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Evergreen *Quercus aquifolioides* remobilizes more soluble carbon components but less N and P from leaves to shoots than deciduous *Betula ermanii* at the end-season

Yu Cong (1),
Ao Wang (2-4-7),
Hong S He (1-3),
Fei-Hai Yu (2-4),
Roberto Tognetti (5-6),
Paolo Cherubini (7),
Xue Wang (2),
Mai-He Li (1-7)

Remobilization is an important mechanism of resource conservation in plants. However, our understanding of whether the responses of resource remobilization to global warming differ between deciduous and evergreen trees remains unclear. We assessed resource remobilization from leaves to 1-year-old shoots in a deciduous (*Betula ermanii*) and an evergreen (*Quercus aquifolioides*) species along elevational gradients (*i.e.*, temperature gradient) at the end of growing season. We aimed to test the hypotheses that the reallocation rate increased with increasing elevation and that more resources were reallocated from leaves to storage tissues in deciduous species than in evergreen species. We analyzed the concentrations of non-structural carbohydrates (NSC), nitrogen (N) and phosphorus (P), and compared the differences in remobilization efficiency of NSC, N, and P between leaves and shoots within each species and between the two species along the elevational gradients. Due to the different strategies of evergreen and deciduous species in nutrients use, the deciduous species had higher N and P remobilization rate, but lower remobilization rate of sugars, starch, and NSC than the evergreen species at the end of growing season. The remobilization rate of NSC, N, and P was significantly higher in trees at their upper limits compared to lower elevations. Our results suggest that trees reallocate resources from leaves to storage tissues before leaf senescence or at the end of growing season, to increase the resource use efficiency and to adapt to the harsh alpine environments. These results contribute to better understanding of the alpine treeline phenomenon in a changing world.

Keywords: Altitudinal Gradient, Non-structural Carbohydrates, Sugars, Starch, Nutrients, Reallocation

Introduction

Plants reallocate resources from leaves to storage tissues prior to senescence, to support growth after dormancy (Aerts 1996, Cherbuy et al. 2001), but also for defense and reproduction (Chapin et al. 1990). To increase the resource use efficiency and to reduce the costs, leaf carbon components, nitrogen (N), and phosphorus (P) are recycled through remobilization from leaves to storage organs with a major pulse shortly before senescence (Chapin et al. 1990). The main reallocatable carbon components in trees are non-structural carbohydrates (NSC), and the NSC concentration is always higher in leaves (carbon source) than in shoots, stem, and roots (carbon sink) of trees (Hoch & Körner 2003, Li et al. 2008a, Gruber et al. 2011). Similarly, leaf nutrient (*e.g.*, N and P) concentration is also always higher in leaves than in shoots (*e.g.*, Körner 1989, Li et al. 2008b). At the end of the growing season, resources can be reallocated from leaves to shoots to increase the resource use efficiency. Resource remobilization efficiency can be higher at low nutrient availability or in low-temperature environment (Pugnaire & Chapin 1992, Bridgham et al. 1995), representing an adaptation to the less suitable habitats (Chapin 1980). A recent review (Etienne et al. 2018) and a research paper (Maillard et al. 2015) indicated that, during leaf senescence, N and P were always remobilized from leaves to shoots, irrespective of plant species, and environmental factors, such as drought and nutrient deficiency, modified the remobilization efficiency. Despite the importance of such processes, it is still unclear whether elevation affects remobilization of nutrients and carbohydrates, and whether

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(1) School of Geographical Sciences, Northeast Normal University, Changchun 130024 (China); (2) Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou 318000, Zhejiang (China); (3) School of Natural Resources, University of Missouri, Columbia, MO 65211 (USA); (4) School of Nature Conservation, Beijing Forestry University, Beijing 100083 (China); (5) Dipartimento di Agricoltura, Ambiente e Alimenti, Università degli Studi del Molise, v. de Sanctis, 86100 Campobasso (Italy); (6) European Forest Institute (EFI) Project Centre on Mountain Forests (MOUNTFOR), v. E. Mach 1, I-38010 San Michele all'Adige (Italy); (7) Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zuercherstrasse 111 CH-8903 Birmensdorf (Switzerland)

@ Hong S He (hehs100@nenu.edu.cn)

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this elevation-remobilization relation differs between deciduous and evergreen species.

The patterns of resource remobilization differ between deciduous and evergreen species (Chapin et al. 1990, Aerts 1996). Larger allocation to storage in deciduous than in evergreen species reflects the more pronounced asynchrony of supply and demand undergone by deciduous species (Chapin et al. 1990), since storage of carbohydrates is particularly important for deciduous trees that enter early spring without photosynthesizing tissue, with later sink to source transition in comparison with evergreen trees (Marchi et al. 2005a, 2005b). In early spring, deciduous species make new leaves at low carbon cost per unit leaf area, but at high cost in terms of carbohydrate reserves (Dickson 1989). In evergreen species, carbohydrates can be supplemented through photosynthesis of leaves from previous-years leaf cohorts (Epron et al. 2012). Carbon construction cost of evergreen leaves, being rich in carbon-based compounds, is relatively high (Pearcy et al. 1987), and only a portion of leaves is renewed annually.

Remobilization of plant's N also differs between leaf habits of the species (Cherbuy et al. 2001). Nitrogen resorption efficiency is much higher in deciduous than in evergreen shrubs and trees, while P resorption from senescing leaves does not significantly differ between evergreen and deciduous species (Aerts 1996). In contrast to deciduous species that recycle N back into storage organs before leaf abscission, evergreen species can directly retain resources in over-wintering leaves (Chapin et al. 1990, Wyka et al. 2015). Indeed, N is stored mainly in the youngest age class of foliage in evergreen species (Millard & Proe 1992). However, exceptions seem to exist, for example, no major differences in patterns of N and P distribution have been shown between deciduous and evergreen Alaskan tree species (Chapin & Kedrowski 1983).

