0.591***	0.683***	-0.063	-0.041	-0.291*	-0.317*	-0.219	0.279*
	Mg	-0.229	-0.233	-0.061	-0.006	0.510***		-0.362**	-0.273*	-0.128	0.349**	-0.164	0.312*	0.155	0.276*	0.110
	C: N	0.257*	-0.960***	0.126	0.455***	0.258*	0.168		0.677***	0.266*	0.089	0.142	-0.566***	-0.419***	-0.394**	-0.183
	C: P	0.339**	0.154	-0.885***	-0.380**	-0.359**	-0.065	-0.092		0.886***	-0.106	0.274*	-0.647***	-0.380**	-0.547***	0.169
	N: P	0.011	0.773***	-0.681***	-0.565***	-0.428***	-0.162	-0.762***	0.708***		-0.191	0.253	-0.508***	-0.265*	-0.474***	0.307*
	Soluble sugar	0.067	-0.102	-0.487***	-0.361**	-0.032	0.185	0.146	0.342**	0.124		-0.158	0.294*	0.144	0.303*	-0.235
	Starch	0.032	0.189	0.040	0.276*	-0.370**	-0.155	-0.187	0.119	0.189	-0.056		-0.419***	0.485***	-0.735***	-0.026
	Sucrose	0.268*	0.615***	-0.335**	-0.629***	-0.380**	-0.279*	-0.567***	0.391**	0.642***	0.230	-0.056		0.591***	0.905***	0.079
	NSC	0.225	0.596***	-0.225	-0.282***	-0.545***	-0.319*	-0.558***	0.378**	0.617***	0.135	0.657***	0.716***		0.219	0.053
	Sucrose:starch	0.286*	0.337**	-0.315*	-0.628***	-0.155	-0.159	-0.287*	0.306*	0.400**	0.243	-0.543***	0.847***	0.260*		0.071
	$\delta^{13}C$	0.144	0.587***	-0.249	-0.522***	-0.275*	-0.109	-0.581***	0.320*	0.592***	-0.089	0.061	0.551***	0.459***	0.408**	

Note: (a) Partial correlation coefficients on removing the effects of both age and altitude (upper triangle). The correlation coefficients without removing the effects of age and altitude (lower triangle).
(b) Partial correlation coefficients on removing the effects of age (upper triangle). The correlation coefficients without removing the effects of altitude (lower triangle).
*, p<0.05; **, p<0.01; ***, p<0.001.
NSC means nonstructural carbon.

as the optimum distribution zone for tree population, which resulting from the higher nitrogen use efficiency (NUE, derived from C: N ratio) at the expense of decreasing WUE. In this case, $\delta^{13}C$ was negatively correlated with C: N ratio in both needles and twigs along elevation (Tables 5 and 6), which follows the theory of trade-off between WUE and NUE (Iii et al. 1990). Therefore, the trade-off between NUE and WUE along elevation may contribute to understand the altitudinal distribution of *A. faxoniana* in relation to moisture and nutrient availability. Moreover, the variation of C: N ratio in twig also supported this point (Appendix 1). In addition, the a sampling bias may be another explanation for this inflection point, a humidity inhibit in the valley near to a river is in favor of tree growth and will show relative low water use efficiency.

The size and variation of NSC pool could mirror the balance between the demand for carbon and its supply for plants (Fajardo et al. 2012; Yan et al. 2012). Compared with other studies, our results indicate that *A. faxoniana* needles and twigs possessed high NSC concentration at the end of growing season (Xu et al. 2013; Yan et al. 2012). An abundant NSC suggests no carbon limitation under present CO_2 status, which is consistent with previous study (Li et al. 2009b; Shi et al. 2006). Moreover, carbon investment in storage (higher NSC) is an advantageous strategy in habitats with frequent stress or disturbance (Poorter and Kitajima 2007). Generally, high elevation are associated with low temperature, in which a decrease in the assimilation of photosynthetic carbon and starch turnover, as well as an increase in the accumulation of soluble sugars, such as sucrose and glucose (Dubey and Singh 1999; Klotke et al. 2004). The higher sucrose concentration may be a physiological adaptive strategy to high elevation for *A. faxoniana* (Wang et al. 2018; Xu et al. 2013). The concentrations of C and N in needles and $\delta^{13}C$ in needles increased in *A. faxoniana* with elevation (Figure 2 and Appendix 1). However, the photosynthetic rate along elevation showed distinct difference among three age cohorts, which could indicate the sensitivity differences for three ages. Both current-year needles and twigs displayed a greater increase in both C: N ratio and $\delta^{13}C$ along elevation (Appendix 2), indicating the current-year tissues have a trade-off between NUE and WUE, and then lead to in a higher photosynthesis for carbon fixation. Therefore, the younger tissues may be able to better cope with changes such as global warming or extreme chilling.

4 Conclusion

The results of this study suggest that, both intrinsic and environmental factor are responsible for the variations in the plant traits of *A. faxoniana* in the Wanglang Natural Reserve. The age, elevation, and their interaction have profound effects on the physiological ecology of trees. The young tissues generally possessed higher concentrations of P, K and $\delta^{13}C$ (only in needles), but lower Ca concentrations and ratios of C: P and N: P than the older ones. The current-year needles and twigs generally had the highest sucrose concentrations and sucrose: starch ratio among those of one-year and two-year old tissues. Our results suggested that *A. faxoniana* may employ more nutrient but not water to young tissues with elevation. Therefore, there are age-related variations in needles and twigs of *A. faxoniana* in nutrient, nonstructural carbon and isotope composition and the younger tissues are more sensitive to elevation than the older.

Acknowledgements

This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA20020401) and the National Natural Science Foundation of China (NO. 31770650).

Electronic supplementary material: Supplementary material (Appendixes 1, 2) is available in the online version of this article at https://doi.org/10.1007s11629-018-5344-0.

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