We investigated differences in resource remobilization between deciduous and evergreen species. Evergreen trees may maintain the resources directly in over-wintering leaves to reduce the costs of resource transport in the fall and next early spring, whereas deciduous trees need to reallocate resources from leaves to storage tissues in the fall to increase the resource use efficiency. In particular, we tested the hypotheses that: (1) both deciduous and evergreen trees reallocate resources from leaves to shoots at the end of growing season, but the former have higher resource remobilization than the latter; and (2) regardless of foliar habit, resource remobilization increases with increasing elevation (*e.g.*, harsher physical environments and earlier leaf senescence). We used elevational gradients as proxy of changed environmental conditions and of shift in leaf phenology (Körner 1999). Two broad-leaved tree species, a deciduous (*Betula ermanii* Cham.) and an evergreen (*Quercus aquifolioides* Rehder & E.H. Wilson) species, growing along elevational gradients up to their upper limits were chosen for the present study. We analyzed: (1) the concentrations of NSC, N and P in 1-year-old leaves and 1-year-old shoots; (2) the differences in NSC, N and P between leaves and shoots; and (3) the remobilization efficiency from leaves to shoots in both tree species along two elevational gradients in China.

Materials and methods

Sites description and species

The study was conducted in west Changbai Mountain, which is located in the Changbai Mountain Natural Reserve (41° 59′ N, 127° 59′ - 128 °E) in Northeast China, and south Balang Mountain, which is located in the Wolong Natural Reserve (30° 53′ N, 102° 57′ E), at the eastern edge of Qinghai-Tibetan Plateau in Southwestern China.

At the Changbai Mountain site, the treeline elevation ranges from 2000 to 2030 m a.s.l., where groups of trees with height greater than 3 m have the deciduous broad-leaved *Betula ermanii* as the dominant species. Above 2030 m, the distribution of *B. ermanii* is scattered and can reach up to about 2200 m (upper limit – Tab. 1). The growing season at the treeline is generally from late May to late September (the first severe frost). According to long-term climate data collected in Tian-Chi Meteorological Station located at 2623 m a.s.l., the climate varies greatly from year to year. For instance, mean growing season temperature ranges from 3.37 to 8.82 °C (mean temperature is 5.87 °C), and average precipitation ranges from 700 to 1400 mm, the annual frost-free period varies from 65 to 70 days, and the snow cover period is about 199-219 days at 2623 m a.s.l. Soils are classified as mountain soddy forest soil.

At the Balang Mountain site, pure evergreen broad-leaved *Quercus aquifolioides* trees range from 2800 to 3600 m a.s.l. (upper limit – Tab. 1). This naturally generated tree stand is 30-35 years old and comprises multi-stemmed clumps. A nature reserve was established in 1976, and the *Q. aquifolioides* stands have not been disturbed since then. The study area has a subtropical inland mountain climate with sunny, dry winter (November to April) and warm, humid summer (June to September). According to long-term climate data collected at 1920 m a.s.l. (Wolong Nature Reserve Authority), the annual mean precipitation is 995 mm, with rainfall mainly occurring from May to September, and the annual mean temperature is 12.8 °C, with the monthly mean temperature of 17.0 °C in July and -1.7 °C in January. Soils are classified as mountain brown soil.

Tab. 1 - Characteristics of the plots and the sampling trees *Betula ermanii* (mean ± standard deviation; *n* = 5 trees), *Quercus aquifolioides* (mean ± standard deviation; *n* = 6 trees) located in the Changbai Mountain (Jilin, NE China) and Balang Mountain (Sichuan, SW China), respectively. (a): Basal diameter (about 1 cm above the ground surface).

Species and Site No.	Elevation (m)	Average		Slope exposure
		DBH (cm)	Height (m)	
Betula ermanii Site 1	2187	1.1 ± 0.5[a]	0.4 ± 0.1	West
	2137	1.6 ± 0.5[a]	0.6 ± 0.1	West
	2097	1.5 ± 0.3[a]	0.9 ± 0.1	West
	2027	4.9 ± 0.7	4.7 ± 0.3	West
	1977	28.4 ± 2.8	14.9 ± 0.9	West
Quercus aquifolioides Site 2	3589	5.5 ± 1.0	1.8 ± 0.6	South
	3441	7.4 ± 1.0	2.8 ± 0.5	South
	3327	5.1 ± 1.7	2.8 ± 0.9	South
	3159	5.6 ± 2.0	2.8 ± 0.9	South
	2978	7.1 ± 2.8	3.1 ± 0.7	South
	2843	8.2 ± 2.0	3.7 ± 0.3	South

Field sampling

To compare the differences in concentrations of NSC and nutrients between leaves and shoots, and then to calculate the end-season remobilization rate between tissues, samples were taken at the end of growing season (September for Changbai Mt. in northeastern China and November for Balang Mt. in southwestern China), because end-season tissue NSC and nutrient concentrations are relatively stable (Hoch et al. 2002). We sampled 1-year-old leaves and 1-year-old shoots from six plots (*n* = 6) for *Q. aquifolioides* at each of six elevations along an elevational gradient, *i.e.*, 2843, 2978, 3159, 3327, 3441, and 3589 (± 5) m a.s.l., and from five plots (*n* = 5) for *B. ermanii* at each of five elevations along an elevational gradient, *i.e.*, 1977, 2027, 2097, 2137, and 2187 m a.s.l. Several healthy trees of similar age, as revealed by their uniformity in height and diameter at breast height (DBH) were selected for tissue collection in each plot. From each selected tree, we collected 4-8 pieces of 1-year-old leaves and 1-year-old shoots from 4 crown directions. Leaves and shoots collected in a plot were pooled for a mixed leaf sample and shoot sample, respectively. To diminish the influence of diurnal temperature range and light differences, all samples were collected in the period between noon and 2 p.m. and immediately stored in a cool box. The samples were heated in a microwave oven at 600 W for 40 s, dried to a constant mass at 65-75 °C (Li et al. 2008b), ground to powder, and stored after being sealed with silica gel at 4 °C for analysis. In each plot, we measured the mean height and DBH of each tree (Tab. 1). For juvenile trees with small DBH, basal diameter (about 1 cm above ground surface) was measured.

Analysis of non-structural carbohydrates (NSC)

To compare NSC and their composition (sugar:starch ratio) at different elevations, we measured the concentration of NSC using the anthrone colorimetric method, because few samples are required for micro-analysis (Dubois et al. 1956). Dried 1-year-old leaves and 1-year-old shoots were respectively ground to pass a 1 mm sieve. For each sample, 0.1 g of the powdered material was placed into a 10 ml centrifuge tube and mixed with 5 ml of 80 % ethanol. The mixture was incubated in a water shaker at 80 °C for 30 min, cooled to ambient temperature, and then centrifuged at 4000 rpm for 10 min. The sediments were re-extracted twice with 80 % ethanol to extract the soluble sugars. The ethanol-insoluble pellet was used for starch extraction, and the combined supernatants retained for soluble sugar determinations by the anthrone method. Glucose was used as a standard. Starch was extracted from the solid residues after placing in water at 80 °C to remove the ethanol by evaporation. The ethanol-insoluble residues were boiled with 2 ml of distilled water for 15 min. After cooling to room temperature, 2 ml of 9.2M $HClO_4$ was added for 15 min to hydrolyze the starch, 4 ml distilled water was added and mixed, and then the mixture was centrifuged at 4000 rpm for 10 min. Subsequently, the solid residues were added with 2 ml of 4.6M $HClO_4$ for a final extraction. Soluble sugars and starch concentrations were determined at 620 nm using a 721 spectrophotometer. The concentrations of soluble sugars, starch, and NSC were calculated on a dry matter basis (% d.m.).

Analysis of nitrogen and phosphorus

Oven-dried samples were ground to pass a 1 mm sieve. For each sample, 0.1 g of plant tissue was digested in 5 ml of H_2SO_4 and heated to boiling for 15 min. After cooling to room temperature, the digestion solution was added 2 ml H_2O_2 and then boiled for 15 min. The procedure described earlier was repeated until a colorless transparent liquid was produced. The total N concentration (% d.m.) was determined on a semi-automatic azotometer, and phosphorus was determined at 450 nm using a 721 spectrophotometer (Wang et al. 2017).

Methods for evaluating resource remobilization

To clarify the remobilization of mobile carbohydrates and nutrients from leaves to shoots differences between deciduous and evergreen species at the end of growing season concentrations were expressed relatively to a constant parameter. Therefore, mobile carbohydrates, sugars, starch, N, and P remobilization efficiency (%) was calculated as (eqn. 1):

$$R\% = \frac{C_l - C_s}{C_l}\cdot 100 = 1 - \frac{C_s}{C_l}\cdot 100 \qquad (1)$$

where C_l and C_s represent concentrations of NSC, N, or P in leaves and shoots at the end of growing season, respectively. A negative R-value ($C_l < C_s$) indicates accumulation of NSC, N, or P in shoots, whereas a positive R-value ($C_l > C_s$) reflects the direction of resource fluxes. The larger the R-value, the lower remobilization efficiency is.

Data analysis

Given the large difference in elevation of the upper limit of the studied species, irrespective of the length of the growing season, the growing season soil (-10 cm) temperatures at their upper limit are the same being 6.5 ± 0.8 °C (Körner & Paulsen 2004), which makes the present north-south comparison reasonable. NSC, soluble sugars, starch, N and P data were confirmed for normality (Kolmogorov-Smirnov test) before statistic analysis. Three-way analyses of variance (ANOVAs) were used to test the effects of elevation, species, and type of tissue, and their two-way and three-way interactions on the concentrations of NSC, sugars, starch, N, and P, as well as on sugars/starch ratio and N/P ratio. At the species level, two-way ANOVAs were repeatedly performed with elevation and tissue type as factors to test the elevational trends in parameters mentioned above. Two-way ANOVAs were performed with elevation and species as factors to identify the trends in the remobilization efficiency of NSC, sugars, starch, N and P. Differences in the studied parameters were tested for significance using Duncan's test or Tukey's HSD test at $\alpha = 0.05$. Since we were interested in the effects of species and elevation on NSC, sugars, starch, N and P, other effects such as the interaction of elevation × tissue type and the interaction of elevation × tissue type × species are not systematically discussed.

Results

Species-specific responses

Elevation significantly affected tissue concentrations of soluble sugars, starch, NSC, N, and P, but it did not influence tissue sugars/starch ($p = 0.463$) and N/P ratios ($p = 0.232$ – Tab. 2). All parameters differed significantly between deciduous and evergreen tree species (Tab. 2). Tissue type had a significant effect on the concentrations of soluble sugars, NSC, and N, as well as on sugars/starch ratio, whereas it had no effects on starch, P concentrations, and N/P ratio (Tab. 2). The elevation × species interaction was significant for soluble sugars, starch, NSC, and P concentrations, but not for N concentration, and sugars/starch and N/P ratios (Tab. 2). The interaction between tissue type and species was significant for all parameters, except for P ($p = 0.763$ – Tab. 2).

Elevation significantly affected the remobilization efficiency of sugars, starch, NSC and N, but not P (Tab. 3). The remobilization efficiency of sugars, NSC, N and P differed significantly between deciduous and

Tab. 2 - Results of three-way nested ANOVAs with elevation, species, tissue type as factors. F and Prob values are given.

Factors	Soluble sugars		Starch		NSC		Nitrogen (N)		Phosphorus (P)		Sugars/ Starch		N / P	
	F	*Prob*	*F*	*Prob*	*F*	*Prob*	*F*	*Prob*	*F*	*Prob*	*F*	*Prob*	*F*	*Prob*
Elevation (E)	2.98	0.015	51.78	<0.001	19.91	<0.001	991.69	<0.001	186.12	<0.001	0.93	0.463	1.40	0.232
Species (S)	494.79	<0.001	64.23	<0.001	205.03	<0.001	258.08	<0.001	10.30	0.002	652.27	<0.001	19.20	<0.001
Tissue type (T)	444.99	<0.001	1.81	0.182	286.78	<0.001	3.11	0.012	0.34	0.886	331.66	<0.001	2.07	0.154
E × S	18.88	<0.001	19.90	<0.001	5.00	0.001	3.04	0.085	30.83	<0.001	1.53	0.199	0.81	0.519
T × S	804.41	<0.001	5.71	0.019	517.92	<0.001	3.28	0.009	0.52	0.763	315.12	<0.001	16.45	<0.001
E × T	17.99	<0.001	9.87	<0.001	20.23	<0.001	5.35	0.001	2.19	0.076	0.82	0.54	0.42	0.832
E × T × S	4.07	0.004	4.34	0.003	5.09	0.001	2.58	0.042	0.89	0.475	0.91	0.46	0.53	0.716

Tab. 3 - Results of two-way nested ANOVAs with elevation and species as factors. The F and Prob values are given. R refers to remobilization efficiency.

Factors	R sugars		R starch		R NSC		R Nitrogen		R Phosphorus	
	F	*Prob*	*F*	*Prob*	*F*	*Prob*	*F*	*Prob*	*F*	*Prob*
Elevations (E)	18.69	<0.001	9.20	<0.001	50.98	<0.001	4.78	0.001	0.74	0.600
Species (S)	239.56	<0.001	0.11	0.742	430.02	<0.001	16.21	<0.001	69.29	<0.001
E × S	20.31	<0.001	8.16	<0.001	46.16	<0.001	2.93	0.030	0.81	0.528

Tab. 4 - Results of two-way nested ANOVAs with elevation and tissue type as factors. The F and Prob values are given.

Species	Factor	Soluble sugars		Starch		NSC		Nitrogen (N)		Phosphorus (P)		Sugars/ Starch		N / P	
		F	*Prob*	*F*	*Prob*	*F*	*Prob*	*F*	*Prob*	*F*	*Prob*	*F*	*Prob*	*F*	*Prob*
Betula ermanii	Elevations (E)	11.93	<0.001	3.47	0.018	12.74	<0.001	3.71	0.013	2.28	0.080	0.60	0.670	4.38	0.006
	Tissue types (T)	925.90	<0.001	23.95	<0.001	557.60	<0.001	59.85	<0.001	6.19	0.018	184.12	<0.001	53.52	<0.001
	E × T	4.54	0.005	0.11	0.979	3.43	0.019	2.31	0.078	0.32	0.861	0.51	0.731	1.92	0.129
Quercus aquifolioides	Elevations (E)	7.91	<0.001	62.72	<0.001	13.48	<0.001	1.52	0.198	1.07	0.384	45.96	<0.001	1.27	0.288
	Tissue types (T)	40.33	<0.001	1.79	0.186	23.37	<0.001	325.37	<0.001	37.67	<0.001	6.25	0.015	13.17	0.001
	E × T	21.36	<0.001	12.00	<0.001	26.79	<0.001	2.57	0.036	0.96	0.448	7.40	<0.001	0.58	0.716

evergreen tree species (Tab. 3). The elevation × species interaction was significant for all parameters, except for P ($p = 0.528$) (Tab. 3).

Betula ermanii on Changbai Mountain

Tissue NSC concentration of *B. ermanii* was significantly affected by both elevation and tissue type (both $p < 0.001$ – Tab. 4). Leaf sugars (Fig. 1a) and NSC (Fig. 1g) decreased significantly with increasing elevation (both $p < 0.05$), except for trees at 1977 m a.s.l. However, the concentrations of sugars (Fig. 1b) and NSC in shoots (Fig. 1h) tended to have lower levels at the mid-higher elevations (2097 and 2137 m a.s.l.). In particular, the sugar and NSC concentrations were always significantly higher in leaves (Fig. 1a, Fig. 1g) than in shoots ($p < 0.001$ – Fig. 1b, Fig. 1h, Tab. 4).

Both elevation and tissue type had significant effect on starch concentration (Tab. 4). Both leaf and shoot starch concentration showed the lowest level in trees at 2137 m a.s.l. (Fig. 1d, Fig. 1e). In contrast to sugars (Fig. 1a, Fig. 1b) and NSC (Fig. 1g, Fig. 1h), the starch concentration was always significantly lower in leaves than in shoots ($p < 0.001$ – Tab. 4, Fig. 1d, Fig. 1e), and thus, leaves had significantly higher sugars/starch ratio than shoots (Fig. 1j, Fig. 1k), and the ratio did not change with elevation within a tissue type (Fig. 1j, Fig. 1k).

Elevation had no effect on tissue N (Fig. 2a, Fig. 2b) and P concentrations (Fig. 2d, Fig. 2e), except for a lower shoot N con-

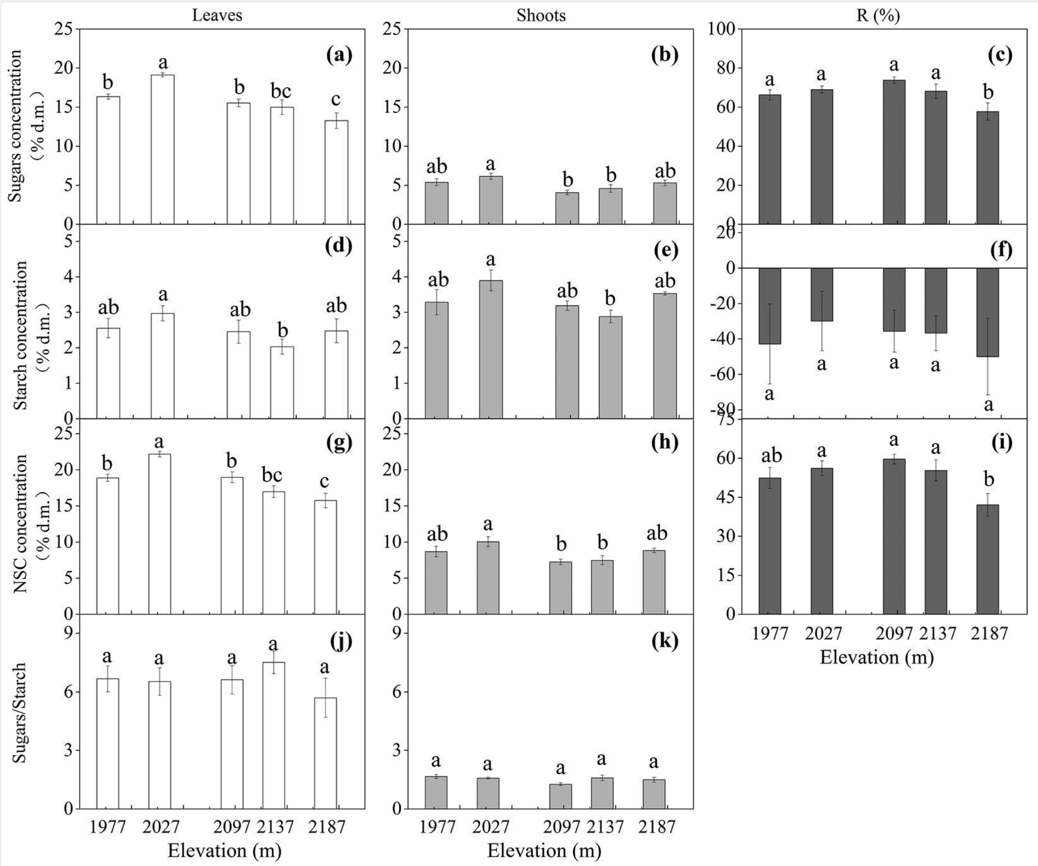

Fig. 1 - Tissue mean concentration (mean ± SE; % of dry matter) of soluble sugars, starch, non-structural carbohydrates (NSC), sugars/starch ratio, and remobilization efficiency (R %) in *Betula ermanii* trees grown along the altitudinal gradients on Changbai Mountain ($n = 5$ for each elevational site and tissue type). Different letters indicate significant differences ($p<0.05$) among elevations as determined by Duncan test single-factor ANOVAs.

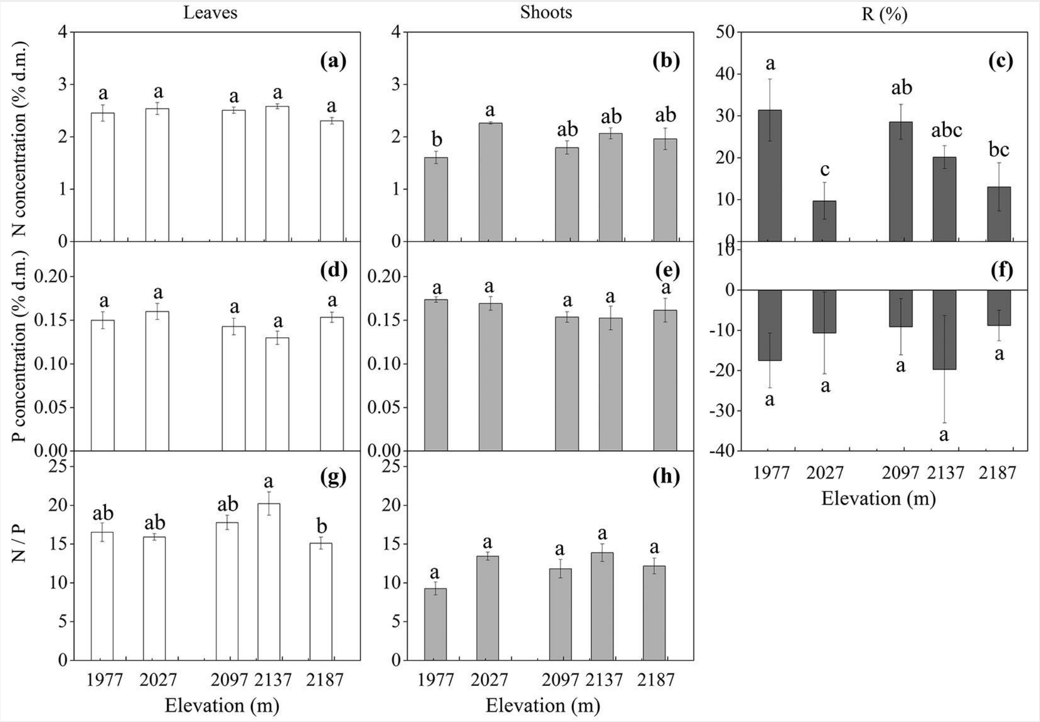

Fig. 2 - Tissue mean concentration (mean ± SE; % of dry matter) of total nitrogen (N) and phosphorus (P), N/P ratio, and remobilization efficiency (R %) in *Betula ermanii* trees grown along the altitudinal gradients on Chanbai Mountain (*n* = 5 for each elevational site and tissue type). Different letters indicate significant differences ($p<0.05$) among elevations as determined by Tukey's HSD test single-factor ANOVAs.

centration in trees at 1977 m a.s.l. (Fig. 2b). Similarly, tissue N/P ratio also did not change with elevation (Fig. 2g, Fig. 2h), except for a lower leaf N/P ratio in trees at 2187 m a.s.l. (Fig. 2g). The N/P ratio was higher in leaves than in shoots (Fig. 2g, Fig. 2h). The mean N concentration was significantly higher in leaves than in shoots ($p < 0.001$ – Tab. 4, Fig. 2a, Fig. 2b). In contrast to N, the mean P concentration was significantly higher in shoots than in leaves ($p = 0.018$ – Tab. 4, Fig. 2d, Fig. 2e).

Trees at the highest elevation (2187 m a.s.l.) remobilized the highest quantities of sugars (Fig. 1c) and NSC (Fig. 1i) from leaves to shoots (both $p < 0.05$), compared to trees at other elevations (Fig. 1c, Fig. 1i). The remobilization efficiency of starch from leaves to shoots did not change with elevation (Fig. 1f). The N remobilization from leaves to shoots tended to increase with increasing elevation (Fig. 2c), whereas the P remobilization did not change with elevation (Fig. 2f).

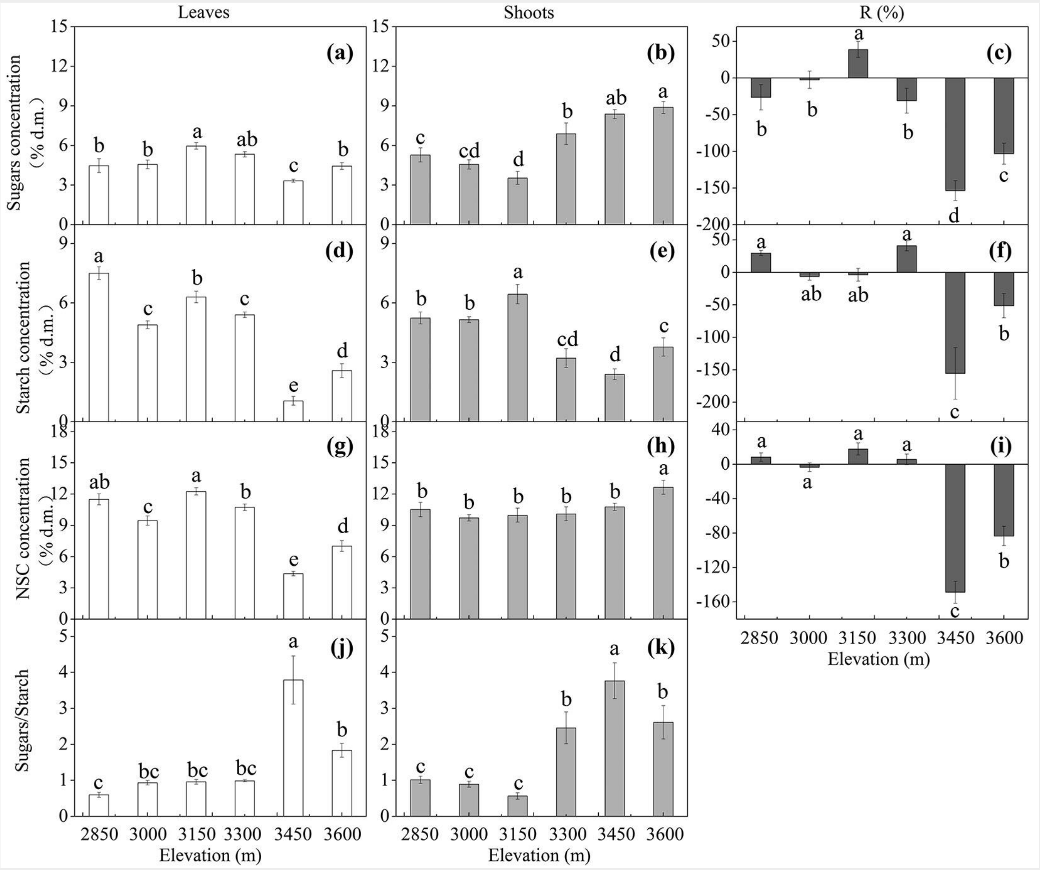

Fig. 3 - Tissue mean concentration (mean ± SE; % of dry matter) of soluble sugars, starch, non-structural carbohydrates (NSC), sugars/starch ratio, and remobilization efficiency (R %) in *Quercus aquifolioides* trees grown along the altitudinal gradients on Balang Mountains (*n* = 6 for each elevational site and tissue type). Different letters indicate significant differences ($p<0.05$) among elevations as determined by Duncan test single-factor ANOVAs.

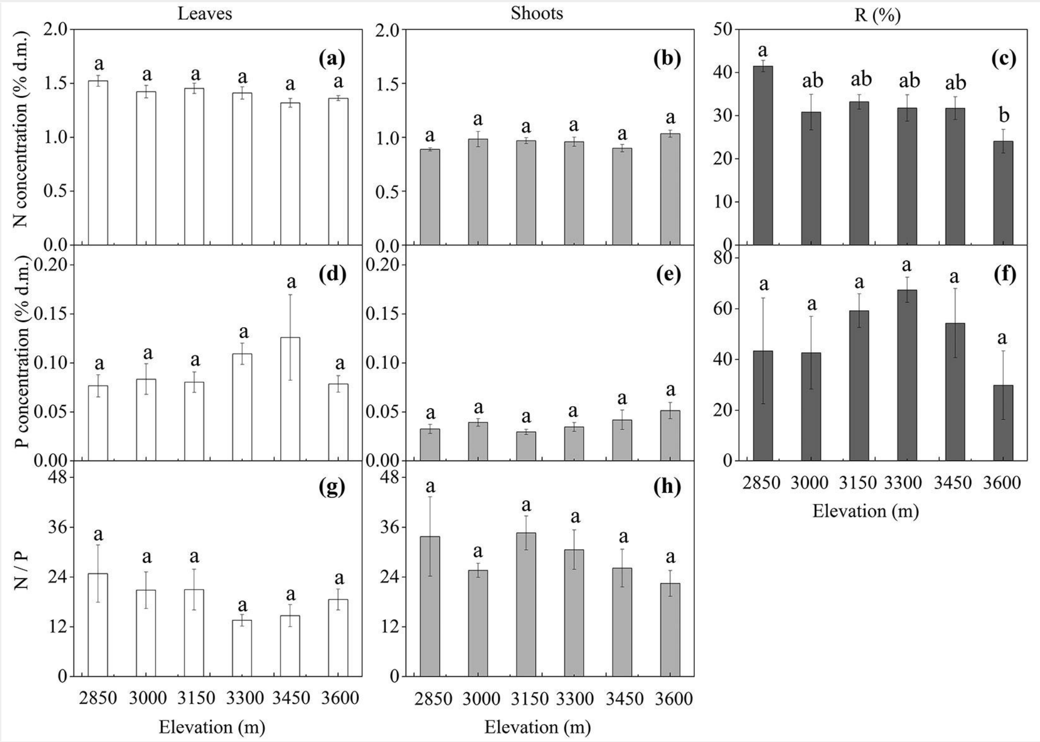

Fig. 4 - Tissue mean concentration (mean ± SE; % of dry matter) of total nitrogen (N) and phosphorus (P), N/P ratio, and remobilization efficiency (R %) in *Quercus aquifolioides* trees grown along the altitudinal gradients on Balang Mountains (*n* = 6 for each elevational site and tissue type). Different letters indicate significant differences ($p<0.05$) among elevations as determined by Tukey's HSD test single-factor ANOVAs.

Quercus aquifolioides on Balang Mountain

The concentration of leaf sugars had a relatively stable level across the elevational gradient, except for a higher level in trees at 3150 m a.s.l. and a lower level at 3450 m a.s.l. (Fig. 3a), whereas the concentration of shoot sugars tended to increase with increasing elevation (Fig. 3b). Leaf NSC concentration decreased with increasing elevation, with a 39 % decrease between the lowest and highest elevation (Fig. 3g). Shoot NSC concentration was relatively stable across the elevational gradient, with an exceptional higher shoot NSC level in trees at the upper limit (Fig. 3h). Mean NSC concentrations were significantly higher in shoots than in leaves ($p < 0.001$ – Tab. 4, Fig. 3g, Fig. 3h). The starch concentration was significantly affected only by elevation ($p < 0.001$ – Tab. 4). The starch concentration in both leaves and shoots tended to decrease with increasing elevation (Fig. 3d, Fig. 3e). The sugars/starch ratio increased significantly with elevation for the two tissue types ($p < 0.001$), and mean sugars/starch ratio was significantly higher in shoots than in leaves ($p = 0.015$ – Tab. 4, Fig. 3j, Fig. 3k).

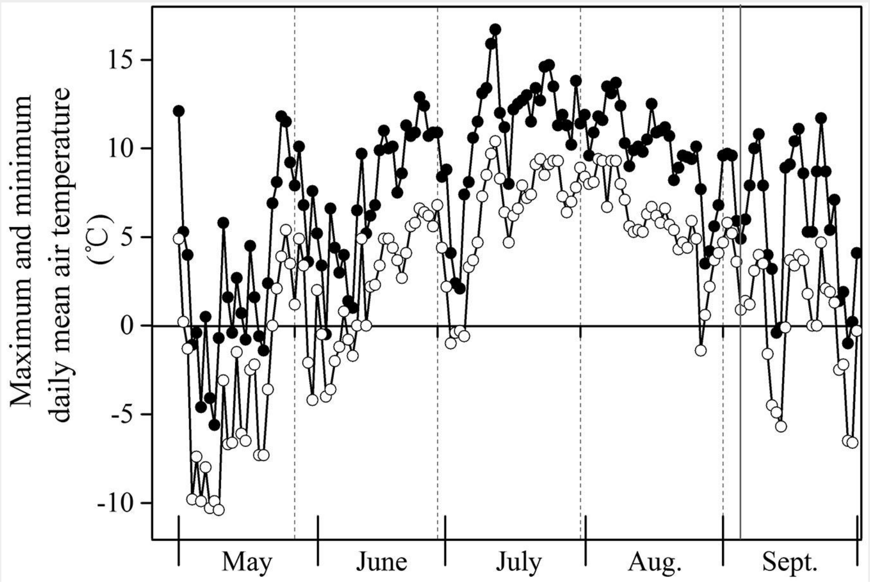

Fig. 5 - Maximum and minimum daily mean air temperature (°C) at Changbai Mountain from late May to late September. Vertical grey line indicates the sampling date.

The N (Fig. 4a, Fig. 4b) and P concentrations (Fig. 4d, Fig. 4e), as well as the tissue N/P ratio (Fig. 4g, Fig. 4h), in both leaves and shoots were not affected by elevation. Only tissue type significantly affected N ($p < 0.001$), P ($p < 0.001$), and N/P ratio ($p = 0.001$ – Tab. 4). The N (Fig. 4a, Fig. 4b) and P concentration (Fig. 4d, Fig. 4e) was significantly higher in leaves than in shoots, whereas the mean N/P ratio was higher in shoots than in leaves (Fig. 4g, Fig. 4h).

The remobilization of sugars, starch, and NSC from leaves to shoots showed similar elevational pattern (Fig. 3c, Fig. 3f, Fig. 3i). Trees at higher elevations (3450 and 3600 m a.s.l.) remobilized more soluble carbon components from leaves to shoots, compared to trees at lower elevations (Fig. 3c, Fig. 3f, Fig. 3i). The N and P remobilization efficiency was relatively stable across the elevational gradient, with an exception for higher N remobilization in trees at the upper limit (3600 m a.s.l.) than in trees at lower elevations (Fig. 4c, Fig. 4f).

Discussion

End-season remobilization of soluble carbon components

Regardless of leaf habit, the concentration of NSC in leaves tended to decrease with increasing elevation (Fig. 1g, Fig. 3g), while being relatively stable across the elevational gradients in shoots (Fig. 1h, Fig. 3h). Richardson (2004) and Li et al. (2008a) showed that plants suffering from environmental stress had lower tissue NSC concentrations. However, Li et al. (2013) found that plants grown with low water availability had higher tissue NSC concentrations, because drought may restrict plant growth rather than reduce the rate of photosynthesis, leading to an accumulation of NSC (Runion et al. 1999). Contradicting results on the variation of NSC, sugars, and starch concentrations with elevation are reported in the literature (increase, steady, decrease – Hoch & Körner 2003, Li et al. 2008a, Gruber et al. 2011).

Our results indicated that mobile carbohydrates for both deciduous and evergreen tree species were remobilized from leaves to shoots at the end of season, especially in the evergreen species (Fig. 1c, Fig. 1f, Fig. 1i, Fig. 3c, Fig. 3f, Fig. 3i). This finding partly confirmed our first hypothesis that both deciduous and evergreen trees reallocate carbon resources from leaves to shoots at the end of growing season. Leaf carbon components decreased at the end of growing season in order to maximize resource allocation, *i.e.*, during leaf senescence, recycling nutrients from assimilating leaves back to storage organs (Chapin et al. 1990). Stored resources may support new leaf and shoot growth after dormancy (Yan et al. 2016), as well as defense and reproduction (Chapin et al. 1990). Many studies have shown that leaves play a major role in resources remobilization (Millard 1996, Eckstein et al. 1998, Cherbuy et al. 2001). However, few studies have revealed that woody tissues of evergreen trees could be potential carbon (Larcher & Thomaser-Thin 1988) or N pools (Roberts et al. 1991). The availability of carbohydrates is strictly dependent on and connected to the growth and development of leaves and shoots, the partitioning of carbohydrates being determined by resource availability, growth capacity and maintenance requirement (Marchi et al. 2007). Several studies have highlighted the function of leaves as storage of nutrients, and resources remobilized from leaves to support new growth (Aerts 1996, Cherbuy et al. 2001). The evidence showing that leaves have a primary role in resource remobilization in comparison with woody tissues seems to be overwhelming.

We expected that the deciduous species had higher resource remobilization efficiency from leaves to shoots at the end of the growing season than the evergreen species. However, our results revealed a significantly lower mean remobilization efficiency of mobile carbohydrates from leaves to shoots in *B. ermanii* than in *Q. aquifolioides* at the end of the growing season (p <0.001 – Fig. 1c, Fig. 1f, Fig. 1i *vs.* Fig. 3c, Fig. 3f, Fig. 3i). In early season, deciduous trees have no photosynthesizing tissues and rely on storage, whereas in evergreen species carbohydrates can be supplied by photosynthesis of previous-years leaf cohorts (Epron et al. 2012). We observed a tendency for pronounced lower remobilization efficiency of soluble sugars in deciduous trees (p <0.001 – Fig. 1a). No significant differences in starch remobilization efficiency were observed between these tree species (p = 0.742 – Tab. 3). Contrary to our results, lower carbohydrate contents allocated to storage were more frequently found in evergreen than in deciduous species (Wyka et al. 2015). A possible reason explaining results of the present study was the failure of the deciduous trees to efficiently remobilize carbohydrates reserves to storage. Previously, Ziegler (1964) found that carbohydrates reserves can be inaccessible to storage organs, since they are in dead cells and, therefore, impossible to be retrieved. Pattern of air temperature at Changbai Mt. confirmed this view (Fig. 5). There was a sudden change in temperature, with minimum daily air temperature below zero on August 26, before our sampling date. Due to the effect of flash freezing in cold temperate mountains, carbohydrates in leaves were difficult to remobilize, and were probably forced to storage. In addition, we found that sugars accounted for a larger proportion of NSC than starch did, in both leaves and shoots of deciduous and evergreen trees (Fig. 1 and Fig. 3). Similar results have been reported for other temperate deciduous and evergreen species (Shi et al. 2008, Yu et al. 2014). It must be bear in mind, however, that concentration of carbohydrates alone does not allow plant allocation patterns to be completely understood, storage tissues being distributed in roots as well as in stems. Yet, it is possible that allocation patterns vary during the season.

The remobilization efficiency of sugars, starch and NSC was significantly higher in trees at their upper limit compared to lower elevations (Fig. 1c, Fig. 1i, Fig. 3c, Fig. 3f, Fig. 3i), except for starch in *B. ermanii* (Fig. 1f). This finding partly confirmed our second hypothesis. The remobilization of soluble carbon components from leaves to shoots increased at the upper limit, which may help trees to withstand low temperature at high elevations. Soluble carbon components may act as a signal molecule to adapt to environmental changes (Sheen et al. 1999), because they participate in cell osmotic regulation and prevent intracellular ice formation through lowering cytoplasm freezing point (Morin et al. 2007), to ensure tree survival in winter. Therefore, trees growing at the treeline depend not only on mobile carbohydrates to maintain respiration and growth (Körner 2003), but also on accumulated soluble sugars to prevent intracellular ice formation in winter, which can severely injure trees (Morin et al. 2007). At the end of season, sugars (and starch to sugar conversion) can be unloaded in the direction of sites of storage within the xylem (Brodersen & Mcelrone 2013), to be used as osmoticum for refilling of freeze-thaw induced embolism when the growth season starts. Accumulation of sugars would prime stems for recovery from winter embolism, when stress is relieved, thus maintaining long-distance and long-lasting water transport tissues, *i.e.*, requiring high carbon investments.

Deciduous trees showed a significantly higher mean sugars:starch ratio in tissues (4.33) than evergreen ones (1.70 – Tab. 2). Moreover, we found that sugars:starch ratio in leaves and shoots of *Q. aquifolioides* increased significantly with elevation. A sugar-starch system that adjusts this ratio in response to low temperature environment can be hypothesized in these trees (Li et al. 2008a). Patton et al. (2007) revealed that the sugar:starch ratio in *Zoysia* spp. was positively correlated with cold hardiness, indicating that sugar:starch ratio actively adapted to environmental conditions.

End-season remobilization of nitrogen and phosphorus

There were no elevational effects on tissue N and P concentrations in both tree species (Fig. 2a, Fig. 2b, Fig. 2d, Fig. 2e, and Fig. 4a, Fig. 4b, Fig. 4d, Fig. 4e), except for a lower shoot N concentration in *B. ermanii* at 1977 m a.s.l., suggesting similar supply and demand ratio. Previous studies have proposed that nutrient accumulation and conservation are adaptive responses that enhance metabolic activity and growth rates in cold climates (Oleksyn et al. 1998, Körner 1999). Kutbay & Ok (2003) reported, however, that absolute and proportion N and P resorption rates did not change significantly along elevational gradients (Körner 1989, Li et al. 2008b, Li et al. 2018), which is consistent with our results.

Nutritional elements are crucial to growth, particularly N and P, which are the most important growth limiting nutrients (Chapin 1980). N and P that support reproduction come largely (about 50 %) from recycling of nutrients from senescing leaves in deciduous as well as evergreen trees (Chapin & Kedrowski 1983, Chapin et al. 1990, Aerts 1996). However, Vergutz et al. (2012) showed, after a leaf mass loss correction, general N and P average resorption of 62 % and 65 %, respectively. We observed a significant difference in N remobilization from senescing leaves to shoots between deciduous and evergreen trees; the N remobilization efficiency was significantly higher in deciduous than in evergreen trees (p <0.001 – Fig. 2c *vs.* Fig. 4c). In line with this result, Aerts (1996) reported that N resorption efficiency in deciduous shrubs and trees was higher than in evergreen ones. The patterns of N remobilization seem to differ between deciduous and evergreen species (Millard 1996). In contrast to deciduous species that recycle N back into storage organs before leaf abscission, evergreen species retain leaf N for newly developing shoots (Chapin et al. 1990, Wyka et al. 2015). The tendency for significantly higher mean P remobilization efficiency in deciduous species was also shown in our study (p <0.001 – Fig. 2f *vs.* Fig. 4f). The patterns of N and P remobilization supported our first hypothesis. Other authors, however, have observed that resorption efficiency does not differ significantly between evergreen and deciduous shrubs and trees (Eckstein et al. 1998).

N/P ratio has been applied to identify thresholds of nutrient limitation (Güsewell & Koerselman 2002). Based on studies of European wetland plants, thresholds of foliar N/P ratios are < 14 for N limitation and > 16 for P limitation (Güsewell & Koerselman 2002). According to this view, both *B. ermanii* and *Q. aquifolioides* canopies were relatively P-limited (Fig. 2g, Fig. 4g), although these N/P thresholds may indicate nutrient limitations when leaf N or P content is sufficient, but not when their content is deficient (Yan et al. 2017). Leaf N/P ratio did not decrease significantly with increasing elevation, suggesting that N and P limitation did not markedly vary within the present altitudinal range at the end of season. Due to the different remobilization strategy between evergreen and deciduous tree species, deciduous trees may remobilize more P from leaves to shoots under P-limited conditions than evergreen ones, which can be ecologically advantageous in soils that are naturally low in bioavailable P.

Conclusions

In line with our hypotheses, we found that mobile carbohydrates and nutrients (N and P), in both the deciduous and evergreen tree species, were remobilized from leaves to shoots at the end of season, and the remobilization efficiency was significantly higher in trees at the upper limit, helping trees to adapt to low temperature at high elevations. We also found that mobile carbohydrates, N and P had different patterns of leaf-to-shoot remobilization between the two species at the end of growing season. Compared to the deciduous *B. ermanii*, the evergreen species *Q. aquifolioides* remobilized more carbon components, but less N and P from leaves to shoots. Differences in resource remobilization between deciduous and evergreen woody species seem to be related to different strategies in using resources (Chapin & Kedrowski 1983), though they can be overridden by interspecies variability. Comparative studies on resource remobilization carried out under similar growth conditions warrant to draw general ecological patterns on species-specific storage processes.

The present study contributes to better understand how trees adapt to growth-limiting temperatures at the alpine treeline, showing that the trees of mountain environments differ in their storage physiology, which may implicate heterogeneous distributional responses to climate warming. A limitation of the present study is that we were not able to quantitatively estimate the pool size of resource remobilization (concentration × biomass) from leaves to shoots (Li et al. 2006), because destructive biomass sampling was not allowed for both tree species in those nature reserves. Further studies may take advantage of stable isotope labeling (*e.g.*, ^{13}C, ^{15}N) to accurately assess carbon and N remobilization from senescing leaves to shoots, and to determine whether other organs (*e.g.*, roots, seeds) contribute to internal resource remobilization (Millard & Proe 1992).

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